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Light is an Important Limiting Factor for the Vertical Distribution of the Largest Extant Benthic Foraminifer *Cycloclypeus carpenteri*

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ABSTRACT: *Cycloclypeus carpenteri* is the largest extant benthic foraminifer, dwelling in the deep euphotic zone (a water depth between 60 and 130 m) of the warm oligotrophic Indo-West Pacific. This foraminifer harbors diatom endosymbionts and the foraminifer-microalgal association acts like a holobiont. To verify that light is an important limiting factor controlling the vertical (depth) distribution of living *Cycloclypeus* holobionts, their physiological responses to light intensity were examined by short-term metabolic measurements and long-term incubations. Net oxygen production (OP) rates measured under different light levels using an oxygen microelectrode indicate that *Cycloclypeus* holobionts are daily net primary producers adapted to low light levels, with slight photoinhibition (reduced net OP rates relative to a light-saturated rate) over 100 μmol photons m^{-2} s^{-1}. Long-term growth increments of asexually reproduced juveniles incubated for two months at different light levels ranging from 0 to 100 μmol photons m^{-2} s^{-1} show that *Cycloclypeus* holobionts are adapted to a low light level (~5 μmol photons m^{-2} s^{-1}), but can be acclimatized to a certain low light ranges (<50 μmol photons m^{-2} s^{-1}). These experimental results confirm that light is an important environmental gradient affecting the vertical distribution of *Cycloclypeus* holobionts.
KEY WORDS: algal symbiosis, diatom, large benthic foraminifer, oxygen production, photosynthesis.

0 INTRODUCTION

Cycloclypeus carpenteri Brady is the largest extant benthic foraminifer (grown up to 10 cm or more in diameter), belonging to the Nummulitidae Family. This species is characterized by developing reduced numbers of nepionic (undivided operculine and divided heterostegine) chambers in the early stage, followed by concentric annular (cyclic) chambers divided into numerous chamberlets in the later stages (Renema, 2015). The species occurs since the Middle Miocene (Langhian) in the Indo-West Pacific (Renema, 2015). This species shows a clear dimorphic life cycle; a microspheric form (agamont) with a larger test size generally grown beyond 5 to 6 cm in diameter, and a megalospheric form (gamont) with a smaller test size grown up to 1 cm in diameter (Krüger et al., 1997). The life span of the gamont is around 1 year, while that of the agamont may be several years or more based on laboratory culturing (Krüger et al., 1997). This species harbors diatom endosymbionts, which live beneath thin transparent calcite windows of many chamberlets (Holzmann et al., 2006; Leutenegger, 1984).

Various environmental factors such as light, temperature, substrate, water motion, water pressure, species competition, and food availability affect the horizontal (geographic, latitudinal) and vertical (depth) distribution of this species (Hohenegger, 2004). The geographic distribution of this species is limited to the tropical Indo-West Pacific (Renema, 2015; Langer and Hottinger, 2000; Koba, 1978), which is mainly controlled by low temperature (Langer and Hottinger, 2000; Koba, 1978) as well as the place of origin and migration history (Renema, 2015). The vertical (depth) distribution of this species is generally within the deep euphotic zone (a water depth between 60 and 130 m), corresponding to the forereef slope and shelf environments (Hohenegger, 2000; Iryu et al., 1995; Koba, 1978). The vertical distribution is mainly controlled by light intensity and water energy (Hohenegger, 2000). Light intensity seems to be the dominant environmental gradient affecting the vertical distribution of *C. carpenteri*, while wave energy may also limit the upper distribution to below the storm wave base (Hohenegger, 2000). However, it has not yet been verified experimentally if light is an important factor controlling the physiology of *C. carpenteri* (including its endosymbiotic diatoms). The purpose of this study is to examine the effects of light intensity on the

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metabolism and growth of *C. carpenteri* with diatom endosymbionts (from here referred to as *Cycloclypeus* holobionts), based on net oxygen production and long-term growth increments.

1 MATERIALS AND METHODS

1.1 Collection of Studied Species

Living *Cycloclypeus* holobionts were collected during two research cruises around the Ryukyu Islands, Southwest Japan. The first samples were collected using an Okean-type grab sampler at site OK-1 located off west of Naha City, Okinawa Island (26°14.907'N, 127°36.949'E, 62 m depth) on 27th May, 2010 during the RN10 cruise (T/S Nagasaki-Maru). Seawater temperature, salinity, and photosynthetically active radiation (PAR) near the sampling site (57 m depth) were 24.47 °C, 34.61, and 17.03 μmol photons m^{-2} s^{-1}, respectively (Toki et al., 2011). The second samples were collected using a K-grab mud sampler at site KG-129 (26°10.2006'N, 127°27.1154'E, 62 m depth) and at site KG-168 (26°15.6793'N, 127°26.2787'E, 72 m depth) off the Kerama Islands on Nov. 7, 2010, during the GH10 cruise (R/V Hakurei-Maru) (Itaki et al., 2011). Seawater temperature and salinity at site KG-129 were 23.66 °C and 34.64, respectively, while those at site KG-168 were 24.97 °C and 34.57, respectively (Amano et al., 2011). Living brown-colored *Cycloclypeus* holobionts found on the surface sediments were transferred using forceps into a small container filled with seawater and maintained in an aquarium on board until brought back to the laboratory.

