Long term photoacclimation responses of the coral *Stylophora pistillata* to reciprocal deep to shallow transplantation: photosynthesis and calcification

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Reciprocal transplantation of *Stylophora pistillata* coral fragments between deep (30 m) and shallow sites (3 m) was conducted gradually and resulted with 100% survival. Photoacclimation of transplants at both depths showed two distinct phases: at the first phase, within 2 weeks, zooxanthellae density decreased below (at 3 m) and increased beyond (at 30 m) these of the control values at the new depth, while chlorophyll a per zooxanthellae cell remained as in the original depths, thereby fully adjusting areal chlorophyll concentration. On the second phase, after 6 months, zooxanthellae chlorophyll and their quantum yield (Fv/Fm) were adjusted at both new depths. Such regulated acclimation was also observed in the maximal photosynthesis rate of both transplants, whereas respiration adjustment was rapid. These results differ from previously reported rapid shade and light acclimation strategies hence we suggest that acclimation mechanism changes when certain symbiont type is exposed to depth out of its boundary zone. Despite seemingly having become physiologically acclimated, calcification at both new depths was only half the rate achieved by the controls, suggesting that the coral host requires even longer time than the symbionts to acclimate.

Keywords: photosynthesis, calcification, photoacclimation, survival, Symbiosis

Introduction

The coral and its symbiotic algae, the zooxanthellae, form a unique holobiont that successfully dominates several niches at diverse conditions along the depth gradient along tropical and subtropical shorelines. Corals of the same species living at the extremes of their depth distribution, at the reef flat or in dimly lit depths are physiologically adapted in order to cope with these two stressful environmental conditions, supraoptimal irradiance and too little of it. The intensity of irradiance (PAR and UV-B) appear to be the most significant factors affecting coral physiology (Cohen et al., 2013) and bathymetric distribution (Achituv and Dubinsky, 1990) throughout depth. Corals minimize the potential for photooxidative damage under excess light in shallow reefs or maximize photosynthesis under dim light in deep water by regulating their tri-dimensional morphologies (Mccloskey and Muscatine, 1984), changes in ratios of chlorophyll a and c, carotenoids, peridinin (i.e., Shick et al., 1995; Brown et al., 1999; Stambler and Dubinsky, 2004)

and photosynthetic unit size (Dustan, 1982). Furthermore, shallow and deep corals were found to harbor different types (clades) of symbionts (i.e., Iglesias-Prieto et al., 2004).

Experimental transfer of colonies from their original location to the opposite end of their light intensity distribution range, subjects them to stressful perturbations of metabolic patterns, which usually lead to their demise. The photoacclimation strategies of the zooxanthellae exposed to different light regimes and the photo adaptive responses to changes in light quantity and quality, have been documented in many papers (Titlyanov et al., 1980; Chang et al., 1983; Mccloskey and Muscatine, 1984; Parker and Muscatine, 1984; Porter et al., 1984; Kaiser et al., 1993; Masuda et al., 1993; Leletkin et al., 1996). Yet, physiological strategies of acclimation applied by deep corals upon exposure to light at shallow depths in comparison to controls remaining at both extremes are poorly understood. Gattuso (1985) showed in a preliminary study that the saturation intensity of photosynthesis (I_k) of *Stylophora pistillata* adjusts quicker following shallow rather than deep transfer. Shallow transplantation of low light corals usually results in bleaching (Baker, 2001; Richier et al., 2008) and high mortality rates (Dustan, 1982; Vareschi and Fricke, 1986; Yap et al., 1998; Iglesias-Prieto et al., 2004). Light harvesting properties of the low light acclimated corals can reach 95% of impinging light (Stambler and Dubinsky, 2005). Sudden exposure to high light causes imbalance between the rate of excitation of reaction centers and the relatively slow rate of the dark reactions, limited by low Rubisco to photosynthetic unit content (Fisher et al., 1989). That results in free radical formation and photodynamic damage. That acute stressor is aggravated by UV-B that likewise can impose oxidative stress which can be fatal (Shick et al., 1995). For example, Lang (1973) observed that corals from 14 to 30 m bleach when transferred to 1 m, but not corals from 25 to 50 m when transferred to 15 m. UV protection and damage repair at shallow depths and extensive production of photosynthetic pigments at deeper depths requires energy diverted from essential metabolic functions (Karentz et al., 1994). Hence some corals fail to modify the concentrations of UV absorbing molecules, hindering their acclimation to shallow water (Scelfo, 1986; Vareschi and Fricke, 1986).

This study, beside its importance for understanding photoacclimative mechanisms, is of ecological interest, since coral reefs, as sessile as they are, may be subjected to alteration in their depth. Since 1833, over 35 tsunamis have been recorded around the Indonesian archipelago (Carey et al., 2001). The earthquakes led to these tsunamis sometimes results in extensive reef upheaval (Moore and Moore, 1984, 1988). Movement of the sea bottom may sink reefs to a deeper location (Brown, 2005), but may also uplift corals to a shallower location (Grigg and Jones, 1997; Carey et al., 2001; Yamano et al., 2001; Brown, 2005; Searle, 2006; Liew and He, 2007). In addition to such natural causes, corals are frequently placed in artificial reefs as part of damaged reef remediation efforts, in coral nurseries, research water tables, and underwater observatories, at depths sometimes different from their original ones.

In the present work, we employ gradual, stepwise transfer of *S. pistillata* from two extreme depths (30 and 3 m) to the reciprocal depth and evaluate the short (14 days) and long (6 months) term acclimative mechanisms of the photosynthetic apparatus and their effect on calcification.

Materials and Methods

Experimental Setup

Five medium sized colonies (15–20 cm diameter) of the hermatypic coral *S. pistillata* (Esper, 1797) were collected from each, 3 and 30 m depth, along the reef adjacent to the Interuniversity Institute for Marine Science (IUI) at the Gulf of Eilat, the northern Red Sea (29° 30′ N, 34° 56′ E). Each colony was fragmented into 80 branches 3–5 cm long, glued onto a plastic tip and returned to its original depth on underwater rectangular tables (1 × 2 m and 1 m above the ground). Twenty days later, half of the fragments from each colony were gradually transferred to the reciprocal depth. Shallow transfer included 10 intermediate stations with 10 day intervals following the method described in Cohen et al. (2013). The slow ascend of deep fragments was required for avoiding the acute oxidative stress and to increase their survival. Deep transfer requires mainly photo-acclimation and not photo-protection processes to evolve, hence included one intermediate station at 10 m for a period of 14 days before final transplantation to 30 m. The shallow transfer of 30 m corals was initiated on November 6th 2006, and the 3 m corals were transferred to the intermediate station at 10 m depth on the 16th of December 2006. Corals were transferred to their final depth at 30 and 3 m on the 1st of January and 10th of February 2007 respectively. By the time shallow corals were placed at 30 m the deep corals were at 7.5 m depth, hence both treatments were not exposed to their final depth the exact time before the first sampling. The process of shallow transfer started before and ended after the process of deep transfer, this however did not affect the results since the first phase of acclimation occurred within this time frame of a few weeks and the second phase was observed only 6 months later. Fragments from both treatments and controls, which remained at their original depth, were analyzed for photoacclimation of photosynthesis and calcification, 14 days (during February 2007) and 6 months (August 2007) after the final transplantation to 3 m. For convenience of the presentation of the results we used this time although final transplantation of shallow corals in 30 m was a month earlier. For the first sampling, 3–4 random fragments from each colony were tested ($n = 16$). On April 2007 half of the remaining fragments were lost due to a storm, hence for the second sampling we used only 1–2 fragments from each colony ($n = 8$). The effect of inner colony variation was tested statistically.

