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Photophysiological Tolerance and Thermal Plasticity of Genetically Different Symbiodiniaceae Endosymbiont Species of Cnidaria

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Coral reefs are endangered by constantly rising water temperature due to global warming. This triggers a breakdown of the nutritional symbiosis between cnidarian hosts and their Symbiodiniaceae symbionts, resulting in the loss of the algal partner. In the Symbiodiniaceae exists a high genetic diversity with broad physiological plasticity within and between species, resulting in large thermal tolerance. While these variations have been studied in individual taxa, comprehensive comparative experimental data on numerous species are still rare. In the present study, the photosynthetic performance and tolerance as function of light and temperature of nine Symbiodiniaceae genetic types of four different clades were determined. The data indicate significant differences in the response patterns. Almost all algal isolates exhibited low to moderate light requirements for photosynthesis without photoinhibition, and a photosynthetic efficiency between 20 and 80% in the temperature range 20–34°C, indicating a broad thermal tolerance to temperature fluctuations in tropical regions. The presented data clearly point to a broad photophysiological tolerance and thermal plasticity of genetically different Symbiodiniaceae, which contributes as an important finding to a better understanding of host-symbiont response to an increasing sea surface temperature.

Keywords: irradiance, photosynthesis, respiration, Symbiodiniaceae, temperature, tolerance

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INTRODUCTION

Coral reefs are one of the most important ecosystems in the marine environment, as they are highly diverse and productive ecosystems (Connell, 1978; Moberg and Folke, 1999). The high productivity of coral reef systems is based on a mutualistic symbiosis between unicellular dinoflagellates (Symbiodiniaceae) and different invertebrate reef species, including corals, sea anemones and sponges among many others (Trench, 1993; Coffroth and Santos, 2005; Decelle et al., 2018).

The success of this symbiosis depends on complementary intracellular solute exchange such as nutrients and carbon compounds. The symbionts can provide up to 95% of photosynthetically fixed organic carbon in the form of glycerol, glucose and alanine to the host (Muscatine and Porter, 1977; Burriesci et al., 2012; Davy et al., 2012). In exchange the host offers protection against environmental stresses (e.g., herbivory) and delivers inorganic nitrogen

(Davies, 1984; Steen and Muscatine, 1984). Despite their immense ecological importance, coral reefs are diminishing worldwide due to various environmental stressors (Hoegh-Guldberg et al., 2007; Hughes et al., 2018, 2020). There are several factors, such as low salinity, water pollution, unusually high or low water temperatures or high solar irradiance causing coral bleaching (Van Oppen and Lough, 2018). When, for instance, the local temperature exceeds the upper physiological threshold of the host, the symbiosis collapses and "bleaching" occurs as a result of the loss of algal endosymbionts (Glynn and D'Croz, 1990). This impact has already been observed globally, with an increasing frequency of these "bleaching events" due to more frequent El-Nino events, particularly in recent years (Glynn, 1993; Eakin et al., 2010; Hughes et al., 2017, 2018, 2020). However, the probability of bleaching depends either on the host characteristics (Baird et al., 2009), on the symbiont ecophysiological traits (Rowan, 2004) or on the prevailing environmental conditions (Hoegh-Guldberg, 1999; Anthony et al., 2009). The flexibility of the coral host, in particular, to associate with different photosymbiont strains at the same time could be critical to cope with rapid environmental fluctuations (Baker, 2001; Berkelmans and Van Oppen, 2006; Abrego et al., 2008; Lewis et al., 2019; Qin et al., 2019).

Several studies suggested that the host might be able to acclimate to higher water temperatures by switching to more temperature-tolerant symbiont species or strains from the local environment or by distributing temperature-tolerant symbionts to make them more abundant (Buddemeier and Fautin, 1993; Baker, 2003; Fautin and Buddemeier, 2004; Correa and Baker, 2011; Cunning et al., 2018). Nevertheless, some investigations revealed that altered host-symbiont pairings often return to their original composition when sufficient time elapsed after a bleaching event (Thornhill et al., 2006; Sampayo et al., 2008). For example, this was found in several coral species at the Great Barrier Reef after a bleaching event in 2002. During bleaching, corals harbored the same Symbiodiniaceae as before and after the thermal stress events (Stat et al., 2009). This suggests that not all symbiotic strains are beneficial to the respective host (Stat et al., 2008; Starzak et al., 2014; Gabay et al., 2018) or that the competitive advantage varies with the environment (Thornhill et al., 2006; Jones et al., 2008; LaJeunesse et al., 2009). The Symbiodiniaceae are highly diverse and combine at least seven different genera, which were recently taxonomically revised (LaJeunesse et al., 2018). The six genera are *Breviolum*, *Cladocopium*, *Durusdinium*, *Effrenium*, *Fugacium*, and *Gerakladium*. Members of the Symbiodiniaceae have a different sensitivity to solar radiation and temperature fluctuations. The underlying mechanisms can be explained by biochemical and molecular processes of the photosynthetic machinery (Takahashi et al., 2008). The key pigments peridinin, chlorophyll *a* and *c*2 are integrated into the chloroplast membrane as the two major antennas *a*-chlorophyll *c*2-peridinin protein complex (acpPC) and peridinin-chlorophyll *a*-binding protein (PCP) (Brown et al., 1999; Takahashi et al., 2008; Niedzwiedzki et al., 2014; Hennige et al., 2019).

High irradiances and temperatures are factors contributing to the phenomenon of coral bleaching. High temperature contributes to an increased production of harmful reactive oxygen molecules (ROS), such as hydrogen peroxide (H_2O_2), singlet oxygen $^1O_2$ or superoxide O_2. ROS originate as a metabolic byproduct and are primarily produced by photosystem I and in parts of photosystem II (Szabó et al., 2020). ROS are chemically highly reactive and hence can, for example, mutate DNA, denature proteins and oxidize lipids as well as cell membranes (Takahashi et al., 2008, 2009; Venn et al., 2008; Lesser et al., 2010). As a result of ROS production and the cellular cascade, this can lead to a disruption of communication and interaction between the host and symbiont. ROS production is considered as the key element for the bleaching process (Weis, 2008; Baird et al., 2009; Szabó et al., 2020). Evidence suggests that the ROS leakage varies between species, as well as depending on temperature and salinity (Gegner et al., 2019). Various species of the Symbiodiniaceae acclimate their photosynthetic performance to different light conditions (Iglesias-Prieto and Trench, 1994; Hennige et al., 2009; Suggett et al., 2015). With elevated temperatures, various Symbiodiniaceae did not grow (Lesser, 1996), while other investigations reported the opposite (Sakami, 2000; Karim et al., 2015; Klueter et al., 2017). Karim et al. (2015), for example, that growth and photochemical efficiency of PSII remained unchanged between 25 and 30°C in various Symbiodiniaceae genera. At temperatures above 33°C, however, the thermal tolerance was exceeded. Further reports point to different genotypes of Symbiodiniaceae which are tolerant to specific temperatures and radiation conditions (Robison and Warner, 2006). Consequently, the tolerance width and upper survival temperature varies from species to species and genotype to genotype (Berkelmans and Van Oppen, 2006; Van Oppen et al., 2009). The genetic identity and ecophysiological capability of Symbiodiniaceae species play a major role in acclimation and adaptation to thermal stress. Several studies identified significant differences in heat tolerance both within a single Symbiodiniaceae genus (Díaz-Almeyda et al., 2017; Bayliss et al., 2019) and between genera (Grégoire et al., 2017). In addition, there are several species, such as *Durusdinum trenchii* (Rowan, 2004; Jones et al., 2008; Bellantuono et al., 2019) and *Cladocopium thermophilum* (formerly Class C, ITS2-"Golf C3") (Hume et al., 2015) which are considered thermotolerant. A genetic subtype of ITS2-D1a (LaJeunesse et al., 2018) within Clade D, gives coral hosts a 1–1.5°C increase in thermal tolerance (Rowan, 2004; Berkelmans and Van Oppen, 2006). A culture of *Cladocopium* C1 in a laboratory selection exhibited better photophysiology and growth at high temperature (31°C) compared to wild type cells (Chakravarti et al., 2017). However, alternating heat-resistant host-symbiont assemblages are associated with metabolic "costs," since the growth rates of the corals often decrease (Abrego et al., 2008).