In the laboratory, each living individual larger than 3 cm in diameter, which corresponds to a microspheric form, was placed in a small clear plastic container filled with natural filtered (0.22 μm) seawater (FSW) collected from Ikei Island (a remotely located area). Living individuals smaller than 1 cm in diameter, which correspond to megalospheric forms, were placed in several large clear plastic containers filled with natural FSW. These containers were placed in an incubator (MIR-153, Sanyo), which was maintained at 25 ± 1.0 ℃, and at a light level of <50 μmol photons m^{-2} s^{-1} with a 12-hr light/dark cycle. The FSW was changed weekly.

1.2 Measurement of Net Oxygen Production

Net oxygen production (OP) rates were measured using a microrespiration system (Unisense AS, Denmark), which was composed of a microrespiration chamber, an oxygen microsensor, a picoammeter (PA-2000), and a data acquisition software (MicOx 3.0). The microrespiration chamber consists of a 2-mL glass-made chamber and a glass-made lid, in which the glass needle of a microsensor can be inserted. An acrylic ring base was placed on the bottom of the chamber. A glass-coated magnetic stir bar was placed in the ring base. A metal mesh was placed on the ring base, then a foraminifer was placed on the mesh. The chamber was filled with sterilized FSW and sealed with a lid. The microrespiration chamber and the oxygen microsensor were immersed in a stainless-steel water bath at a constant water temperature (25 ± 0.1 °C). The chamber was rested on a waterproof magnetic stirrer at the rotation speed of 450 rpm to maintain well-mixed seawater inside the chamber. This system enables to measure the dissolved oxygen (DO; nmol O_2 mL^{-1}) of seawater in a closed chamber with a foraminifer. The DO was recorded at every 10 seconds.

To determine effects of light intensity on the metabolism of *Cycloclypeus* holobionts, net OP rates were measured at eight levels of light intensity ranging from 0 to 200 μmol photons m^{-2} s^{-1} (0, 5, 10, 20, 30, 50, 100, 200). Five megalospheric individuals with an average diameter of ~8 mm collected from site KG-168 were used for measurements (Table S1). The measurements were done within two weeks after collection. A halogen lamp was used as a light source. The light was provided through a fiber with a neutral density filter attached to the irradiation port. The light intensity near the chamber was measured using a photon meter (LI-250 Light Meter, LI-COR). The system was entirely covered with a light shielding curtain during dark measurements. For each trial, the measurement started from a dark period, proceeding from the lowest to the highest light intensities, with an interlude of a dark period before starting next light period. At each light level, changes in DO concentration were recorded for 10 minutes twice.

After the completion of all measurements, chlorophyll (Chl) from the *Cycloclypeus* holobionts was extracted following Hosono et al. (2012). Briefly, a specimen was placed into a glass vial filled with 90% acetone (1 mL) to extract pigment. The vial was shielded from light and stored for 24 h at 5 °C, after which 0.7 mL of extraction liquid was taken, and the absorbance at 630, 663, and 750 nm (A630, A663, A750) were measured using a spectrophotometer (U-2001, Hitachi). The Chl *a* contents from the extracts were calculated using the dichromatic equation developed by Jeffery and Humphrey (1975)

$$\text{Chl } a = 11.43 \times (\text{A663} - \text{A750}) - 0.64 \times (\text{A663} - \text{A750})$$

After Chl extraction, specimens were dried for a period of three days in a desiccator and the dry weights were measured using a microbalance with a precision of $\pm 1.0 \times 10^{-4}$ mg (MX5, Mettler Toledo). Net OP rates were obtained as nmol O_2 mL^{-1} for 10 minutes, and finally converted to nmol O_2 μg^{-1} Chl *a* min^{-1} or nmol O_2 μg^{-1} Chl *a* hr^{-1} to compare with published results (Oron et al., 2018; Stuhr et al., 2017; Schmidt et al., 2016, 2014; Fujita et al., 2014; Sinutok et al., 2014, 2011; Reymond et al., 2013; Hosono et al., 2012; Uthicke and Fabricius, 2012; Uthicke et al., 2012; Vogel and Uthicke, 2012; Walker et al., 2011; Ziegler and Uthicke, 2011; Fujita and Fujimura, 2008; Nobes et al., 2008; Köhler-Rink and Kühl, 2001; Lee et al., 1980). In addition, daily net OP rates were calculated following Walker et al. (2011), assuming that saturation photosynthesis which occurs 10 hours per day and respiration over the entire 24 hours is equivalent to that measured at the initial dark period.