Data Collection

Irradiance and sea temperature were recorded at 3 to 30 meters, at each sampling point (**Table 1**) by a Pulse Amplitude Modulated instrument (diving- PAM, WALZ instruments, S.N: UWFA0221A, Effeltrich, Germany). The light sensor connected to the PAM was previously calibrated to a Li- Cor-1000 light meter.

TABLE 1 | Physical parameters at 3 and 30 m depth measured 14 days (February) and 6 months (August) after relocation next to the experimented corals.

Time after transfer (m)	ambient irradiance (μmol photons m^{-2} s^{-1})		Sea temperature (°C)	
	14 days	6 months	14 days	6 months
3	825	900	20	27
30	94	133	20	25

Quantum Yield of Fluorescence and Non-photochemical Quenching

Maximal quantum yields (Fv/Fm) were measured with the diving PAM at 3 and 30 m depth-*in situ*, on the same day 20 min after sunset. According to a diel quantum yield cycle made for several days on 3 and 30 m treatments and controls during both sampling points- a few minutes after sunset Fv/Fm reaches a maximal peak which is stable for an hour before a gradual night decline. Since this is the highest Fv/Fm of the day, corals were already dark adapted. In the gulf of Eilat the sun sets behind 200 m high mountains, hence there is still a dim light that enables diving without external light source. In order to accurately measure all samples in one dive only 3 repeats were measured in each treatment. Initial fluorescence (F_0) is emitted by the zooxanthellae after weak flashes of wavelength $\lambda > 670$ nm, maximal fluorescence (F_m) is achieved after saturating pulse of 5000 μmol photons m^2 s^{-1} generated by the diving PAM for 0.8 s. Equation 1:

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m}$$

Similar F_v/F_m measurement was performed also at noon in order to calculate NPQ (Equation 2)- the relative decrease in maximal fluorescence during the day (F_m') as a result of shift in dissipation mechanism to heat, compared to night (F_m). Equation 2:

$$NPQ = \frac{F_m - F_m'}{F_m'}$$

Photosynthesis

For the first and second sampling points, 4 and 2 respectively, random fragments from each colony were retrieved from their experimental depth covered with an opaque plastic strap to avoid light stress and within 2 min placed in running seawater aquaria, shaded according to the treatment, for a maximum of 4 h before the measurement of photosynthesis. Each specimen was placed in a sealed metabolic chamber containing 150 ml filtered sea water (G/FC Whatman) to prevent non coral related respiration or photosynthesis. The water was thermostatted according to the respective seasonal temperature (20°C on February and 26°C on August) using a circulating water bath (NESLAB, RTE 210). Oxygen electrodes (Oxi 323, WTW), connected to a digital data logger, recorded the changes in dissolved oxygen concentrations. The electrodes were calibrated for their minimal and maximal readings by bubbling N_2 or O_2 respectively. Since this electrode is temperature compensated we calibrated it in both seasons to

air saturation according to 6.6 mg/L at 25°C. The electrodes had minimal oxygen consumption that was corrected by measuring oxygen change of filtered seawater without corals (blank control). The chambers were cleaned each time before placing a new sample in order to prevent biofilm formation. Fragments were dark adapted for 15 min in order to fully oxidize the PQ pool and avoid a time lag in photosynthesis (Moberg et al., 1997). Changes in dissolved oxygen indicated dark respiration during the first 20 min and net photosynthetic rates at a sequence of 9 increasing irradiances at 10-min intervals. Photosynthesis normalized to chlorophyll *a* was fitted to hyperbolic tangent function (Equation 3) according to Chalker (1981) using nonlinear least squares (nls) of the statistical program Rstudio (version 2.15.0). For this fit we removed all data points that were in photoinhibition since it is not accounted in this model. This formula calculates gross photosynthesis (P_G); however for biological interpretations it was easier to compare net photosynthesis between the treatments, so the data in **Table 2** are calculated after subtraction of respiration of each specimen. Equation 3:

$$P_G = P_{max} X \frac{\text{hyperbolic tangent (Irradiance)}}{I_k} - \text{respiration}$$

The initial slope- α, compensation irradiance- I_c, P_{max} and the saturation irradiance- I_k were calculated using Equation 3.

Zooxanthellae Density and Chlorophyll *a* Concentration

After the photosynthesis vs. irradiance measurement, tissue of the fragments was removed by a jet of compressed air and filtered (GF/C Whatman) seawater using an artist's air brush. The tissue slurry was homogenized and stirred. Zooxanthellae of each sample were counted 4 times on a hemocytometer grid under light microscope (400×). Number of cells was multiplied by 10^4 in order to receive the amount in 1 ml, multiplied again by 50 to match the dilution volume and divided by surface area to calculate their areal density as number of zooxanthellae per cm^2. The surface area of each fragment was estimated from the weight of aluminum foil covering its surface area according to Marsh (1970).

Chlorophyll *a* was extracted by filtering 5 ml of the tissue slurry on a GF/C filter. The filter was then homogenized with 5 ml of acetone 90% and kept for 24 h in the dark at 4°C. Chlorophyll *a* concentration was evaluated with a spectrophotometer (ultrospec 2100 pro, Amersham Pharmacia Biotech) following the equations described by Lorenzen (1967). Concentration of chlorophyll *a* per zooxanthella cell was

TABLE 2 | Photosynthetic parameters (Mean ± SE) of *S. pistillata* from 3 and 30 m, and after 14 days (during February) and 6 months (during August) of their relocation to the reciprocal depth (3→30 m and 30→3 m).