The objective of this study was to examine the photosynthetic performance [net primary production], hyperthermal tolerance, acclimation capacity of several genotypes (strains), species, and genera of Symbiodiniaceae using oxygen optodes. Based on previous studies (Rowan, 2004; Jones et al., 2008; Bellantuono et al., 2019), in which *Durusdinium trenchii* exhibited a pronounced heat tolerance, we hypothesized that *D. trenchii* outperforms the other strains in terms of photosynthetic

efficiency as no loss of photosynthetic function occurs at higher temperature. A total of nine algal strains belonging to four genera and four species of Symbiodiniaceae were comparatively examined under controlled manipulative conditions: *Breviolum minutum* (4 strains), *Breviolum psygmophilum* (2 strains), *D. trenchii* (one strain), *Effrenium voratum* (one strain), and *Symbiodinium linucheae* (one strain).

MATERIALS AND METHODS

Origin and Maintenance of Symbiodiniaceae

The photophysiological properties and thermal tolerance of clonal cultures under controlled conditions were investigated in nine different Symbiodiniaceae genetic types from four different clades (A, B, D, and E) (LaJeunesse et al., 2018). These strains originated from the culture collection of the IMaGeS Lab (Dr. Rodriguez-Lanetty; originally started by Dr. Mary Alice Coffroth) of Florida International University Miami. The investigated isolates were isolated from four host species (seven individual hosts) collected in three biogeographic regions [Hawaii (Pacific), Florida (Caribbean), and Panama (Caribbean)]. In addition, *Effrenium voratum* was utilized as a free-living species from New Zealand waters (West Pacific) (**Table 1**). These lineages originate from locations with distinct thermal profiles and thus, have likely acquired specific adaptations to their local environments. For example, the strain *Breviolum minitum* SSB 01 from Hawaii (Pacific) is more temperature sensitive (Dang et al., 2019) compared to heat tolerant *Durusdinium trenchii* CCMP2556 (Bellantuono et al., 2019) from the Caribbean. All Symbiodiniaceae strains were kept as unialgal cultures under controlled conditions (21° ± 0.6°C, approx. 35–40 μmol photons m^{-2} s^{-1} at 16:8 h light dark cycle, provided by Lumilux Cool Daylight L18W/865; OSRAM, Munich, Germany) for at least 6 months in Rostock. The clonal cultures were grown in natural, filtered (0.2 μm, Sartorius, Germany) Baltic Sea water (absolute salinity, 14 S_A), where the salinity was adjusted to 33 S_A by adding artificial sea salt (hw Marinemix professional–HW Wiegandt Aquaristik, Krefeld, Germany) and enriched with f/2 medium (Guillard's Medium, type G0154, Sigma Aldrich, Germany). The stock culture media were refreshed monthly. The medium was refreshed 3–4 days prior each experiment to ensure always growth in the log phase.

Genetic Identity

Genetic identity of the Symbiodiniaceae cultures was confirmed and evaluated by amplification and sequencing of the hypervariable region of domain V of the chloroplast 23S ribosomal DNA gene (cpr23S), which is considered as a *Symbiodinium*-specific molecular marker (Granados-Cifuentes et al., 2015; Bonthond et al., 2018). This region was identified with the forward primer 23S_F-Forward_Overhang (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAATAA CGACCTGCATGAAAC, Invitrogen) and reverse primer 23S_R-Reverse_Overhang (GTCTCGTGGCTCGGAGATGTGTATA AGAGACAGGCCTGTTATCCGTAGAGTAGC, Invitrogen). The PCR profile was as follows: initial denaturation for 2 min at 95°C, 35 cycles at 95°C for 35 s, 35 cycles 55°C for 30 s, 35 cycles 72°C for 60 s, followed by a final extension of 5 min at 72°C. The PCRs contained 1X GoTaq GreenMaster Mix (Promega), 0.625 μl per primer and nuclease-free water in a final volume of 25 μl. For each sample 1 μl of template DNA was used. Purified PCR product were sent to Eurofins for sequencing using the Sanger-O Primer. Received dinoflagellates sequences were adjusted and aligned with the program SnapGene Viewer. Sequences were BLASTn search against the GenBank from National Center for Biotechnology Information (NCBI) for species identification.

Photosynthesis and Respiration Measurements

The photosynthesis irradiance curves (PI curve) reflect the measured relationship of oxygen production per chlorophyll *a* as function of increasing photon fluence densities (PFD). For this purpose, the PI curves were generated in four separate water-surrounded oxygen electrode chambers (Hansatech Instruments, King's Lynn, United Kingdom), each filled on top of a magnetic stirrer (Hansatech Instruments, King's Lynn, United Kingdom). The chambers were connected to a water supply (K10, Thermo Haake, Karlsruhe, Germany) and a thermostat (DC10, Thermo Haake, Karlsruhe, Germany) to keep the temperature constant (± 0.1°C). LEDs (LUXEON Rebel1 LXML-PWN1-0100, neutral white, Phillips, Amsterdam) were used as light sources, which were implemented in the PI-Box. To reduce the photon flux densities (PFD), neutral density filters were placed between the

TABLE 1 | Culture and taxonomic assignment of the investigated Symbiodiniaceae genotypes, along with information on origin, lifestyle, and isolator.

Culture	Symbiodiniaceae type	Host	origin	Lifestyle	Isolated by	18S rDNA	cp-type
Mf 1.05 b	*Breviolum minutum*	*Orbicella faveolata*	Caribbean	Symbiotic	M.A. Coffroth	B	B184
SSB 01	*Breviolum minutum*	*Aiptasia pulchella*	Pacific	Symbiotic	Xiang	B	
RT-002	*Breviolum minutum*	*Aiptasia pallida*	Caribbean	Symbiotic	R.K. Trench	B	
MAC 703	*Breviolum minutum*	*Plexaura kuna*	Caribbean	Symbiotic	M.A. Coffroth	B	B211
CCMP 421	*Effrenium voratum*	–	W. Pacific	Free-living	Bigelow	E	E202
MAC HIAP	*Breviolum psygmophilum*	*Aiptasia pulchella*	Pacific	Symbiotic	R.A. Kinzie	B	B224
1046	*Breviolum psygmophilum*	–		Symbiotic		B	
CCMP 2556	*Durusdinium trenchii*	*Orbicella faveolata*	Caribbean	Symbiotic	M.A. Coffroth	D	D206
SSA 01	*Symbiodinium linucheae*	*Aiptasia pallida*	Caribbean	Symbiotic	T. Bieri	A	

LEDs and the cuvette to generate different PFDs (0–1,400 μmol photons m^{-2} s^{-1}). Each cuvette was equipped with an integrated oxygen immersion probe (optode) (PreSens Precision Sensing GmbH, Regensburg) and connected via a fiber optic to an oxygen transmitter (Oxy 4-Mini, PreSens Precision Sensing GmbH, Regensburg). For each cuvette, the oxygen concentrations were displayed and recorded with the computer program OXY4v2_30 (PreSens Precision Sensing GmbH, Regensburg) and later calculated based on the chlorophyll *a* content. To calibrate the oxygen dip probes, each cuvette was treated at 0 and 100% oxygen saturation at 20°C. Oxygen saturation at 100% was done by aeration of the culture media for 15 min. Sodium dithionite ($Na_2S_2O_4$) was used to achieve oxygen free media.