A production (*P*)-irradiance (*I*) curve was fitted with a following non-linear function

$$P = P_{\max}\left(1 - e^{\left(-\frac{\alpha I}{P_{\max}}\right)}\right)e^{\left(-\frac{\beta I}{P_{\max}}\right)} + C_0$$

where P_{max} is the light-saturated production rate, α is the photosynthetic efficiency (i.e., the initial slope of the light curve), β is the rate of decline at supra-optimal irradiance levels, and C_0 is the compensation irradiance (i.e., irradiance where a gross production rate equals a respiration rate). The saturation irradiance (E_k) is given as intercept between α and P_{max}. Statistical analyses were conducted in R 4.1.1 (R Core Team, 2021).

1.3 Long-Term Incubation

To determine effects of light intensity on the growth of *Cycloclypeus* holobionts, asexually reproduced megalospheric juveniles were maintained in the incubator at seven light levels (0, 5, 10, 20, 30, 50, 100 μmol photons m^{-2} s^{-1}) with a 12-hr interval light/dark cycle for approximately two months (56 days). Light sources are three tubes of a 20-W blue fluorescent light (Caribbean Blue, Sudo Co. Ltd., wavelength: 400–560 nm), fixed at the roof of the incubator. Seven light levels were determined based on a preliminary experiment and the depth distribution of *Cycloclypeus* holobionts in their natural environment (Hohenegger, 2000). Temperature in the incubator was maintained at 25 ± 1.0 ℃, which was close to those at the sampling sites.

Megalospheric juveniles used for the experiments were obtained from two agamont *Cycloclypeus* holobionts collected at site OK-1, which asexually reproduced on Aug. 17, 2010. These two populations of asexually reproduced megalospheric juveniles were maintained in the incubator for a week until the start of experiment (Aug. 24–25, 2010), and referred to as population 1 and 2. A-week-old juveniles from each population were divided into seven groups of 20 individuals. Each group was placed in a sterile petri dish filled with natural FSW, which was changed twice per week until the end of experiment (Oct. 21, 2010; 8 weeks). Petri dishes were sealed with a transparent wrap to prevent evaporation and placed in the incubator. The light levels of each petri dish were adjusted by changing a vertical distance from the light sources above.

Each individual was photographed under a binocular microscope initially and weekly. The digital images were used to measure the shell surface area of each individual using Image J (NIH), and the average surface area was calculated per group. Images at the end of experiment were also used to count the number of chambers, conditions of the symbiont color (categorized into “entirely brown”, “brown and granulated”, “pale”, and “colorless”), and conditions of algal overgrowth (categorized into “entirely covered”, “fringed”, and “no”) for each individual.

Surface area data were analyzed by a linear model with interactions, using populations, time (days) and light levels as explanatory variables. The surface area data were square root transformed before statistical analysis. Residual and normality (normal Q-Q) plots indicated that assumptions of normality and equal group variance for linear models were not violated. These statistical analyses were conducted in R 4.1.1 (R Core Team, 2021).

2 RESULTS

2.1 Net Oxygen Production Rates

Net OP rates of *Cycloclypeus* holobionts increased with light levels up to 50 μmol photons m^{-2} s^{-1}, and were saturated between 50–100 μmol photons m^{-2} s^{-1}, and slightly decreased above 100 μmol photons m^{-2} s^{-1} (Fig. 1, Table S2). The fitted *P-I* curve indicates the α of 0.018 ± 0.003 7 (mean ± SE, $p < 0.001$) and the P_{max} of 0.42 ± 0.062 nmol O_2 μg^{-1} Chl *a* min^{-1} (mean ± SE, $p < 0.001$) at the saturation irradiance (E_k) of 23.5 μmol photons m^{-2} s^{-1} (Table 1). The β was 0.000 3 ± 0.000 46 (mean ± SE, *ns*). The C_0 was -0.02 ± 0.022 μmol photons m^{-2} s^{-1} (mean ± SE, *ns*). Calculated daily net OP rates were 880 ± 187 nmol O_2 $individual^{-1}$ (mean ± SE).