	Time after relocation	30 → 3/3 m	30 → 3 m relocated	3 m control	3/30 m	30 m control	3 → 30 m relocated	3 → 30/30 m
Rd - dark respiration	14 days	0.42**	−21.8 ± 3.5	−52 ± 5	2.29*	−22.7 ± 6.3	−71 ± 14.8	3.1*
μmol O$_2$ h^{-1} Chl a^{-1}	6 months	0.77	−46.5 ± 9.1	−60.8 ± 6	1.85	−32.8 ± 10.9	−67.3 ± 15.3	2.1
	Ratio		2.1	1.2		1.4	0.9	
α - initial slope	14 days	0.5	0.56 ± 0.14	1.02 ± 0.13	0.42	2.4 ± 0.67	1.23 ± 0.6	0.5*
mol O$_2$ cm^{-2} (mg Chl)$^{-1}$	6 months	1.5	1.4 ± 0.49	0.99 ± 0.14	0.89	1.1 ± 0.25	0.95 ± 0.2	0.9
(mol photons)$^{-1}$	Ratio		2.6 (P = 0.06)	1.0		0.5	0.8	
I$_c$ - compensation point	14 days	0.95	59.5 ± 14*	62.6 ± 5.5	426***	14.7 ± 2.1	440 ± 156	29.9***
μmol photons m^{-2} s^{-1}	6 months	0.7	45.2 ± 8.3	64 ± 3.8	1.61	39.7 ± 13.5	76.8 ± 7.4	1.9
	Ratio		0.8	1.0		2.7**	0.2*	
I$_k$ - light saturation	14 days	2.1	159.7 ± 27*	103.4 ± 8.7	2.54**	40.6 ± 8.4	227 ± 88	5.6*
μmol photons m^{-2} s^{-1}	6 months	0.8	127.3 ± 24	169 ± 8.8	1.47	115.1 ± 18.1	205 ± 28	1.8*
	ratio		0.6	1.6*		2.8*	0.9	
Potential net P$_{max}$	14 days	1.0	48.7 ± 7.2	47.6 ± 9	1.6	29.7 ± 4.5	18.2 ± 12.8	0.6
μmol O$_2$ h^{-1} Chl a^{-1}	6 months	0.7*	75.6 ± 7.9	109.5 ± 10	1.51	72.5 ± 8.5	90.9 ± 13	1.3
	Ratio		1.6*	23***		5**		
Actual net P$_{max}$	14 days	0.4*	19 ± 2.6	47.6 ± 9	1.8	26.4 ± 4.4	15.7 ± 12	0.6
μmol O$_2$ h^{-1} Chl a^{-1}	6 months	0.54*	58.7 ± 7.6	109.5 ± 10	1.85*	59.1 ± 7.4	90.7 ± 12.9	1.5
	Ratio		3.1*	2.3*		2.2*	5.8*	
Pmax:R ratio	14 days	2.8*	2.6 ± 0.58	0.94 ± 0.13	0.43	2.19 ± 0.48	0.58 ± 0.35	0.3
	6 months	1.12	2.0 ± 0.4	1.8 ± 0.2	0.43	4.2 ± 0.6	2.2 ± 0.6	0.5
	Ratio		0.8	1.9*		1.9	3.7	
Photoinhibition	14 days	0.53	−32.6 ± 6.9	−62 ± 9.9	1.03	−49 ± 9.6	–	–
μmol O$_2$ h^{-1} Chl a^{-1}	6 months	2.71	−56 ± 17.8	−20.6 ± 3.9	0.41	−50.2 ± 8.9	−19.8 ± 9.5	0.4
	Ratio		1.72	0.33**		0.98	–	
Photoinhibition	14 days	1.16	891 ± 104	766 ± 106	2.6***	292 ± 84	–	–
μmol photons m^{-2} s^{-1}	6 months	0.64*	722 ± 94	1130 ± 70	1.6*	693 ± 89	990 ± 102	1.42
	Ratio		0.81	1.47		2.37	–	

Data calculated from hyperbolic tangent function (Chalker, 1981). Differences between controls and transferred corals are shown as ratios in bold and statistical differences are marked by *<0.05, **<0.01, ***<0.001. Potential net P$_{max}$- maximal photosynthesis along the P vs. I curve; actual net P$_{max}$- photosynthesis calculated according to the highest irradiance available at each depth. Two parameters were calculated for Photoinhibition: photosynthesis at 1200 μmol photons m^2 s^{-1} subtracted by Pmax, and the average irradiance at which photoinhibition initiates. Red and blue colors represent values closely similar to 3 and 30 m control respectively. Values that substantially changed during the long term acclimation are colored with darker shade.

calculated by dividing chlorophyll a concentration (μg ml^{-1}) by the number of cells ml^{-1}. Concentration of chlorophyll a cm^{-2} was calculated by multiplying chlorophyll a (μg ml^{-1}) by the volume of the sample (50 ml) and dividing by surface area of the fragment.

Calcification

Daily percent growth was calculated using the buoyant weight technique (Jokiel et al., 1978). Specimens were weighed with a semi analytic digital scale (SnowRex, LB300 300 × 0.01 g) on December 20th 2006 and at each sampling time (14 days and 6 months after final relocation). During December 2006 the deep corals were still in the process of the gradual shallow transfer and were at 7.5 m, this was necessary however in order to get a good

signal when calculating growth rates. The fragments used for each sampling were sacrificed for the rest of the measurements hence the results were independent.

Statistical Analyses

All statistical analysis was done with Rstudio software. Within the repeats of all treatments we measured several fragments from each of the five mother colonies extracted from each 3 and 30 m depth. To assess the significance of the effect of inter-colony variation as well as season and depth on calcification, photosynthesis and symbiosis we used permutated mixed effects model. The permutation test was chosen due to the non-normality of the data according to Shapiro-Wilk normality test. In order to examine the statistical differences between the

combine groups of depth and seasons we tested the normality of the mixed effects model residuals using the Shapiro-Wilk normality and performed pairwise Wilcoxon rank sum test. Bonferroni correction was used to account for the multiple comparisons. In each test the fix factors were colony number (1–5), season (winter- 14 days and summer- 6 months) and treatment (3 m, 3→30 m, 30 m, 30→3 m).
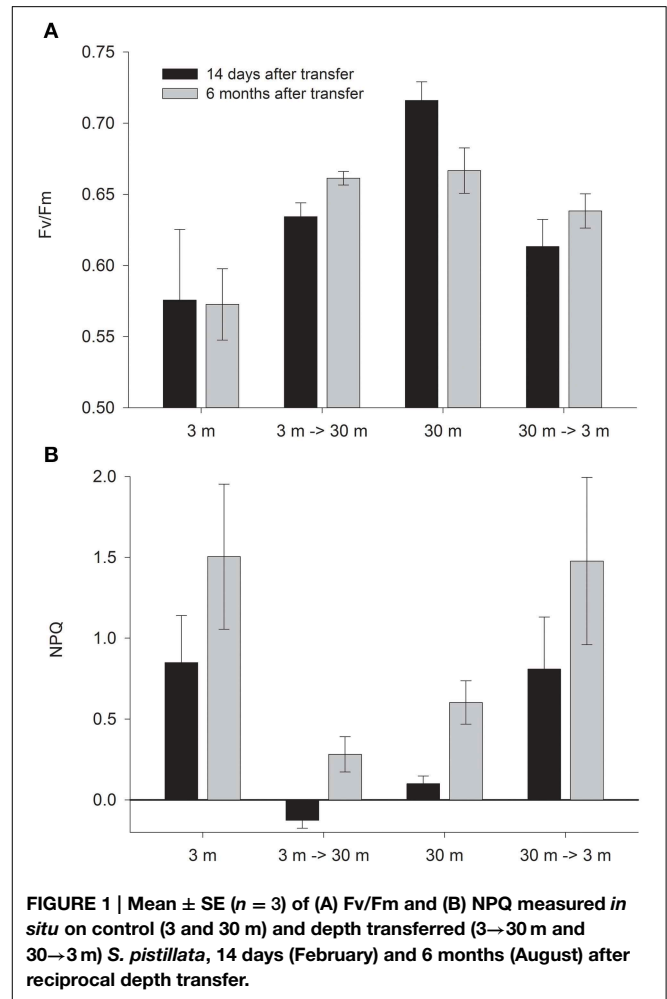
Results

The slow and gradual transplantation to both depths resulted in 100% survival of coral fragments, with no partial mortality and no significant sign of stress as excessive mucus release. This allowed us to relate to the results of the treatments as corals in a process of acclimation and not in a process of degradation. All of the parameters used for the statistical tests, except one (I_c) showed that the difference between repeats of the same colony was larger than in between colonies, hence all fragments are accounted as true repeats.

Irradiance levels at noon were 8.8 fold higher at 3 m than at 30 m during February (winter), 14 days after transfer, and only 6.8 fold higher during August (summer) 6 months later (**Table 1**). Irradiance during August, compared to February, was only 9% higher at 3 m in respect to 41% at 30 m. That difference corresponds to the documented seasonal changes in phytoplankton densities between the stratified, oligotrophic summer and the nutrient enriched winter and results in the vertical light attenuation k_d at 30 m increase from its summer minimum of $0.45 \, \mathrm{m}^{-1}$ to $0.8 \, \mathrm{m}^{-1}$ in winter (Dishon et al., 2012). Temperature mostly changed between seasons: 20°C at both depths during February and between 25 and 27°C during summertime seawater stratification in August.