In each measurement, four replicates of 3 ml pre-incubated log-phase suspension of Symbiodiniaceae were loaded into the cuvette. All replicates were enriched with sodium bicarbonate ($NaHCO_3$, 2 mM final concentration) before the measurements to ensure sufficient carbon supply. Each PI measurement started with a 30 min respiration phase in darkness, followed by an always 10 min photosynthesis phase at each of 10 different light level, at a constant temperature of 20°C. After each PI curve measurement, the algal suspension was filtered from each cuvette onto an individual Whatman GF/6 glass fiber filter (Ø 25 mm) for chlorophyll *a* determination. Chlorophyll *a* was extracted with 3 ml 96% ethanol (v/v), thoroughly vortexed and incubated in the dark for 24 h at 4°C. Afterward the extracts were centrifuged at 5,000 rpm for 10 min (Heraeus Megafuge, Hanau, Germany) to reduce turbidity. The extinction of the supernatants were measured at 665 and 750 nm in a spectrophotometer (Shimadzu UV-2401 PC, Kyoto, Japan). The concentration of chlorophyll *a* was calculated according to a protocol of the Baltic Marine Environment Protection Commission (1988) (Helcom, 1988).

To generate the PI curve, the chlorophyll *a* content and the measured data were fitted using the mathematical photosynthesis model of Platt et al. (1980). Several photosynthesis parameters were estimated from the least square's regression curves, which were adjusted to the measured values using the solver function of MS Office excel 2013. Based on these curves, the maximum rate of net primary production (NPP_{max}), respiration (R), light utilization coefficient (α), photoinhibition coefficient (ß), light saturation point (I_k) and light compensation point (I_c) were calculated.

Temperature Dependent Photosynthesis and Respiration

To investigate the temperature requirements of photosynthesis and respiration, two different temperature treatments were applied. The first and broader experiment aimed to define the upper and lower temperature tolerance along with the optimum for each strain in the temperature range from 10°C up to 40°C and down to 10°C in 5°C increments.

The second temperature experiment was carried out based on the previously gained data with smaller temperature range and steps to precisely identify the upper temperature limits for both photosynthesis and respiration. The responses between the temperatures 20°C up to 34°C in 2°C steps were measured. Both experiments were performed using the oxygen-optode system described above with four replicates of 3 ml pre-incubated log-phase suspension of Symbiodiniaceae at the same time. Each sample was incubated in the dark for 20 min at each experimental temperature, starting at the respective starting temperature (10°C/20°C), before respiration was monitored for an additional 10 min. This was followed by the photosynthesis phase for an additional 10 min at an exposure of ∼340 μmol photons m^{-2} s^{-1}. This light level was held constant for all photosynthesis measurements. After determining photosynthetic oxygen evolution, the temperature was increased by 5°C/2°C and a new incubation period was started after reaching the new temperature in the thermostat chamber. The O_2 consumption and production per unit time were related to the concentration of total Chl *a* per sample as described above.

Statistics

All values shown represent mean values and standard deviation (n = 4), unless otherwise stated. For statistical analysis, IBM's SPSS Statistics 25 computer program was applied. Significance levels were calculated using one-way ANOVAs with *post hoc* Tukey tests to show significant differences between the different temperature levels. For the assumptions, normality was previously tested with the Shapiro-Wilk test and for homogeneity of variances, the Levene's test ($p > 0.05$) was applied. For violation of assumptions, if Levene's test was significant, Welch-ANOVA and Games-Howell *post hoc* test were performed instead of a one-way ANOVA. For all ANOVAS, "photosynthesis" and "respiration" were used as independent variables (= levels).

RESULTS

Photosynthesis and Respiration Measurements

Photosynthetic oxygen production in all Symbiodiniaceae cultures at 20°C showed a similar PI curve shape with increasing PFD, followed by rarely perceived photoinhibition (**Figure 1**). The average respiration rate at 20°C ranged from −38.33 μmol O_2 mg^{-1} Chl *a* h^{-1} in strain SSB 01 to −127.40 μmol O_2 mg^{-1} Chl *a* h^{-1} in strain SSA 01, while the maximum photosynthesis rate NNP_{max} in the light saturated range was between 47.21 μmol O_2 mg^{-1} Chl *a* h^{-1} in strain SSA 01 and 233.45 μmol O_2 mg^{-1} Chl *a* h^{-1} in strain 1,046 (**Figure 1**). Even under the highest tested photon fluence density (1,400 μmol photons m^{-2} s^{-1}), only in SSB 01 small photoinhibition could be demonstrated (**Figure 1B**). The Symbiodiniaceae genotypes showed an alpha value (photosyntethic efficiency) between 2.66 and 6.09 μmol O_2 mg^{-1} Chl *a* h^{-1} (μmol photons m^{-2} s^{-1}) $^{-1}$, whereas SSA 01 exhibited a very high alpha value with 39 μmol O_2 mg^{-1} Chl *a* h^{-1} (μmol photons m^{-2} s^{-1}) $^{-1}$. The values of the light saturation point (I_k) ranged from 4.48 μmol photons m^{-2} s^{-1} in strain SSA 01–110.26 μmol photons m^{-2} s^{-1} in strain MAC HIAp, and those of the light compensation point (I_c) between 5.84 (strain SSA 01) and 35.73 μmol photons m^{-2} s^{-1} (strain 1,046) (**Figure 1** and **Table 2**). All these values indicate low to moderate light requirements for photosynthesis at 20°C, with the

