frontiers in Marine Science

ORIGINAL RESEARCH
published: 08 October 2021
doi: 10.3389/fmars.2021.712214

Physiological Responses of *Pocillopora acuta* and *Porites lutea* Under Plastic and Fishing Net Stress

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Marine debris has become a global problem affecting coral health around the globe. However, the photophysiological responses of corals to marine debris stress remain unclear. Therefore, this study firstly investigated transparent and opaque plastic bag shading and fishing nets directly contacting the coral. Photosynthetic performance, pigment content, symbiont density, and calcification rate of a branching coral *Pocillopora acuta* and a massive coral *Porites lutea* were investigated after 4 weeks of exposure to marine debris. The results show that the maximum quantum yield of PSII significantly decreased in *P. lutea* with all treatments, while *P. acuta* showed no effect on the maximum quantum yield of PSII from any treatments. Transparent plastic bag shading does not affect *P. acuta*, but significantly affected the maximum photochemical efficiency of *P. lutea*. Photoacclimation of cellular pigment content was also observed under opaque plastic bag shading for both species at week 2. Fishing nets had the strongest effect and resulted in *P. acuta* bleaching and *P. lutea* partial mortality as well as a decline in zooxanthellae density. Calcification rate of *P. acuta* significantly decreased with treatments using opaque plastic bag and fishing net, but for *P. lutea* only the treatment with fishing net gave any observable effects. This study suggests that the sensitivities of corals to marine debris differ strongly by species and morphology of the coral.

Keywords: marine debris, plastic litter, fishing nets, ecophysiology, zooxanthellae, photoacclimation, corals

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Aquatic Physiology, a section of the journal Frontiers in Marine Science

Received: *20 May 2021*
Accepted: *30 August 2021*
Published: *08 October 2021*

Citation:
Ying L, Sinutok S, Pramneechote P, Aiyarak P, Ralph PJ and Chotikarn P (2021) Physiological Responses of Pocillopora acuta and Porites lutea Under Plastic and Fishing Net Stress. Front. Mar. Sci. 8:712214. doi: 10.3389/fmars.2021.712214

INTRODUCTION

Coral reefs are the most biologically diverse ecosystems in the world, and provide a variety of valuable ecosystem services to global communities (Moberg and Folke, 1999; Harrison and Booth, 2007). However, global warming, acidification of our oceans, and sea level rise can severely affect the survival of corals worldwide (Marshall and Baird, 2000; Orr et al., 2005; Anthony et al., 2008). Fishing gear found across shallow reefs can damage coral reef structure, destroying both benthic flora and fauna, as well as entangling macrofauna (Abu-Hilal and Al-Najjar, 2009). In addition, the anthropogenic activities over the last century have made marine debris a global problem (Ryan, 2015). It is estimated that about 4.8–12.7 MT of marine debris is discharged into the oceans every

year (Jambeck et al., 2015), with 5.25 trillion plastic particles weighing 268,940 tons floating in the oceans (Eriksen et al., 2014). Plastic waste, fishing gear and metals are the dominant three types of debris observed in the Red Sea (Abu-Hilal and Al-Najjar, 2009). Marine debris can be found in every marine habitat, from surface water down to the deep sea (Dameron et al., 2007; Barnes et al., 2009; Buhl-Mortensen and Buhl-Mortensen, 2017), and it affects hundreds of marine species worldwide (Schipper et al., 2008; Barnes et al., 2009; Todd et al., 2010) as well as disrupts whole reef ecological systems (de Carvalho-Souza et al., 2018) and causes loss of coral structural complexity and diversity (Figueroa-Pico et al., 2020). Marine debris has detrimental effects on coral and coral reef ecosystems both directly and indirectly (Donohue et al., 2001). Large debris items physically damage corals through abrasion, suffocation, or starvation (Richards and Beger, 2011). Lamb et al. (2018) found more than 11 billion plastic items entangled with coral reefs in the Asia-Pacific, and suggested that coral disease outbreaks increase when corals are entangled with plastic. Decreased light intensity from being covered by plastic debris has been documented to effect branching coral health and lead to coral bleaching (Muhammad et al., 2021). Field research in Kho Tao, Thailand, found that coral underneath a fishing net suffered the most damage (Valderrama Ballesteros et al., 2018). Massive and branching coral types are the most susceptible when compared with colonies of other morphologies (Putra et al., 2021). In addition, corals are particularly sensitive to abrasion (Tanner, 1995) and abrasion responses in corals are common with plastics. Marine debris has the potential to accumulate bacteria (Zhang et al., 2017), and coral health is controlled by the microbiome within the muco-polysaccharide layer (Kline et al., 2006). Rapid changes in environmental conditions alter coral microbiome, causing bleaching, and disease susceptibility (Bourne et al., 2016). Coral requires carbon both as an autotrophic and a heterotrophic nutrition source (Anthony and Fabricius, 2000; Richards and Beger, 2011). However, macro- and microplastics affect cold-water coral skeleton growth rate, as microplastics interfere with polyps capturing food particles (Chapron et al., 2018). Microplastics are found wrapped in mesenterial tissue within the coral gut cavity, and ingesting high concentrations of microplastics could potentially damage the health of corals (Hall et al., 2015). A number of studies have confirmed that there is abundant marine debris in the oceans, and that it devastates coral reefs. However, there are limited studies quantifying the damage from different types of debris on photosynthesis and productivity of corals and zooxanthellae. Therefore, this study aimed to investigate how *Pocillopora acuta* and *Porites lutea* coral species respond to different types of marine debris.