Quantum Yields

Corals transferred to deep water have increased Fv/Fm values; however values attained 14 days after final transplantation (and 3 months after the gradual transplantation had started) were still below these of the native deep controls. Only 6 months later, Fv/Fm in the deep transplants reached values equal to these of the 30 m controls. Fv/Fm values of the shallow transferred corals decreased already after 14 days ($P < 0.05$, **Figure 1**), however, these never reach the low values of the controls. A similar trend emerged in the NPQ values (**Figure 1**) which changed significantly in both treatments ($P < 0.05$). It is not surprising that shallow corals decreases NPQ at greater depth, however, these corals attained lower values even than the controls at 30 m after 14 days and 6 months ($P < 0.05$). During August all corals dissipated a higher percentage of the impinging irradiance (significant only in 30 m and 3→30 m, $P < 0.05$).

The formula provided for calculation of NPQ can lead, in some cases, to over or under estimation of values, since it is based on the variable fluorescence proxy values. During an August day, all shallow corals reduced their Fm' to less than one half of those measured at night. As a result NPQ is calculated to be higher than 1- more than 100% of the light appears to dissipate. Conversely at deep water, Fm' values of 3→30 climbed during the day above the values measured in the dark, therefore NPQ seems

FIGURE 1 | Mean ± SE (n = 3) of (A) Fv/Fm and (B) NPQ measured in situ on control (3 and 30 m) and depth transferred (3→30 m and 30→3 m) *S. pistillata*, 14 days (February) and 6 months (August) after reciprocal depth transfer.

negative even though it is impossible to release negative amount of heat. We discuss possible reasons for this phenomenon, and explain why although we cannot rely on the PAM NPQ values, the trend of the difference between treatments can be considered genuine.

Photosynthesis and Respiration

Oxygen consumption via respiration of *S. pistillata* corals was more than twice faster at 3 m than at 30 m ($P < 0.05$, **Table 2**), and became greatly modified upon both shallow and deep transfers ($P < 0.01$ and $P < 0.05$ respectively). The compensation intensity (I_c) and saturation intensity (I_k) of photosynthesis were 4.3 and 2.5 fold higher respectively at 3 m compared to 30 m corals during February, whereas the irradiance difference during that month at noon was 8.8 fold higher at 3 m. During August, I_c and I_k were only 1.6 and 1.5 fold higher at 3 m compared to 30 m corals whereas irradiance was 6.8 fold higher. Hence photosynthesis, in regard to the available light, is consistently lower in shallow compare to deep corals. Such discrepancy suggests that photoacclimation over a depth range is not linear with light intensity. Compensation irradiance changed in both shallow and deep transfers but was far from attaining the optimal values of controls by the end of the first 2 weeks.

Photosynthesis among deep transferred corals compensated their respiration at higher irradiance compared to the 30 m controls, although similar respiration was measured. Shallow transferred corals utilized oxygen at a rate higher than it was produced by the symbionts under most of the light intensities along the P vs. I curves (**Figure 2**) and reached compensation at irradiance 7 fold higher than was measured for 3 m controls. Only 6 months later, compensation irradiance and the photosynthesis curves under both treatments came to closely resemble the controls at the new depth.
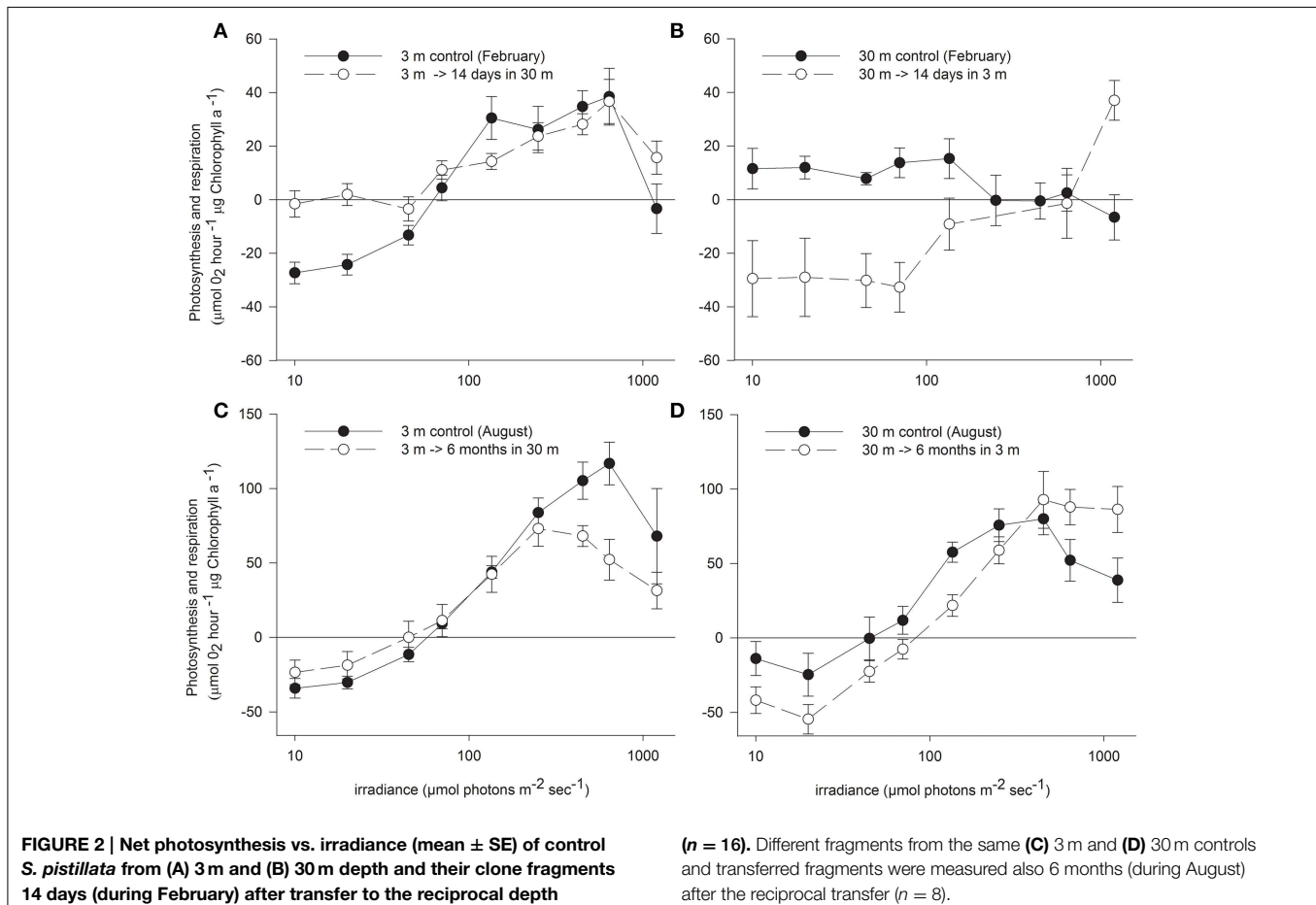
Maximal rates of photosynthesis according to the P vs. I curves were 1.6 fold higher at 3 m than at 30 m regardless of the season, however this represent a potential P_{max} at intensities up to 1200 μmol photons m^{-2} s^{-1}. Irradiance levels at 3 m are higher than the I_k measured for shallow corals, hence the actual photosynthesis of these corals are similar to their potential P_{max}. Conversely, *S. pistillata* at 30 m can increase photosynthesis at irradiances beyond their availability attaining only 0.88 of the actual P_{max} in February and 0.8 in August. Hence the actual differences in P_{max} are higher in shallow corals than usually calculated. While the potential Pmax is not significantly different between shallow and deep control, the actual Pmax is significantly higher in shallow corals. Two weeks after shallow or deep transfer the potential P_{max} was not influenced; significant
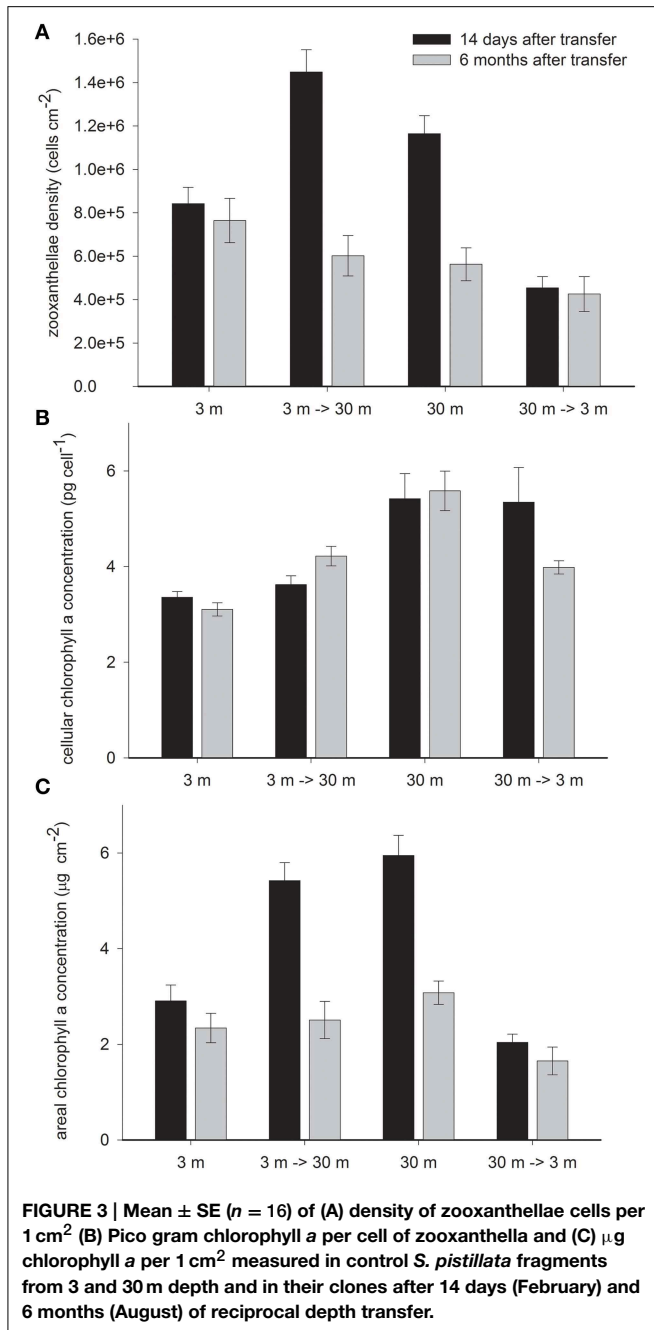
change within the deep transferred corals was observed only after 6 months.