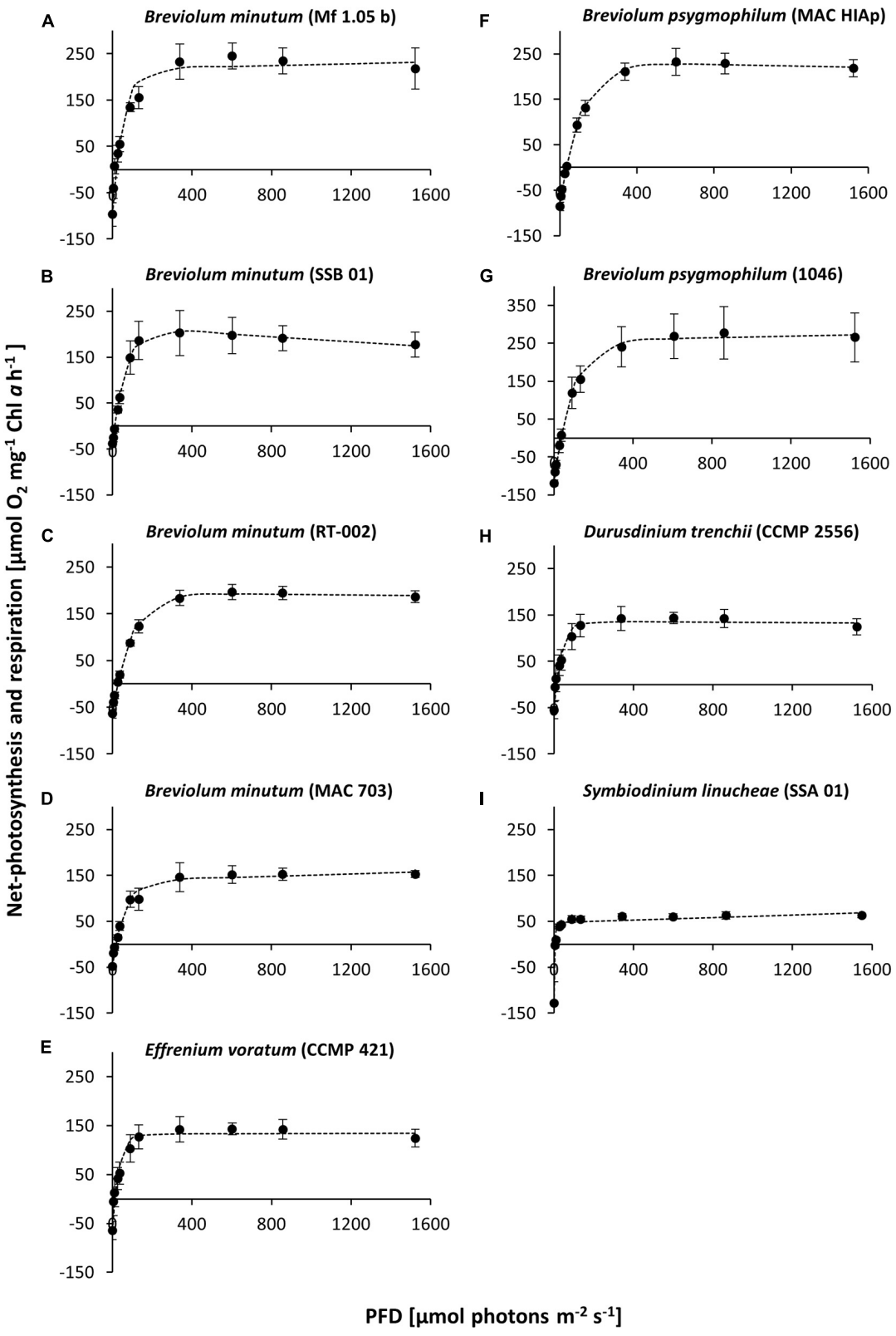

FIGURE 1 | Net primary production [NPP] rates [µmol O_2 mg^{-1} Chl *a* h^{-1}] in relation to increasing photon fluence density (PFD) [µmol photons m^{-2} s^{-1}] of nine Symbiodiniaceae cultures at 20°C, kept in f/2 Baltic Sea water medium, $33S_A$, measured by oxygen production with optodes. Data represent a mean value ± SD (n = 4). Dotted line: fitted according to Platt et al. (1980). **(A–I)** represent the studied Symbiodiniaceae genotypes as shown in **Table 1**.

TABLE 2 | Parameter of respective PI-curves (**Figures 1A–I**) of nine Symbiodiniaceae cultures (n = 4, mean value ± SD) at 20°C kept in a f/2 Baltic Sea water medium, $33S_A$.

Strains	NPP max [μmol O_2 mg^{-1} Chl *a* h^{-1}]	Respiration [μmol O_2 mg^{-1} Chl *a* h^{-1}]	α [μmol O_2 mg^{-1} Chl *a* h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$]	β [μmol O_2 mg^{-1} Chl *a* h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$]	I_k [μmol photons m^{-2} s^{-1}]	I_c [μmol photons m^{-2} s^{-1}]
Mf 1.05b	212.56 ± 46.98	−97.17 ± 25.78	5.73 ± 1.72	−0.01 ± 0.04	54.08 ± 16.99	20.36 ± 3.12
SSB01	218.28 ± 57.05	−38.33 ± 5.90	3.73 ± 0.45	0.03 ± 0.03	68.79 ± 11.69	11.02 ± 2.30
RT-002	194.92 ± 23.41	−63.36 ± 9.93	2.66 ± 0.24	0.00 ± 0.01	97.06 ± 9.94	27.26 ± 3.67
MAC 703	133.34 ± 38.73	−47.56 ± 4.39	3.01 ± 0.26	−0.01 ± 0.02	60.05 ± 11.41	18.34 ± 3.10
CCMP 421	132.49 ± 21.37	−64.51 ± 18.07	6.09 ± 1.15	0.00 ± 0.01	32.35 ± 5.06	12.83 ± 5.47
MAC HIAp	233.45 ± 29.59	−85.69 ± 9.33	2.89 ± 0.48	0.01 ± 0.01	110.26 ± 8.78	34.34 ± 3.21
1046	249.35 ± 57.35	−119.19 ± 5.47	4.03 ± 1.05	−0.01 ± 0.01	91.53 ± 17.85	35.73 ± 5.08
CCMP 2556	136.30 ± 16.26	−55.14 ± 18.22	5.16 ± 2.64	0.00 ± 0.01	37.07 ± 17.85	12.59 ± 5.08
SSA01	47.21 ± 7.93	−127.40 ± 19.36	39.00 ± 19.38	−0.01 ± 0.01	4.48 ± 1.49	5.84 ± 1.03

NPP_{max} represents the maximum oxygen production rate, alpha (α) the initial slope of oxygen production in the light limited range, beta (β) the final slope of oxygen production in the light saturated range (photoinhibition), I_c the light compensation point where respiration and photosynthesis are equal, I_k the light saturation point.

exception of *Symbiodinium linucheae* which revealed a strong increase with a rather low maximum oxygen production rate (NNP_{max}) (**Figure 1I**).

Overall, the data document genotype-specific similarities and differences between the nine Symbiodiniaceae isolates. While, for example, similar PI curves concerning the NNP_{max} values were recorded for members of the different genera *B. minutum*, *D. trenchii,* and *E. voratum* (MAC 703, CCMP 2556, and CCMP 421), the PI curves within the genus *Breviolum* varied widely. Both isolates of *B. psygmophilum* showed a remarkably similar NNP_{max}, as well as light compensation and light saturation point. On the other hand, all *Breviolum minutum* strains exhibited a wide range of NNP_{max} values. In addition, SSB 01 showed a slight photoinhibition (β). Furthermore, *S. linucheae* (SSA 01) had the lowest NNP_{max}, while *B. psygmophilum* (1046) showed the highest NNP_{max} (**Figures 1I,G**).

Temperature Dependent Photosynthesis and Respiration

The effects of temperature on the production of photosynthetic oxygen and respiratory oxygen consumption in Symbiodiniaceae cultures displayed strong differences within and between the strains with an increasing temperature gradient.

A broad temperature experiment (**Figure 2**) was conducted from 10°C up to 40°C, with a recovery phase decreasing the temperature back to 10°C. All isolates showed a weak photosynthetic and respiratory rate at 10°C. Increasing temperature stimulated photosynthesis and respiration to a species-specific maximum, followed by a decrease under the highest temperature conditions. While optimal photosynthesis was measured between 20 and 30°C in all investigated strains, the highest respiration occurred between 30 and 40°C (**Figure 2**). Furthermore, after a complete inhibition of photosynthesis at 40°C, no recovery was observed when the temperature was subsequently lowered, indicating a damage to the photosynthesis apparatus. This was illustrated by a two-color triangle in the graph (A-I). All cultures showed still respiration in the dark at 40°C (**Figure 2**). Subsequently, four (**Figures 2B,E,I,H**) out of nine cultures exhibited a complete inhibition of photosynthesis along with high respiration rates from 30 to 35°C. The remaining five strains (**Figures 2A,C,D,F,G**) showed still a partly functioning photosynthesis at 35°C. Both strains of *B. psygmophilum* reached the highest values in photosynthetic oxygen evolution (MAC HIAp: ∼264.76 μmol O_2 mg^{-1} Chl *a* h^{-1}; strain 1046: ∼201.70 μmol O_2 mg^{-1} Chl *a* h^{-1}) (F, G). In contrast, *B. minutum* (SSB 01) showed the lowest maximum value at 20°C with ∼59.98 μmol O_2 mg^{-1} Chl *a* h^{-1} (B). Therefore, SSB 01 was the only strain with maximum photosynthetic oxygen evolution at 20–25°C, while all other isolates had their maxima between 25 and 30°C (**Figure 2**). At 35°C photosynthesis of *E. voratum* was completely inhibited. In contrast, the other eight cultures showed only slightly decreased or constant respiration during the light phase at 35°C (**Figures 2A–I**).