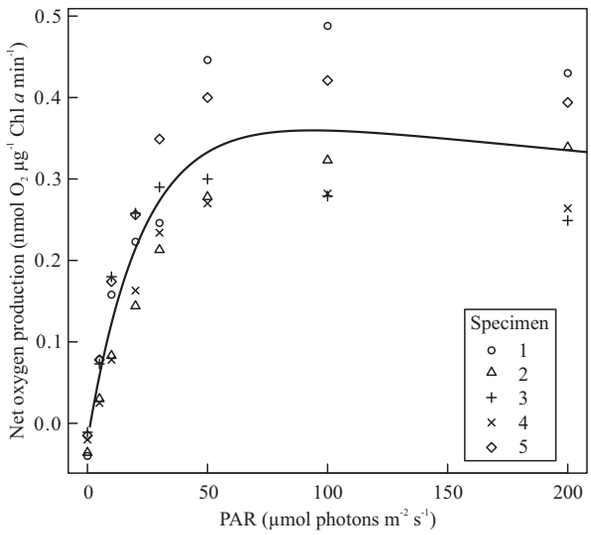

Figure 1. Net oxygen production (*P*)-irradiance (*I*) curve for *Cycloclypeus* holobionts. The fitted *P-I* curve indicates the α of 0.018 ± 0.003 7 (mean ± SE, $p < 0.001$) and the P_{max} of 0.42 ± 0.062 nmol O_2 μg^{-1} Chl *a* min^{-1} (mean ± SE, $p < 0.001$) at the saturation irradiance (E_k) of 23.5 μmol photons m^{-2} s^{-1}. The β was 0.000 3 ± 0.000 46 (mean ± SE, *ns*). The C_0 was -0.02 ± 0.022 μmol photons m^{-2} s^{-1} (mean ± SE, *ns*).

Table 1 Results of the non-linear model for light response parameters of *Cycloclypeus* holobionts

	Estimate	SE	*t*	*p*
P_{max}	0.418 485	0.062 427	6.704	8.06×10^{-8}
α	0.017 826	0.003 65	4.884	2.14×10^{-5}
β	0.000 334	0.000 464	0.719	0.477
C_0	-0.021 52	0.022 247	-0.967	0.34

P_{max} is the light-saturated production rate, α is the photosynthetic efficiency (i.e., the initial slope of the light curve), β is the rate of decline at supra-optimal irradiance levels, and C_0 is the compensation irradiance (i.e., irradiance where a gross production rate equals a respiration rate).

2.2 Growth Increment

The two *Cycloclypeus* holobiont populations exhibit different growth increment curves due to the different light levels (Fig. 2, Table S3). Average surface areas at all light levels increased until 28 days, then stabilized at all light levels. Linear models indicate that surface area was significantly affected by populations, time and light levels (all $p < 0.001$, Table 2). In addition, the interactions between populations and light and between light and time were also significant ($p < 0.01$), indicating that the effect of light conditions also varies between populations and time.

At the end of the two-month incubations, the highest average surface area for population 1 was observed at 5 μmol photons m^{-2} s^{-1}, while the lowest average surface area was observed at 100 μmol photons m^{-2} s^{-1} and in the dark. On the other hand, the highest average surface area for population 2 was observed at 30 and 50 μmol photons m^{-2} s^{-1}, while the lowest average surface area was observed at 100 μmol photons m^{-2} s^{-1}. Average surface areas at 5, 10 and 20 μmol photons m^{-2} s^{-1} were lower than those at higher light levels, and similar to those in the dark. *Cy-*

cloclypeus holobionts added several heterostegine and cyclic chambers, ranging from 2.85 to 4.50 chambers on average (Fig. 3, Table 3). Individuals with higher surface areas generally build more chambers. Conditions of algal symbiont color were likely related to light levels (Fig. 3, Table 3). For population 1, the cytoplasm inside the chamberlets was generally granulated with algal symbionts at various light levels except for 100 μmol photons m^{-2} s^{-1}. At 5 μmol photons m^{-2} s^{-1}, more than half of the individuals were entirely brown and filled with algal symbionts. On the contrary, individuals at higher light levels (>30 μmol photons m^{-2} s^{-1}) tended to be pale or colorless. Most individuals were colorless at 100 μmol photons m^{-2} s^{-1}. Results for population 2 were generally similar to those for population 1, but pale or colorless individuals were more common at various light levels, particularly those higher than 10 μmol photons m^{-2} s^{-1}. Algal overgrowth was commonly observed for *Cycloclypeus* holobionts subjected to higher light levels (Fig. 3, Table 3). No algal overgrowth was observed for individuals at 5 μmol photons m^{-2} s^{-1} and in the dark for both populations, while more than half of the individuals at light levels higher than 10 μmol photons m^{-2} s^{-1} were either fringed or entirely covered with algae. Assuming that individuals with pale or colorless algal symbiont color in addition to those fringed or entirely covered by algal overgrowth are either unhealthy or dead individuals, several individuals at 30 or 50 μmol photons m^{-2} s^{-1} and almost all individuals at 100 μmol photons m^{-2} s^{-1} in population 1 were either unhealthy or dead at the end of incubations, while more than half of individuals at >10 μmol photons m^{-2} s^{-1} were either unhealthy or dead in population 2.