Photosynthesis was inhibited in shallow fragments at higher irradiance compared to deep fragments at both seasons. However, rate of photosynthesis under the highest experimental illumination decreased similarly in both controls. The resilience to photoinhibition under high irradiance within shallow corals remained high also after 2 weeks of deep transplantation, but resembled the deep control corals after 6 months. In August, both control and transferred corals at 3 m were less photoinhibited by the high light of the P vs. I cure than control and transferred corals at 30 m.

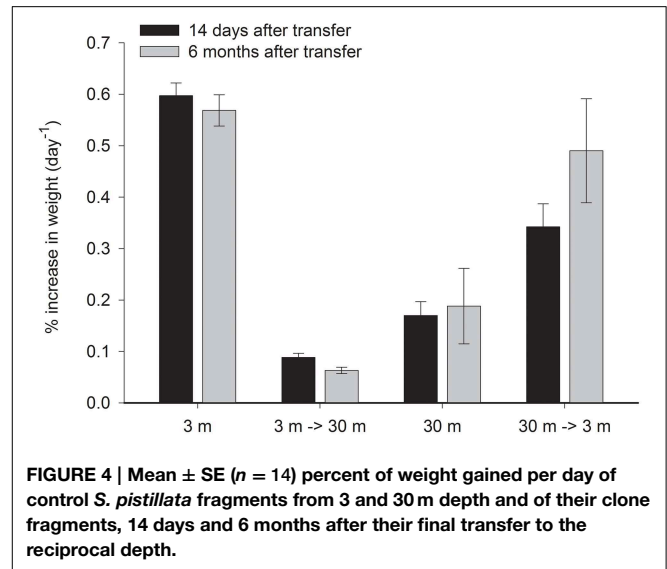## Zooxanthellate Density and Chlorophyll *a* Concentration

Deep control corals harbor 28% higher zooxanthellae density per cm^2 (**Figure 3A**) and 38% higher chlorophyll a concentrations per zooxanthella cell than shallow corals in February (**Figure 3B**) This led to twice higher chlorophyll *a* cm^{-2} at 30 m than at 3 m controls ($P < 0.01$). In both treatments, 14 days subsequent to their final depth transfer, a dramatic change was observed in the abundance of zooxanthellae cells whereas chlorophyll *a* per zooxanthellae cell did not change and resembled the control colonies at the original depth. The 3 → 30 m transfer resulted

FIGURE 2 | Net photosynthesis vs. irradiance (mean ± SE) of control *S. pistillata* **from (A)** 3 m **and (B)** 30 m **depth and their clone fragments 14 days (during February) after transfer to the reciprocal depth** (*n* = 16). Different fragments from the same **(C)** 3 m and **(D)** 30 m controls and transferred fragments were measured also 6 months (during August) after the reciprocal transfer (*n* = 8).

FIGURE 3 | Mean ± SE (*n* = 16) of (A) density of zooxanthellae cells per 1 cm^2 (B) Pico gram chlorophyll *a* per cell of zooxanthella and (C) μg chlorophyll *a* per 1 cm^2 measured in control *S. pistillata* fragments from 3 and 30 m depth and in their clones after 14 days (February) and 6 months (August) of reciprocal depth transfer.

FIGURE 4 | Mean ± SE (*n* = 14) percent of weight gained per day of control *S. pistillata* fragments from 3 and 30 m depth and of their clone fragments, 14 days and 6 months after their final transfer to the reciprocal depth.

36% increase by deep and 29% decrease by shallow transferred corals closed only some of the gap with the controls in their new habitat. Superimposed on the above described depth related changes, areal chlorophyll concentration decreased in all corals during summer, however significantly only in 30 m (−58%) and 3→30 m (−55%) fragments (both $P < 0.001$) as a result of substantial decrease in zooxanthellae density ($P < 0.05$), whereas with no significant concomitant decrease in cellular pigmentation.