A second experiment was carried out to identify more precisely the temperature requirements of photosynthesis and respiration in the critical range between 20 and 34°C (**Figures 3A–I**). The data confirmed in principle the previous experiment. Eight out of nine strains showed photosynthesis and respiration between 20 and 34°C, although at the highest temperature some inhibition in oxygen production could be observed. *B. minutum* (SSB 01) was the only exception as photosynthesis >28°C was fully inhibited (**Figure 3B**). To determine the optimum temperature for photosynthesis, we applied the widely used model of Blanchard et al. (1996) (**Figure 4** and **Table 3**). It shows the species-specific tolerance range of temperature for photosynthesis and respiration in both temperature experiments. For all cultures percentiles of <20%, 20–80%, and >80% were determined. In the broad temperature approach, all Symbiodiniaceae exhibited a wide temperature tolerance, with almost all values above the 20% percentile. At the lowest tested temperature (10°C), four isolates (Mf 1.05 b, MAC 703, MAC HIAp, CCMP 2556) showed efficiency below 20%. All other strains had efficiency between 20 and 80%. Four isolates (Mf 1.05 b, 1046, SSA 01, CCMP 421) exhibited the largest temperature range in the upper percentile by covering three temperature levels from 20 to 30°C. In comparison,

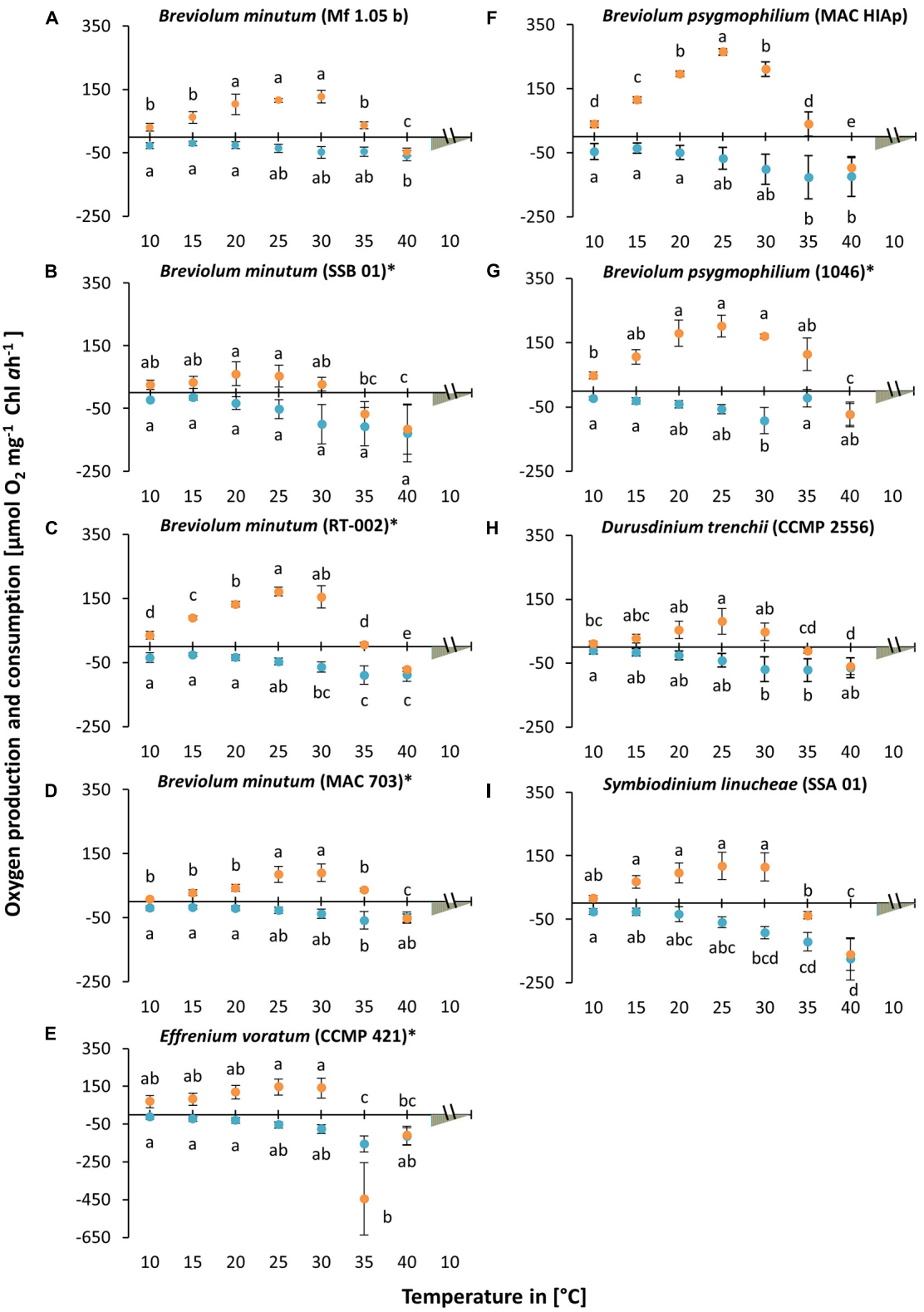

FIGURE 2 | Oxygen production (orange) and consumption rates (blue) [µmol O_2 mg^1 Chl *a* h^1] in relation to increasing temperature [10–40°C] at a saturating photon fluence density (PFD) of approx. 350 µmol photons m^{-2} s^{-1} in nine Symbiodiniaceae cultures **(A–I)**, kept in f/2 Baltic Sea water medium, ~ 33 S_A, measured by oxygen evolution with optodes. Data represent a mean value ± SD (n = 4). Lowercase letters at photosynthesis and respiration indicate significantly means ($p < 0.05$; one-way ANOVA with *post hoc* Tukey-HSD test; * indicate Welch-ANOVA (significant: $p < 0.05$) with Games-Howell *post hoc* test).