Table 2 Results of the linear model for growth increments (measured as surface area) of *Cycloclypeus* holobiont populations

	DF	SS	MS	*F*	*p*
Population	1	0.516	0.516	34.663 0	4.451×10^{-9}
Light	1	0.956	0.956	64.180 9	1.730×10^{-15}
Time	1	38.971	38.971	2 616.853 4	$<2.2 \times 10^{-16}$
Population × light	1	0.154	0.154	10.334 7	0.001 322
Population × time	1	0.002	0.002	0.126 4	0.722 196
Light × time	1	0.154	0.154	10.369 1	0.001 298
Residuals	2 478	36.903	0.015		

Surface area data were analyzed by a linear model with interactions, using populations, light levels and time (days) as explanatory variables.

3 DISCUSSION

The *P-I* curve shows that *Cycloclypeus* holobionts are daily net primary producers adapted to low light levels, with slight photoinhibition (i. e., reduced net OP rates compared with P_{max}) beyond optimal irradiances (over 100 μmol photons m^{-2} s^{-1}). The daily net OP rates of *Cycloclypeus* holobionts are higher than those of other forereef-dwelling taxa, and comparable to those of shallow reef-dwelling taxa (Stuhr et al., 2017; Schmidt et al., 2016, 2014; Fujita et al., 2014; Sinutok et al., 2014, 2011; Reymond et al., 2013; Hosono et al., 2012; Uthicke and Fabricius, 2012; Uthicke et al., 2012; Vogel and Uthicke, 2012; Walker et al., 2011; Ziegler and Uthicke, 2011; Fujita and Fujimura, 2008; Nobes et al., 2008; Köhler-Rink and Kühl, 2001; Lee et al., 1980), likely due to their large and flat shells with dense diatom endosymbionts. Although P_{max} is difficult to compare among different studies due to different units used, the rates converted per hour is comparable to those of forereef-dwelling, diatom symbiont-bearing *Amphistegina lessonii*, and higher than those of other two amphisteginids (*A. gibbosa* and *A. radiata*), both of which are daily net consumers (Walker et al., 2011). The E_k of *Cycloclypeus* holobionts is much lower than that of shallow reef-dwelling taxa, but similar to that of forereef-dwelling taxa (amphisteginids, calcarinids, nummulitids), which also show photoinhibition at lower light levels (Walker et al., 2011; Ziegler and Uthicke, 2011; Fujita and Fujimura, 2008; Nobes et al., 2008; Köhler-Rink and Kühl, 2001; Lee et al., 1980). *Heterostegina depressa*, the nummulitid species mainly dwelling in forereef environments, shows slightly higher E_k values of 27–42 μmol photons m^{-2} s^{-1} with photoinhibition over 100 μmol photons m^{-2} s^{-1} (Ziegler and Uthicke, 2011; Nobes et al., 2008; Lee et al., 1980). Slightly

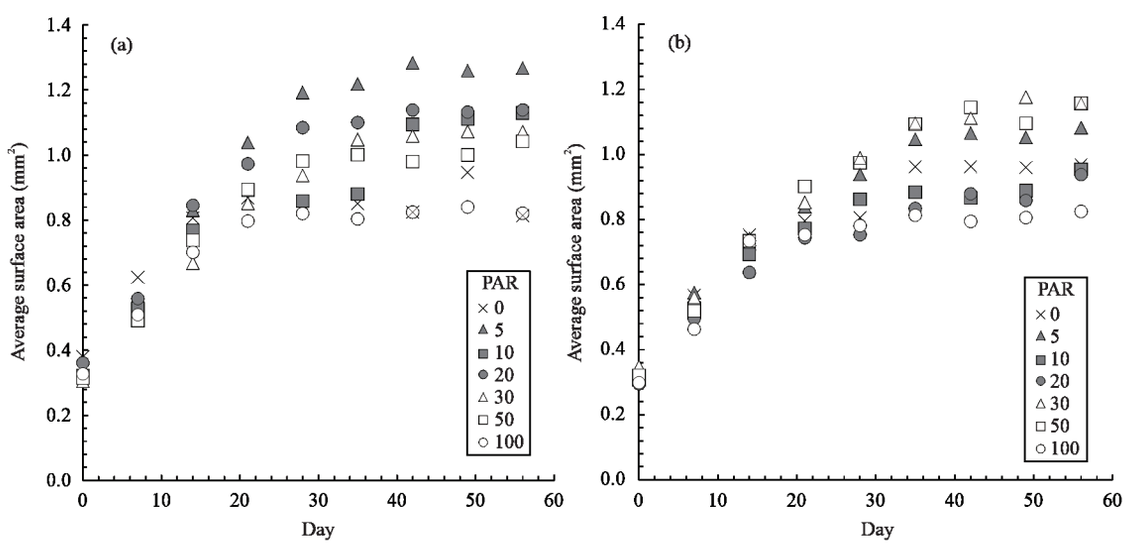

Figure 2. Growth increment curves expressed as average surface area of the two populations of *Cycloclypeus* holobionts subjected to seven different light levels. (a) Population 1, (b) population 2.