Calcification

S. pistillata in this study grew 3.5 times faster at 3 m than at 30 m depth ($P < 0.01$, **Figure 4**). The shallow corals reduced growth rate by 86% ($P < 0.01$) when transferred to 30 m, 48% lower than the deep control after 14 days and remained lower than the deep control even after 6 months. Conversely, deep coral fragments increased their growth rate by 100% ($P < 0.05$) above their original growth rates when transferred to 3 m. However, they were still 43% lower than the 3 m controls ($P < 0.01$). Six months later, 30→3 m transplants increased the daily average growth by a further 43%, but were still slightly growing slower (by 14%) than the rates of the 3 m control.

Discussion

The depths chosen for this study (3 and 30 m) represent two extremes of their bathymetric range- where corals withstand either too much or very low light. Corals grow ideally at 5–10 m depth (Erez, 1978; Gattuso, 1985) which provides 45–60% of subsurface irradiance in the Red Sea (Cohen et al., 2013). Depth acclimation of *S. pistillata* was evident within all physiological parameters measured in this study however following transfer to a new depth some of these parameters change partially after a few weeks and complete their change only after a few months. Although shallow transfer started earlier than the deep transfer, both treatments were still in the short term acclimation process during our first sampling- zooxanthellae chlorophyll

in 71% increase in zooxanthellae ($P < 0.05$), 24% higher than in the 30 m control and the 30→3 m samples lost 69% of their zooxanthellae ($P < 0.001$), remaining with 47% lower than the shallow controls ($P = 0.07$). Increasing densities of the low chlorophyll zooxanthellae with deep transfer and dilution of the high chlorophyll zooxanthellae upon shallow transfer resulted in significant change in chlorophyll *a* cm^{-2} ($P < 0.001$ with shallow and $P < 0.5$ with deep transfer), which attained similar values to the controls at both new depths (**Figure 3C**).

Changes in cellular chlorophyll were observed only 6 months after transplantation of both treatments- becoming significantly different from their original depth control ($P < 0.05$). The

concentrations and the potential Pmax are still similar to their original depth controls.

Quantum Yield and Photosynthesis

Lower respiration with depth and the concomitant dim light, which was shown in many studies (Dustan, 1982; Mccloskey and Muscatine, 1984; Gattuso, 1985; Masuda et al., 1993; Fitt et al., 2000), was one of the initial responses to depth relocation of *S. pistillata* (**Table 2**). Respiration can be stimulated by the high photosynthesis and growth rates (Mccloskey and Muscatine, 1984) as seen in shallow corals. However, the high respiration observed in the corals transferred to 3 m could result from chronic photoinhibition (Hoegh-Guldberg and Jones, 1999; Warner et al., 1999) and may assist in avoiding dangerous increase in oxygen concentration in the tissue (Shick et al., 1991).

Unlike respiration, a two step, slow process of acclimation was observed in all parameters of photosynthesis. While differences in α, I_c, I_k, and P_{max} in this study were consistent with corals at different depth (Chalker et al., 1983; Gattuso, 1985) or shading (Falkowski and Dubinsky, 1981), some discrepancies were found in the kinetics of depth and shade acclimation. Unlike the rapid photosynthetic adjustment between reciprocal shade and light acclimation reported by Falkowski and Dubinsky (1981) we observed a much longer term process with depth acclimation.

Shallow corals maintain the high reduction potential of the plastoquinone pool by not optimizing the compensation irradiance (I_k) after 14 days at 30 m, resulting in 2.5 fold higher potential P_{max} compared to actual P_{max} under the intensity of 30 m. This mechanism can prevent further stress under natural cases when corals experience temporarily changes in light conditions. Such mechanism was also responsible for the observed resilience to photoinhibition of the deep transferred corals under the high experimental intensities, therefore indicates that photoacclimation mechanisms, especially to high light, can be highly conservative. After 6 months, deep transferred corals became more susceptible to photoinhibition and their I_k was only slightly higher than the 30 m control (**Table 2**).

Conversely, I_k and I_c were the first parameters to change upon shallow transfer, probably due to the increased respiration and was even higher than the shallow control. I_k is commonly used as a measure of the adaptation of a plant to its light regime (Steemann Nielsen, 1975). At irradiance levels above I_k, like that which deep corals experience when transferred to shallow water, more energy is trapped by the light reaction centers than can be used by photochemical processers or be disposed harmlessly. This imbalance may lead to photodynamic damage to components of the photosynthetic apparatus (Kyle et al., 1984; Hoegh-Guldberg and Jones, 1999) and to non-photosynthetic components of the electron transport chain (Figueroa et al., 2003). Since photosynthesis among 30 m controls was inhibited at intensity of ~290 μmol photons m^{-2} s^{-1}, shallow transferred corals would be expected to experience photoinhibition under intensity that can reach ~825 μmol photons m^{-2} s^{-1} at 3 m depth. However, the low photosynthesis observed among the shallow transferred corals resulted only from its limited photoacclimation mechanisms to increase net photosynthesis above the vast increase in respiration resulting in photosynthesis

below the compensation point throughout most of the P vs. I curve. The dark adapted Fv/Fm of this treatment remained higher than the shallow control, suggesting that the turnover time of PSII repair was higher than the immediate damage. In fact, the I_c and I_k of these corals calculated by the hyperbolic tangent underestimate the real measurements shown in **Figure 2** since the increase in oxygen occurred only under high intensities, roughly equivalent to the peak irradiance at 3 m.

Furthermore, the shallow transferred *S. pistillata* dissipated the excess energy via NPQ at efficiency similar to the shallow controls (**Figure 1**). High NPQ may indicate on a functional photosynthesis since epoxidation of diadinoxanthin to diatoxanthin during light depends on a pH gradient developed by pumping protons, via non damaged PSII, across the thylakoid membrane into the lumen. However, even damaged reaction centers can continue to trap excitation energy and NPQ may occur simultaneously (Krause, 1988; Falkowski and Kolber, 1995).

Fm' values decreases during the day with activation of NPQ (Gorbunov et al., 2001), however over-reduction of Fm' in corals at 3 m during August resulted in NPQ values higher than 1. This was also shown for plants under high light (Ralph and Gademann, 2005). In contrast, Fm' slightly increased in the deep transferred zooxanthellae hence negative NPQ values were calculated. In such case where the difference in Fm is lower than the error of measurement- the NPQ value is counted as 0 (Schreiber, 2004). Fluorescence may account at most for a few percentage of harvested energy; Therefore, Equation 2 can only approximate thermal dissipation. Although these assessments do not represent the correct absolute values, we presume that the differences between treatments are reflecting their factual behavior.

Potential (and actual) P: R ratios are consistently higher in deeper corals compared with shallow ones. While during winter P_{max} is almost equal to respiration of shallow corals, and hence daily photosynthetic energy input is limited. The opposite happens in summer, when the net energy input from photosynthesis is even higher at 3 m than at 30 m, although P: R ratios are lower, since photosynthesis rates are much higher (**Table 2**, Falkowski et al., 1984).

Zooxanthellate Density and Chlorophyll *a* Concentration

Chlorophyll per zooxanthellae was higher in deep *S. pistillata* (**Figure 3B**) like in other deep corals (Titlyanov et al., 1980; Mccloskey and Muscatine, 1984; Porter et al., 1984; Kaiser et al., 1993; Masuda et al., 1993; Leletkin et al., 1996; Mass et al., 2007; Winters et al., 2009). However, zooxanthellae density with depth increase in some corals (**Figure 3B**, Drew, 1972; Winters et al., 2009) and decrease in others (Dustan, 1979; Mccloskey and Muscatine, 1984; Masuda et al., 1993; Fitt et al., 2000). Such variation is not specie specific, for example zooxanthellae density in *S. pistillata* can either increase (**Figure 3**, Winters et al., 2009) or decrease (Mccloskey and Muscatine, 1984) with depth. However, there might be a correlation between zooxanthellae density and the strategy of acclimation to depth. In studies where zooxanthellae density decreases with depth they were

harbored in 4–5 million cells cm^{-2} in shallow water, and only 2–3 million at greater depth. Mccloskey and Muscatine (1984) suggested that lower zooxanthellae densities in *S. pistillata* under dim light may minimize self-shading. Conversely, when the density was below 1 million cells cm^{-2} in shallow thereby higher zooxanthellae densities would assist rather than obstruct light capture.