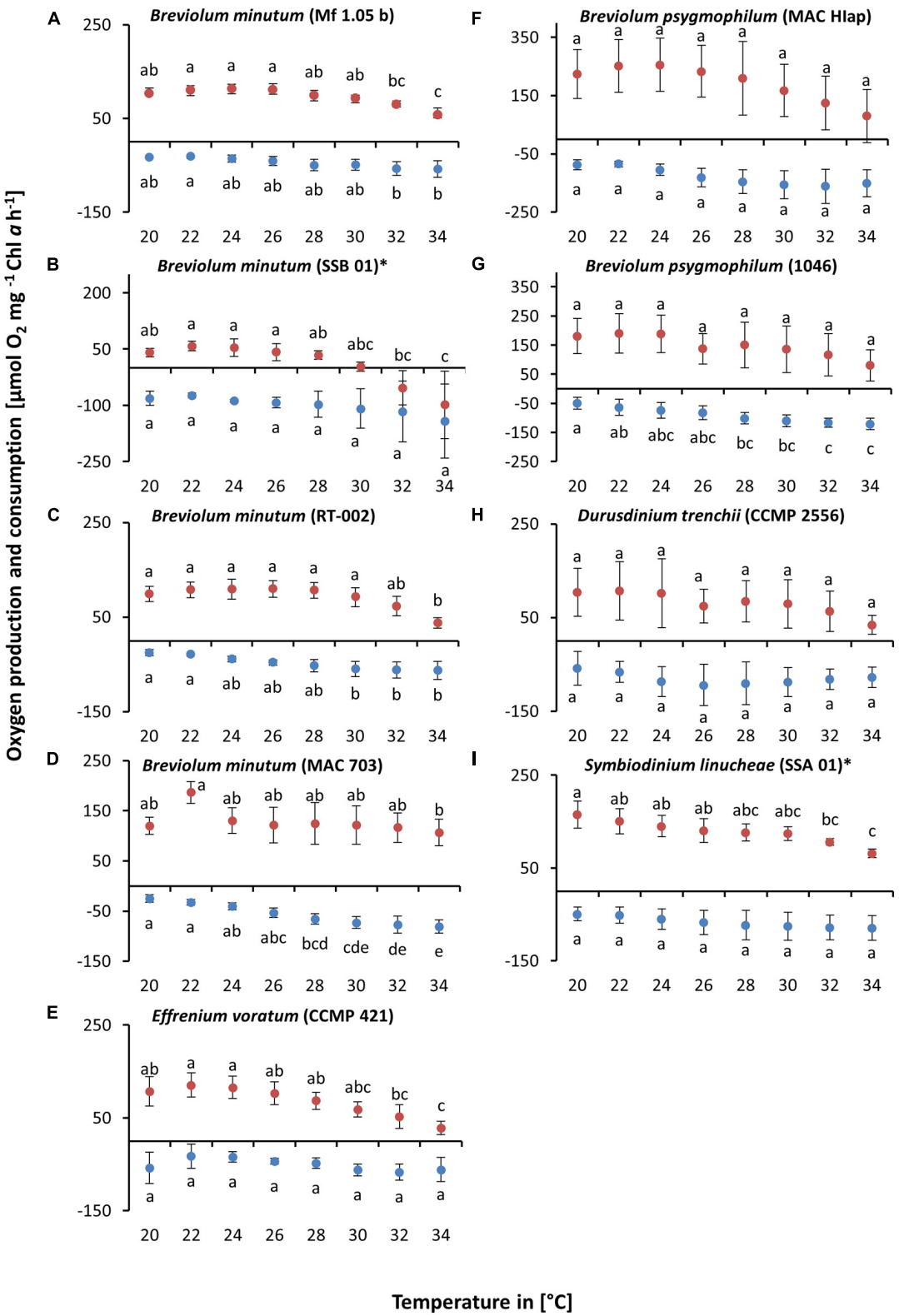

FIGURE 3 | Oxygen production (red) and consumption rates (dark blue) [µmol O_2 mg^{-1} Chl *a* h^{-1}] in relation to increasing temperature [20–34°C] at a saturating photon fluence density (PFD) of approx. 350 µmol photons m^{-2} s^{-1} in nine Symbiodiniaceae cultures **(A–I)**, kept in f/2 Baltic Sea water medium, ~ 33 S_A, measured by oxygen evolution with optodes. Data represent a mean value ± SD (n = 4). Lowercase letters at photosynthesis and respiration indicate significantly means ($p < 0.05$; one-way ANOVA with *post hoc* Tukey-HSD test; * indicate Welch-ANOVA (significant: $p < 0.05$) with Games-Howell *post hoc* test).

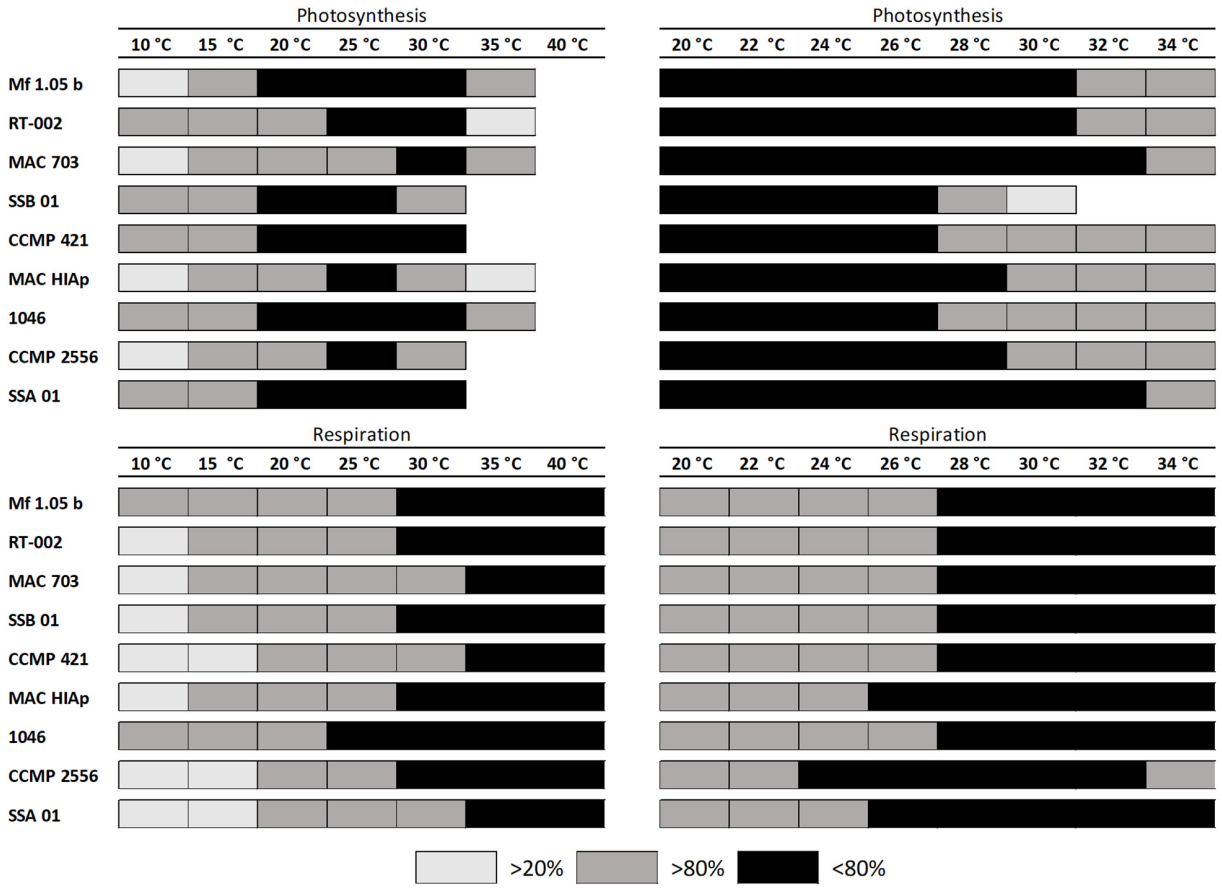

FIGURE 4 | Influence of temperature on photosynthetic oxygen production and respiratory oxygen consumption of nine tested Symbiodiniaceae strains using the fit of Blanchard et al. (1996). The black box represents area of highest photosynthesis more than >80% percentile, dark gray symbols within 20 and 80% percentile light gray symbols <20% percentile and white spaces no photosynthesis or respiratory signal. The data represent mean values (n = 4).

several strains showed a small range for photosynthetic efficiency, MAC HIAp and CCMP 2556 at 25°C and MAC 703 at 30°C. Photosynthesis was not detectable at 35°C in four of nine strains (SSB 01, CCMP 2556, SSA 01, CCMP 421). At 35°C the other cultures showed different efficiencies, for RT-002 and MAC HIAp the efficiency ranged below 20%, for the remaining isolates the efficiency reached 20–80%. The highest respiration values were measured between 30 and 40°C. The respiration increased above the 80% for different strains at different temperatures (**Figure 4** and **Table 3**).