Table 3 Number of chambers, symbiont color and algal overgrowth of the two populations of *Cycloclypeus* holobionts after 56 days of incubation at different light levels (PAR; μmol photons m^{-2} s^{-1})

Population		PAR	0	5	10	20	30	50	100
1	Number of individuals		20	20	18	19	20	20	20
	Number of chambers	Median	3	5	4	4	4	4	3
		Mode	2	5	4	4	4	5	3
		Mean	2.85	4.50	3.78	3.84	4.10	4.05	3.44
		SD	1.23	1.00	0.81	0.60	0.85	0.83	0.70
	Symbiont color	Entirely brown	0	12	1	2	0	0	0
		Granulated	17	8	17	17	12	13	0
		Pale	1	0	0	0	7	2	2
		No	2	0	0	0	1	5	17
	Algal overgrowth	Entirely covered	0	0	0	0	4	0	10
		Fringed	1	0	9	12	7	15	10
		No	19	20	9	7	9	5	0
	Unhealthy individuals		1	0	0	0	3	6	19
2	Number of individuals		20	19	20	20	19	19	19
	Number of chambers	Median	3	4	4	4	5	5	4
		Mode	3	5	4	4	6	5	4
		Mean	3.25	4.32	3.83	3.90	4.47	4.47	3.47
		SD	0.64	1.06	0.79	1.37	1.47	1.12	0.80
	Symbiont color	Entirely brown	0	4	0	0	1	1	1
		Granulated	17	12	6	9	4	6	1
		Pale	0	2	8	4	2	3	0
		No	3	1	6	6	12	9	16
	Algal overgrowth	Entirely covered	0	0	5	9	1	1	4
		Fringed	0	0	12	9	13	14	11
		No	20	19	3	2	5	4	4
	Unhealthy individuals		0	0	13	9	11	10	14

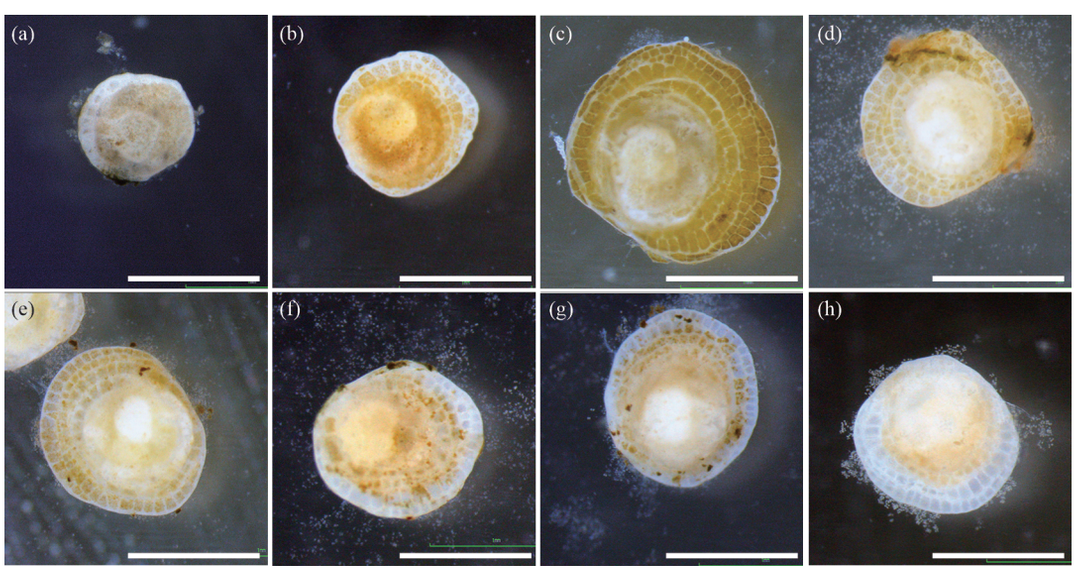

Figure 3. Images of typical individuals of *Cycloclypeus* holobionts (from population 1) after 56 days of incubation at different light levels. (a) Individual at the start of experiment, (b) individual in darkness, (c) individual at 5 μmol photons m^{-2} s^{-1}, (d) individual at 10 μmol photons m^{-2} s^{-1}, (e) individual at 20 μmol photons m^{-2} s^{-1}, (f) individual at 30 μmol photons m^{-2} s^{-1}, (g) individual at 50 μmol photons m^{-2} s^{-1}, (h) individual at 100 μmol photons m^{-2} s^{-1}. Scale bar = 1 mm.