Optimizing light capture at depth by increasing zooxanthellae densities becomes jeopardize when encountering sudden increase in PAR and UV-B by transfer to shallow depth. ROS could be induced in the host (Nii and Muscatine, 1997) and in the zooxanthellae (Lesser, 1996) and lead to zooxanthellae expulsion (Lesser, 1996; Smith et al., 2005), and trigger cell death (Dunn et al., 2002; Apel and Hirt, 2004; Franklin et al., 2004; Lesser and Farrell, 2004). Such reduction in zooxanthellae densities upon shallow transfer of *S. pistillata* from 30 m is enhanced by the high UV-B levels (Cohen et al., 2013). Dramatic reductions in algal densities can occur within a very short time scale (24 h) after exposure to high solar radiation (Brown et al., 1994) or within seasons (**Figure 3**, Mass et al., 2007). In the present study, 14 days after final transplantation of deep *S. pistillata* to 3 m, zooxanthellae densities were lower than in the control at 3 m, conversely, 3–30 m transfers had higher zooxanthellae densities than the controls at that depth. Overshooting numbers of zooxanthellae in both treatments compensate for the unaffected cellular chlorophyll content, which remained high in the shallow transferred corals and low in the deep transferred corals. As a result, appropriate areal chlorophyll concentrations are maintained in the new light fields (**Figure 3C**).

The remaining symbionts within shallow transferred fragments were able to efficiently dissipate the excess energy (as explained before) however the inadequate cellular chlorophyll may have impeded optimal photosynthetic capability. Six months after shallow transfer, zooxanthellae densities were still different from control values at the new habitat, however at lower discrepancies. This associates with partial, however significant, change in cellular chlorophyll toward control values at both relocation depths which was probably still an ongoing process. Cellular chlorophyll adjustments in zooxanthellae were complete after 1 month of shade to light acclimation and 3 months of light to shade acclimation (Falkowski and Dubinsky, 1981), and were as fast as 24 h in the free living chlorophyte alga *Dunaliella salina* (Berner et al., 1989). Hence such progressive response of cellular chlorophyll to depth relocation, observed in this study, probably occurs at a different mechanism. We suggest that the reason for the prolonged two step acclimation observed lays in the difference in zooxanthellae types, which is known to have distribution boundaries set by depth (Iglesias-Prieto and Trench, 1997a; Baker, 2001; Iglesias-Prieto et al., 2004; Winters et al., 2009). Dustan (1979) suggested that corals originating from different depths respond differently to the same conditions in part due to the inherent characteristics of several populations of symbionts. This could explain the differences in zooxanthellae densities and their chlorophyll content between transferred corals and the control at the new depth to adjust areal chlorophyll concentrations. Differences in zooxanthellae

densities of control and transferred corals at the same depth resemble the different densities *Symbiodinium* clades were found under several temperatures (Gillette, 2012). Also, Chang et al. (1983) and (Iglesiasprieto and Trench, 1994, 1997b) isolated several strains of *Symbiodinium microadriaticum* and showed that their mechanisms of photoadaptation are different although they were kept under similar high or low light conditions. One of these mechanisms is a distinct variation among the chlorophyll-protein complexes (Iglesias-Prieto and Trench, 1997b) as would affect the chlorophyll per cell concentrations suggested here. Hence, while corals transferred to shade or light rapidly adjust densities of zooxanthellae and chlorophyll per cell (Falkowski and Dubinsky, 1981), depth relocation is prolong.
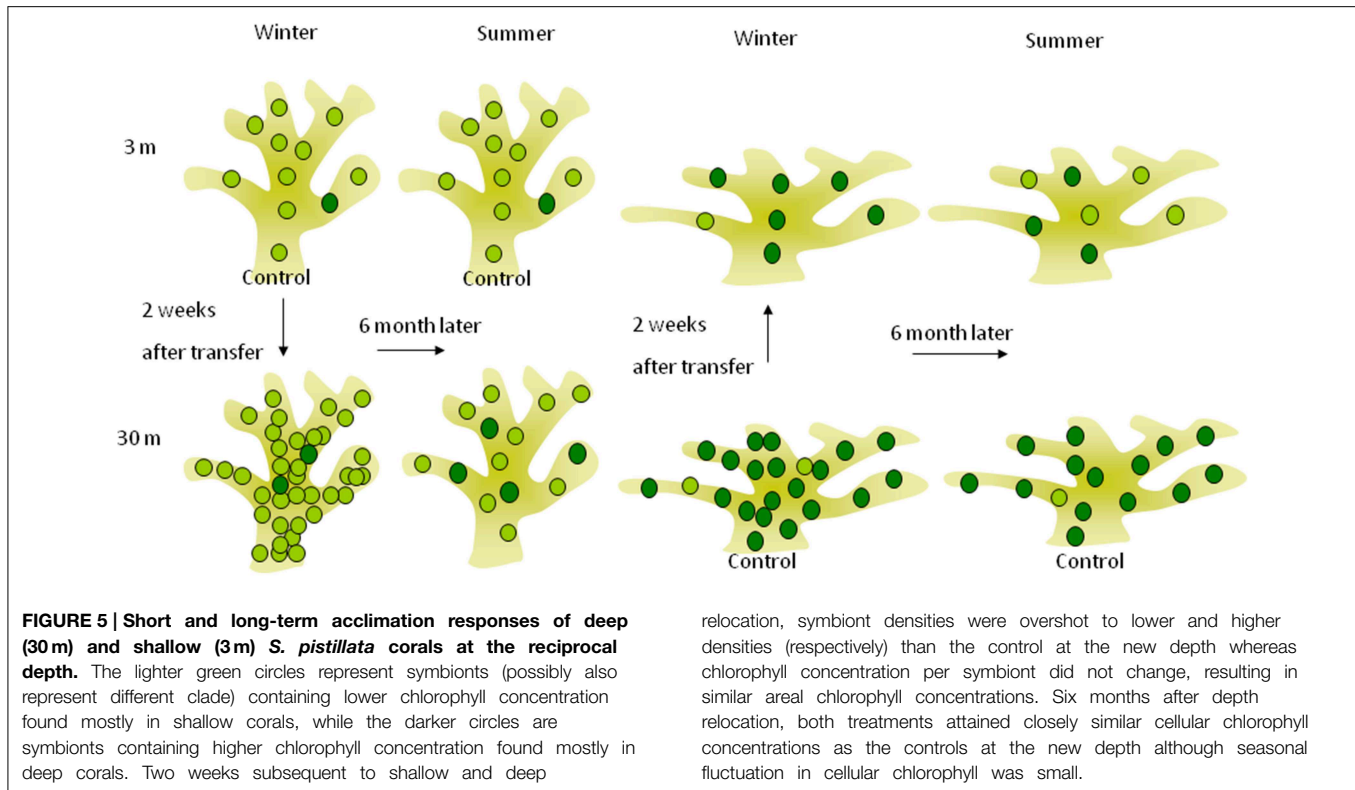
Earlier studies on *Montastraea annularis* (Rowan et al., 1997) showed that depth relocation caused shift in the clades after 6 months- corresponding to the time response for cellular chlorophyll to adjust (**Figure 3**). *P. verrucosa* harboring clade A responded to depth transfer by changing cellular chlorophyll and not symbiont density (Ziegler et al., 2014) as was observed for shade and light acclimation of shallow *S. pistillata* in the Red Sea (Falkowski and Dubinsky, 1981) which harbors clade A in the shade and also in high light (Winters et al., 2009). In deeper water *S. pistillata* (20 m), clade A co-exists with the C type (Winters et al., 2009) which responds to higher light by dramatic reduction in its densities (**Figure 3**). Similar results were observed when *P. verrucosa* harboring clade C zooxanthellae were transferred to shallow depth (Richier et al., 2008).