The critical temperature levels from the experiments were analyzed in more detail and all cultures without a "cold stress" had a photosynthetic efficiency in the upper percentile of over 80% from 20°C up to 26°C. MAC 703 and SSA 01 exhibited largest temperature tolerance as reflected in the upper percentile >80% up to 32°C. In contrast, SSB 01, 1046, and CCMP 421 showed highest photosynthetic efficiency in the upper percentile up to the 26°C range. The different strains tested showed an increased respiration at 28°C with more than >80%, while CCMP 2556, MAC HIAp, and SSA 01 exhibited lower temperature values (24 and 26°C, respectively) (**Figure 4** and **Table 3**). The results indicate that all investigated strains have a broad temperature tolerance for photosynthesis and respiration.

DISCUSSION

The susceptibility of corals is influenced by a variety of abiotic factors such as increased light and temperature conditions, and the physiological and genetic diversity of the endosymbionts strongly contribute to different response patterns of the host (Berkelmans and Van Oppen, 2006; Robison and Warner, 2006; Baird et al., 2009). In the present study the experiments focused on the physiological tolerance and plasticity of genetically different Symbiodiniaceae strains in response to elevated temperatures for a better understanding of their photosynthetic traits as a function of light and temperature.

The Symbiodiniaceae used were grown as unialgal cultures under identical and controlled conditions for at least 1 year, which might explain relatively similar alpha, I_c and I_k values derived from the respective PI curves. Symbiodiniaceae species acclimate their photosynthetic performance to various light levels (Iglesias-Prieto and

TABLE 3 | Calculation of temperature effects on photosynthetic oxygen production and respiratory oxygen consumption in nine tested Symbiodiniaceae strains using the fit of Blanchard et al. (1996).

		Photosynthesis			Respiration		
		Temperature [°C]			Temperature [°C]		
	Strains	100%	>80%	>20%	100%	>80%	>20%
10–40°C	Mf 1.05 b	24.7	19.4–30.1	10.4–35.4	38.7	27.0–(49.9)	(5.9)–(67.9)
	RT-002	26.4	20.6–31.2	(8.3)–37.3	40	30.0–(49.0)	10.9–(61.9)
	MAC 703	28.5	24.0–32.1	14.1–36.3	38.9	30.5–(47.9)	13.1–(62.2)
	SSB 01	22.4	17.7–26.8	(6.2)–32.4	36.4	27.5–(44.9)	12–(58.6)
	CCMP 421	28.2	19.0–34.9	(4.6)–(41.6)	38.9	32.7–(44.9)	21.7–(54.5)
	MAC HIAp	25.9	21.0–29.8	10.7–34.5	38.2	29.5–(46.0)	13.2–(57.7)
	1046	25.1	19.0–31.1	(8.9)–41.2	32.9	22.9–(42.8)	(6)–(59.4)
	CCMP 2556	24.2	20.2–28.2	13.5–35.0	35.4	28.8–(40.9)	15–(47.8)
	SSA 01	27.2	19.9–33.8	(5.8)–(43.5)	40	33.2–(46.5)	21.2–(56.9)
20–34°C	Mf 1.05 b	24	17.9–30.1	(7.4)–(40.0)	34	26.6–(41)	(13.1)–(51.9)
	RT-002	25.2	16.9–30.6	(4.9)–(36.7)	33	27.2–(37.9)	(15.3)–(42.2)
	MAC 703	26	4.6–33.9	(−71)–(35.2)	33.7	28–(39.2)	(17.8)–(47.8)
	SSB 01	23.1	18.8–26.4	(9.7)–29	34	26.9–(40.7)	(14)–(51)
	CCMP 421	22.6	17–27.6	(6.3)–(36.8)	32.5	27.8–(37.2)	(19.9)–(45.1)
	MAC HIAp	23	19.1–28.8	(10.7)–(36.8)	33.5	25.6–(41.1)	(11.4)–(53)
	1046	20.3	20.0–27.9	(−0.4)–(40.4)	33.6	27.2–(40.7)	(15.5)–(51.8)
	CCMP 2556	20.8	13.3–28.1	(10.6)–(40.2)	27.5	22.2–32.7	(13)–(41.1)
	SSA 01	21.9	18–32.8	(−81)–(35)	34	25–(43.4)	(8.5)–(58)

The data represent mean values (n = 4) of maximum photosynthesis and respiration (100%) and the upper 80 and 20% percentiles. The data in brackets represent temperature calculations that exceed the measured temperature.

Trench, 1994; Anthony and Hoegh-Guldberg, 2003; Roth, 2014). The applied light conditions under which the cultures were grown are comparable to the shady *in vivo* conditions inside the coral tissue (Anthony et al., 2005). Furthermore, Symbiodiniaceae maximize light absorption and utilization under low light conditions by increasing the concentration of photosynthetic pigments thereby improving photosynthetic efficiency (Anthony and Hoegh-Guldberg, 2003).

The photosynthetic optimum under the prevailing environmental conditions is represented by the maximum rate of photosynthesis (NPP_{max}) (Raven and Geider, 2003). The Symbiodiniaceae cultures investigated in this study revealed different type-specific eco-physiological response patterns regarding NPP_{max} and respiration. These patterns are mainly caused by type-specific traits rather than as a result of the stable cultivation conditions. The data displayed a similar NPP_{max} for various genera (MAC 703, CCMP 2556, and CCMP 421), which was distinct to both genotypes of *Breviolum psygmophilum* MAC HIAp and 1046. Furthermore, the NPP_{max} of *Symbiodinium linucheae* SSA 01 was much lower in contrast to the other eight strains. This strain is also considered "heat tolerant" (Swain et al., 2017; LaJeunesse et al., 2018; Gegner et al., 2019) but might not be so beneficial for the coral host (Stat et al., 2008; Herrera et al., 2020). Although only minor photoinhibition was detected in the studied strains we cannot completely exclude an experimental effect as longer exposure to high irradiances could have resulted in a stronger photoinhibitory response. The data from this study, clearly indicate that SSA 01 has a much lower photosynthetic rate compared to the other strains than, for example, strain *B. psygmophilum* (1046), which had the highest average photosynthetic rates per chlorophyll *a*. Therefore, SSA 01 likely transfers less important photosynthetically fixed organic carbon to the host (Stat et al., 2008). The photosynthetic rates of the present study are in agreement with data of Grégoire et al. (2017), who used two identical genotypes for their experiments. The photosynthesis performance between the species were different under the same cultivation conditions (Iglesias-Prieto and Trench, 1994), pointing to genotypic traits. Previous studies indicate an important role of the genetic identity of Symbiodiniaceae strains as well as their respective eco-physiological capabilities in acclimation and adaptation to thermal stress in the reef environment (Baker, 2003; Abrego et al., 2008; Frade et al., 2008a,b). Both the endosymbiont and the host are affected by elevated temperatures. Host genetics have been identified as an important trait in the response of the symbionts (Cunning et al., 2015; Howells et al., 2016). In order to determine how the genetic identity of the symbiont is affected by elevated temperatures, the cultures were exposed to different thermal stress scenarios.