different light responses between *Cycloclypeus* holobionts and other taxa are partly attributed to symbiotic diatom compositions. Among taxa with diatom endosymbionts, the species compositions vary with host taxa, depth and locations (Lee et al., 1989). Although there are still incongruences concerning the symbiont type specificity between culturing isolation and molecular sequencing studies, molecular studies showed different symbiont sequences between shallow-water and deep-water nummulitids (Holzmann et al., 2006). Diatom symbionts in *C. carpenteri* are likely adapted to low light (Lee et al., 1982), receiving ambient low light by living beneath thin, transparent calcite windows of chamberlets (Leutenegger, 1984). It also seems that diatom symbiont-bearing species can be adapted to a very low light environment, compared to dinoflagellate-rhodophyte- and chrolophyte-bearing species (Hallock and Seddighi, 2022; Prazeres and Renema, 2019; Hallock, 1988). In addition to different symbiont compositions, recent culturing studies suggest that foraminiferal light responses vary with conditions of ocean warming, acidification and eutrophication (Narayan et al., 2022; Kawahata et al., 2019; Stuhr et al., 2017; Prazeres et al., 2016a, b; Schmidt et al., 2016, 2014; Doo et al., 2014; Fujita et al., 2014; Sinutok et al., 2014, 2011; Reymond et al., 2013; Uthicke and Fabricius, 2012; Uthicke et al., 2012; Vogel and Uthicke, 2012).

Our long-term growth studies indicate that light intensity affects the growth and mortality of *Cycloclypeus* holobionts. Lower growth increments were observed in the dark and at the highest light level (100 μmol photons m^{-2} s^{-1}) for both investigated populations. Most individuals subjected to the highest light level were either unhealthy or dead. The lower growth in darkness may be partly due to their dormancy states (Ross and Hallock, 2019). Optimal light levels differed between the two investigated populations, which are also likely attributed to health conditions of *Cycloclypeus* holobionts, determined by algal symbiont color and algal overgrowth (Nobes et al., 2008; Hallock et al., 1995). In population 1, unhealthy individuals increased with higher light levels. In population 2, even though individuals at 30 and 50 μmol photons m^{-2} s^{-1} showed higher growth, more than half of the individuals were either unhealthy or dead. Most individuals under 10 and 20 μmol photons m^{-2} s^{-1} grew faster than those in darkness and also showed unhealthy conditions. Therefore, results of population 1 are more reliable than those of population 2, suggesting that *Cycloclypeus* holobiont populations grew better under a low light level of ~5 μmol photons m^{-2} s^{-1}. However, our results also suggest that *Cycloclypeus* holobionts can be acclimatized to a certain level of low light ranges (<50 μmol photons m^{-2} s^{-1}) by changing symbiont compositions and photopigment concentrations (Lee et al., 1982). It must also be mentioned that those growth responses to light are affected by recent ocean warming, acidification and eutrophication (Narayan et al., 2022; Kawahata et al., 2019; Stuhr et al., 2017; Prazeres et al., 2016a, b; Schmidt et al., 2016, 2014, 2011; Doo et al., 2014; Sinutok et al., 2014, 2011; Reymond et al., 2013; Uthicke and Fabricius, 2012; Uthicke et al., 2012; Vogel and Uthicke, 2012). Growth increment data also indicate a certain amount of growth during the first 28 days regardless of light levels, suggesting that nutrition was initially provided from a parent to asexually reproduced juveniles.

Previous studies also demonstrated a preference of lower light levels for the growth of forereef-dwelling diatom symbiont-bearing taxa. Hallock et al. (1986) showed that growth of *A. gibbosa* and *A. lessonii* increased with increasing light intensity ranging between 1.6 and 40 μmol photons m^{-2} s^{-1}. Subsequent studies showed that the optimal PAR is 6–8 μmol photons m^{-2} s^{-1} for *A. gibbosa*; higher PAR produced bleaching in a higher percentage of individuals (Williams and Hallock, 2004). Nobes et al. (2008) showed that growth of *H. depressa* significantly increased with a decrease in light intensity. Highest growth rates were observed in low light conditions (<60 μmol photons m^{-2} s^{-1}). Mortality in *H. depressa* reached close to 90% in high light treatment (>1 250 μmol photons m^{-2} s^{-1}).

Our experimental results are generally consistent with optimal levels as well as upper and lower limits of light for living *Cycloclypeus* holobionts, based on the depth distributions in the Northwest Pacific (Hohenegger, 2000; Fig. 4). The optimum light level of the depth distribution is 2.8% surface PAR, which corresponds to 14 μmol photons m^{-2} s^{-1} if average daytime surface PAR is 500 μmol photons m^{-2} s^{-1} (Fujita, personal observations). This light level is within a range between the optimal light level for growth and the saturation irradiance for metabolism in this study. The upper limit of light levels of the depth distribution is 10% surface PAR, which corresponds to 50 μmol photons m^{-2} s^{-1} if average daytime surface PAR is 500 μmol photons m^{-2} s^{-1}. This is consistent with the upper limit of light levels for growth in this study. The lower limit of light levels of the depth distribution is 0.4% surface PAR, which corresponds to 2 μmol photons m^{-2} s^{-1} if average daytime surface PAR is 500 μmol photons m^{-2} s^{-1}. This is generally consistent with the threshold light levels for *Cycloclypeus* holobionts as daily net primary producers. Therefore, our experimental results generally support the conclusion by Hohenegger (2000) that light intensity is the dominant environmental gradient affecting the vertical (depth) distribution of *Cycloclypeus* holobionts.