Based on these former observations and our results, we suggest in **Figure 5** a mechanistic model for short and long term depth acclimation. Days to weeks photoacclimation of the indigenous zooxanthellae clades of the depth of origin is limited by the boundaries of phenotypic flexibility inherent to each clade. The ensuing, months-long complete "acclimation" we documented, is likely to have resulted from the replacement of the zooxanthellae clades by those native to the "new" depths.

Natural seasonal bleaching and recovery were repeatedly observed in deep (40–63 m) *S. pistillata* in the Red Sea from April to September (Nir et al., 2014). This phenomenon was apparent with the 52 and 59% reduction in zooxanthellae cells of control and transplant corals at 30 m, in regard to only 6–10% reduction at shallow. This would suggest that the bleaching phenomenon responds to conditions in the deep environment and is not intrinsic only to corals originated at the mesophotic zone. However, according to the model we suggest, zooxanthellae clade after deep transfer could already be replaced by August, hence the bleaching might still result from an intrinsic response of the deeper clade C to the increased light and temperature of summer. Downs et al. (2002) correlate the drastic decline in cell density at greater depths with increase in levels of oxidative damage products as consequence of raising water temperature.

Calcification

The symbiotic relationship between coral (or Foraminifera) and zooxanthellae is not always reflected by linear rates of

FIGURE 5 | Short and long-term acclimation responses of deep (30 m) and shallow (3 m) *S. pistillata* corals at the reciprocal depth. The lighter green circles represent symbionts (possibly also represent different clade) containing lower chlorophyll concentration found mostly in shallow corals, while the darker circles are symbionts containing higher chlorophyll concentration found mostly in deep corals. Two weeks subsequent to shallow and deep relocation, symbiont densities were overshot to lower and higher densities (respectively) than the control at the new depth whereas chlorophyll concentration per symbiont did not change, resulting in similar areal chlorophyll concentrations. Six months after depth relocation, both treatments attained closely similar cellular chlorophyll concentrations as the controls at the new depth although seasonal fluctuation in cellular chlorophyll was small.

their most prominent biological activity- host calcification and symbiont photosynthesis. Understanding the physiological interrelationship between these organisms is a prerequisite for understanding the constraints of this interaction and the conditions where it is most efficient. This notion was already discussed when Goreau and Goreau (1959) and Colombo-Pallotta et al. (2010) showed that calcification of bleached corals can be activated by light, although in reduced rates. In Foraminifera, LEC can proceed in normal rates for 5 h although photosynthesis is inhibited with the PSII blocker DCMU (Erez, 1983). These responses of the host are due to light activation of Ca^{2+} uptake from the seawater and elevation of pH at the calcifying site (Al-Horani et al., 2003). Photoacclimation of photosynthesis as observed in this study may influence coral calcification by optimizing energy supply (Muscatine et al., 1984) for such active biological processes. Transport of Ca^{2+} (Chalker, 1976) and carbon (Lucas and Knapp, 1997) are energetically expensive although these ions can also arrive to the site of calcification by a paracellular pathway (Tambutte et al., 2012). Several of the carbohydrates synthesized by the symbionts are transported to growing parts of the polyp to generate ATP (Pearse and Muscatin, 1971; Fang et al., 1989). A good correlation was found between calcification rates in this study: 3 (August) to 3.5 (February) fold higher at 3 m over 30 m fragments (**Figure 4**), and the translocation of photosynthetically fixed carbon to the *S. pistillata* host: 38% at 3 m and 11% at 35 m depth (Mccloskey and Muscatine, 1984). Low light corals use most of the limited carbon to sustain essential metabolic functions (Scharf, 1982), whereas at shallow depths, photoinhibition (Muscatine, 1990) in

addition to UV light (Jokiel and York, 1982) may also lead to reduced growth rates. *S. pistillata* grow ideally at 5–10 m depth (Erez, 1978; Gattuso, 1985), however specie specific responses to such perturbations alters the optimum depth for growth (Huston, 1985). Carricart-Ganivet et al. (2007) for example showed that massive Porites corals did not change significantly with increasing depth up to 20 m. Without acclimation, *Montastrea annularis* collected from 33.5 m decreased calcification when incubated at 9 m for 24 h (Barnes and Taylor, 1973). Physiological acclimation, as observed in chlorophyll concentrations of corals exposed to changes in irradiance can minimize decreases in growth rate. Indeed, calcification was significantly enhanced upon transfer to shallow depth, although the P: R ratio in the transferred corals was much lower (0.58) in respect to the deep control (2.2) during February. This indicates that light can enhance calcification even when photosynthesis is much lower than respiration, a conclusion deviating from the stoichiometric relationship implied by the "light enhanced calcification" model. Mass et al. (2007) also found evidence for such discrepancies in corals at 50–65 m depth. During the first 2 weeks after depth transfer, the corals in both treatments grew only half the rate of the control at their new depth. We suspect that the lower calcification rates of the deep transferred corals (also observed by Yap et al., 1998) stemmed from energy diverted to acclimation process. Interestingly, 6 months after relocation, growth of both transplants was still lower than the control at the new depth although P_{max} and P: R ratios were similar, suggesting that acclimation processes of the host are longer than those of the zooxanthellae.

Summary and Conclusions

In this study, reciprocal shallow (3 m) and deep (30 m) transplantation of *S. pistillata* fragments was performed gradually and resulted with 100% survival rate. Photoacclimation of photosynthesis and calcification to the new depth revealed differences from previously known shade and light acclimation at the same depth and suggest a different mechanism. This acclimation was prolonged and included two distinct phases. The initial response to depth relocation results is a vast change of zooxanthellae densities and not in chlorophyll concentration per cell as would be expected from shade to light acclimation. Such changes were not efficient in modifying photosynthesis at both depths during the short acclimation time but only after 6 months, when cellular chlorophyll concentrations were almost adjusted. Conversely respiration rapidly increased with shallow transfer and decreased with deep transfer. The faster, incomplete adjustment to the "new" depth is assumed to be within the phenotypic plasticity range of the original zooxanthellae population. The subsequent, long-term full adjustment to values in the unmoved controls is likely the result of a slow replacement in symbionts' clade. Such photosynthetic acclimation did not alleviate the drastic reduction in calcification of both transferred treatments. Deep transferred corals grew only half of the unmoved deeper corals, even after 6 months suggesting that the host requires longer than its symbionts for a complete depth acclimation. Interestingly, calcification within shallow transferred corals did increase under the high irradiance although the P_{max}: R ratio of these corals was 0.58 during the first 2 weeks after transfer. This suggests that light has a larger role on calcification than photosynthesis.

Acknowledgments

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