The results of this study indicated a clear species-specific threshold of upper temperature tolerance, in the range of 30°–40°C for all tested species, and the Symbiodiniaceae cultures demonstrated the strongest stress response with a reduced or an inhibition of photosynthesis and strongly increased respiration rate. At 35°C, only five of nine species showed a reduced but measurable photosynthesis (**Figure 4**). Above 35°C, none of the

strains exhibited any photosynthetic activity. It can be assumed that the Symbiodiniaceae were not dead due to the continuing respiratory activity. Moreover, the results of the Symbiodiniaceae cultures showed clearly a tolerance to short-term cold stress at 10°C and the most efficient photosynthesis performance was measured on average at 25°C (**Figure 4**). The different Symbiodiniaceae species are classified based on a variation in thermal tolerances, which is also reflected in the results of this study (Robison and Warner, 2006; Suggett et al., 2008; Díaz-Almeyda et al., 2017; Bellantuono et al., 2019). While any oxygen production at 35°C was not traceable in the isolates SSB 01, CCMP 2556, SSA 01, and CCMP 421, the other strains still showed a reduced activity. *Durusdinium trenchii* is described as heat tolerant species (Bellantuono et al., 2019) but demonstrated in culture a similar pattern as the other tested isolates up to 34°C. The hypothesis that *D. trenchii* is more heat tolerant than the other tested strains could not be confirmed in this experiment. Thus, all tested Symbiodiniaceae exhibited quite similar temperature tolerance with an optimal photosynthetic performance between 20° and 26°C. In addition, the strains MAC 703 and SSA 01 showed an "optimal" photosynthetic capacity up to 32°C. The upper temperature tolerance for photosynthesis of the investigated strains was between 34 and 35°C, that of respiration even higher. Consequently, it is reasonable to assume that the increased consumption of oxygen under elevated temperatures might favor anoxic conditions within the holobiont, which can cause symbiont loss and bleaching. The results of the present study are comparable to those of other studies, which demonstrated a decrease in the maximum quantum yield of PSII and the rate of gross photosynthesis under heat stress in different zooxanthellae in culture. Impairment of photosynthesis was mainly measured at 32–34°C in cultivated zooxanthellae (Iglesias-Prieto et al., 1992; Warner et al., 1996; Iglesias-Prieto and Trench, 1997; Jones et al., 1998; Brown et al., 1999). In *Symbiodinium microadriaticum*, gradual inhibition of photosynthesis occurred at temperatures above 30°C, followed by complete inhibition at higher temperatures of 34–36°C (Iglesias-Prieto et al., 1992). The reason why so many zooxanthellae exhibit a similar upper temperature tolerance for photosynthesis might be related to the fact that dinoflagellates have a Type II Rubisco, which differs in many properties including heat sensitivity from Type I, the dominant form in other algal groups (Tabita et al., 2008).

In the second temperature experiment, it became clear that *Breviolum minutum* SSB 01 was much more susceptible to enhanced temperatures starting at 30°C. This demonstrates different temperature thresholds between strains of *B. minutum*, also within the genus *Breviolum* the species-specific tolerances differ. This is in line with another study, where closely related *Breviolum* species revealed significant functional variation against elevated temperature (Bayliss et al., 2019; Herrera et al., 2020). The different genotypes exhibited decreasing photosynthetic activity at elevated temperatures, which could be an important feature for the strength of the relationship between the symbiont and the host. Some studies concluded that thermotolerance is not species- or clade-specific but is widespread and diverse among members of the genus *Symbiodinium* (Suggett et al., 2008; Díaz-Almeyda et al., 2017). Considering the photosynthetic machinery of coral symbionts as very sensitive to changes in the environment, it was observed that exposure of Symbiodiniaceae to light and temperature caused an impairment of the photosynthetic apparatus by PSII photoinhibition (Iglesias-Prieto et al., 1992). The level of photoinhibition is partly determined by elevated temperatures or light, as these factors can accelerate PSII photodamage and inhibit repair processes (Brown et al., 1999; Takahashi et al., 2009). But other studies report that the temperature-induced inhibition of the dark reactions (Calvin-cycle) in zooxanthellae does not exclusively contribute to coral bleaching, while the concomitant ROS formation could be the main trigger (Hill et al., 2014).

The results of Mansour et al. (2018) found intra- and interspecific differences in the melting points of thylakoid membranes of Symbiodiniaceae species. Such process could explain the inhibition of photosynthetic oxygen production under elevated temperatures during the experiments. A still ongoing respiration was measured at higher temperatures, indicating that the repair mechanisms are working and that the experimental cooling time was too short to be used as proxy for recovery. However, in the experiment 20–34°C the thermal capacity of the photosynthetic membrane was probably not exceeded and hence significant differences between the strain-specific response patterns could be measured. A certain, genetically determined temperature threshold must be reached to harm the photosynthetic activity. The loss of photosynthetic function at extremely high temperatures occurs in a short time period in isolated Symbiodiniaceae cultures. The results of this study clearly indicated that the physiological properties of Symbiodiniaceae at elevated temperatures vary even within the same taxon, i.e., genotypic differentiation even on taxonomically lower rank levels plays a key role for temperature acclimation/adaptation, with consequences for the endosymbiont-host interactions (Bayliss et al., 2019). However, the physiological temperature response patterns of Symbiodiniaceae might differ between culture conditions and in symbiosis as documented for *D. trenchii* (Bellantuono et al., 2019). These authors compared *in hospite* and free-living transcriptomes, and reported strong alteration of transcriptional activity *in hospite* under increased temperature conditions, indicating that symbiotic interactions elicited an exacerbated stress response compared to free-living cells.

Although mainly vegetative (clonal) reproduction occurs within the Symbiodiniaceae, mutation rates can be high, resulting in new variants of individual genotypes. These genetic variations are further increased or maintained by transposons, retrotransposons, tandem repeats, or recombination during sexual reproduction (Shoguchi et al., 2013). Even hosts harboring only a single symbiont genotype can quickly accumulate genetic variation due to this high mutation rate in Symbiodiniaceae (van Oppen et al., 2011). Natural selection is likely to lead to a higher temperature tolerance in the symbiont population, since many temperature tolerance traits are inherited (Császár et al., 2010; Quigley et al., 2016). This selection in the symbiont population also strengthens the holobiont, allowing the host to survive periods of warming (Chakravarti and Van Oppen, 2018).

Nevertheless, previous work suggests that the physiology of symbionts actually differs in culture and host (Ralph et al., 2001; Bhagooli and Hidaka, 2003; Howells et al., 2012; Chakravarti et al., 2017; Ravelo and Conaco, 2018).

Intact relationships between host and symbionts are essential for the ecological health of coral reefs, as they represent one of the most important ecosystems in tropical marine waters. Coral reefs provide many ecosystem services such as high biodiversity and productivity, habitat, fishery, water quality and biogeochemical cycling, and coastal protection (Moberg and Folke, 1999), which are negatively influenced by anthropogenic global warming.

CONCLUSION

In this study we demonstrate a necessity for a comprehensive comparative eco-physiological analysis at intra-specific taxonomic level to fully understand the underlying genetic traits responsible for the variation in thermotolerance. The results clearly demonstrate that there are significant eco-physiological differences among the tested species but also within a species, regarding light affinity and temperature tolerance. Although the short-term responses shown here do not necessarily reflect the long-term response of corals in the reef, this study highlights that symbionts play an important role in the response of holobionts to temperature increases within their average summer maxima. The results showed clearly that *B. minutum* (SSB 01) is much more sensitive to heat in comparison to the strain *B. minutum* (MAC 703) and to the species *S. linucheae* (SSA 01). Furthermore, *S. linucheae* (SSA 01) exhibited a lower photosynthetic rate, suggesting that this strain in symbiosis probably transfers less photosynthetically fixed organic carbon to the host (Stat et al., 2008). Therefore, future studies in which specific symbiont genotypes are introduced into the same host (e.g., Starzak et al., 2014; Hoadley et al., 2015; Herrera et al., 2020) are required to determine the role of host and symbiont in the response patterns. The different response patterns suggest that in symbiosis with a host, the carbon supply from the symbiont to the host might be affected by light and temperature. Presumably, thermal stress leads to an unbalanced bi-directional flow of metabolites between host and endosymbiont which should be further investigated in future studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

VR and UK planned and designed the ecophysiological experiments. VR conducted the ecophysiological characterization of the algal strains, and processed the data. MR-L and VR planned molecular analysis, which was conducted by VR. VR wrote the first draft of the manuscript, which was edited by MR-L and UK. All authors edited and approved the final version of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.657348/full#supplementary-material

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