However, other environmental factors such as water energy, nutrients, foods and water temperature also limit the distribution and abundance of *C. carpenteri*. On the shelf of the Ryukyu Islands, living *C. carpenteri* individuals are attached on the surface of loose coarse-grained carbonate sand and gravels (Iryu et al., 1995; Koba, 1978). Tsuji (1993) reported strong semi-diurnal tidal currents (approx. 30–40 cm s^{-1}) on an island shelf off the Miyako Islands, in the southern Ryukyu Islands, forming mud-free, coarse sediments with rhodoliths and larger foraminifers. Nutrients are likely supplied on the shelf by topographically induced upwelling along the shelf slope. Therefore, a warm, low light environment with moderate water energy and nutrient supply would be necessary for *Cycloclypeus* holobionts.

4 CONCLUSION

Net OP measurements of diatom symbiont-bearing *C. carpenteri* individuals under eight light levels showed that *Cycloclypeus* holobionts are daily net primary producers adapted to low light levels. Long-term incubations using two asexually reproduced populations indicated that *Cycloclypeus* holobiont populations grew better at a low light level (~5 μmol photons m^{-2} s^{-1}), but can be acclimatized to a certain level of low light ranges (<50 μmol photons m^{-2} s^{-1}). These experimental results

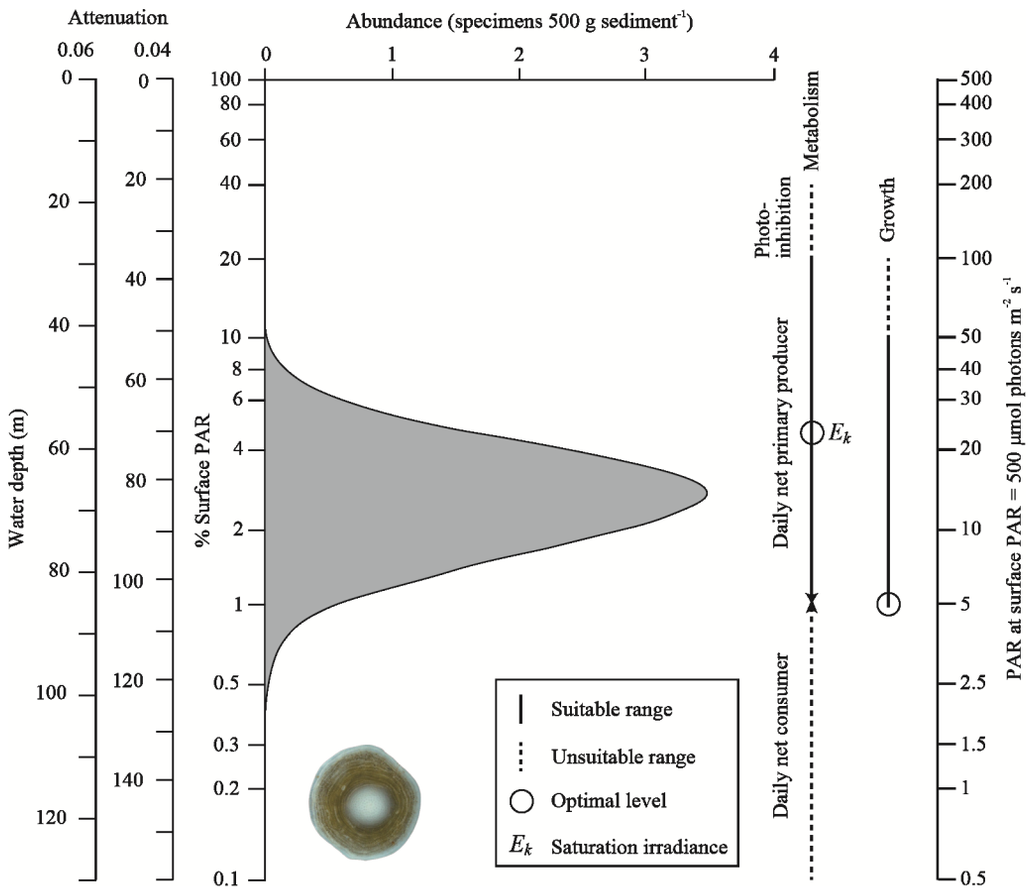

Figure 4. Comparisons of the range and optimal level of light between the vertical distribution (theoretical frequency distribution data from Hohenegger, 2000), metabolism (net oxygen production rates) and growth increments of *Cycloclypeus* holobionts examined in this study.

verified that *Cycloclypeus* holobionts are physiologically adapted to a low light environment of a deep euphotic zone. This study suggests that light is an important environmental gradient affecting the vertical (depth) distribution of *C. carpenteri*.

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