MATERIALS AND METHODS

Experimental Design

Six colonies each of the coral specie *P. acuta* and *P. lutea* were collected from Panwa Cape, Phuket Thailand (7°48′06.9″N 98°24′24.4″E) and transferred within 24 h to aquarium tanks at Prince of Songkla University, HatYai, Thailand, for acclimation. Temperature, salinity and light intensity from collection site were recorded. Every colony was acclimated in a holding tank for a week under 120 μmol photons m^{-2} s^{-1} light, 29°C, 33 psu salinity, and pH 8.20. After that, the coral colonies were cut into 192 nubbins (*P. acuta* with 3 cm length, *P. lutea* with size around 1.5 cm × 1.5 cm) and acclimated in holding tanks for another week, after which the nubbins were randomly placed to four sets (24 nubbins/specie/treatment) of experimental tanks and acclimated for 1 week. After acclimation, the tanks were subjected to four treatments. Control had no marine debris (Treatment 1); opaque plastic (High Density Polyethylene) bag-located 2 cm above nubbins (15% Light reduction, Treatment 2); transparent plastic (Low Density Polyethylene) bag-located 2 cm above nubbins (3% light reduction, Treatment 3); and Fishing net (13% light reduction and abrasion, Treatment 4). There were 6 replicate samples per treatment per species (**Figure 1**). The whole experiment was performed over 4 weeks to test the effects of marine debris on coral. We sampled nubbins for the zooxanthellae density and chlorophyll concentration analysis in week 2 and week 4. Heater chiller (JMC-02, JBA, China), COB light (TS-A600, Aquarium lamp, China), and LED light (A601, Chihiros, China) were, respectively, used to control water temperature and light intensity in the experimental tanks. Light intensity, temperature, salinity and pH in each experimental tank were set for 120 mmol photons m^{-2} s^{-1}, 29°C, 33 psu and pH 8.20, respectively. Light was turned on at 10:00 and turned off at 22:00 (12:12 hr light:dark period). Nitrate and phosphate concentrations were controlled below 0 mg L^{-1} and 2 mg L^{-1}, respectively. Aquariums were filled with natural seawater filtered with a nomex filter bag and treated with chlorine [50 mg chlorine L^{-1} (50 ppm)]. Thirty percent of the aquarium water was replaced every week, and all physical and chemical parameters were measured every 2 days. Photosynthetic performance of zooxanthellae and health of corals were monitored using chlorophyll *a* fluorescence, and this was randomly measured with the Diving-PAM at the top of nubbins. Pigment concentration and symbiotic cell density were investigated for supporting photosynthetic performance of zooxanthellae. Growth of corals was investigated using buoyant weight technique (Jokiel et al., 1978).

Chlorophyll *a* Fluorescence

Chlorophyll *a* fluorescence of *P. acuta* and *P. lutea* in each treatment was quantified every 2 days over the 4 weeks of experiment by using a Diving-PAM fluorometer (Walz GmbH, Germany) connected to a 6-mm diameter fiber-optic probe (6 replicates per species per treatment per time). Photochemical efficiency of PSII was described by maximum quantum yield (F_v/F_m) or active PSII centers (F_v/F_0) and measured at 08:00 (before light was turned on). Reduction of PSII reaction center number can provide photoinhibitory indication (Behrenfeld et al., 1998). Rapid light curves (RLCs) were measured at 13:00. Maximum relative electron transport rate ($rETR_{max}$), minimum saturating irradiance (E_k), and initial slope (alpha) were derived from curve fits (Platt et al., 1980; Ralph and Gademann, 2005).

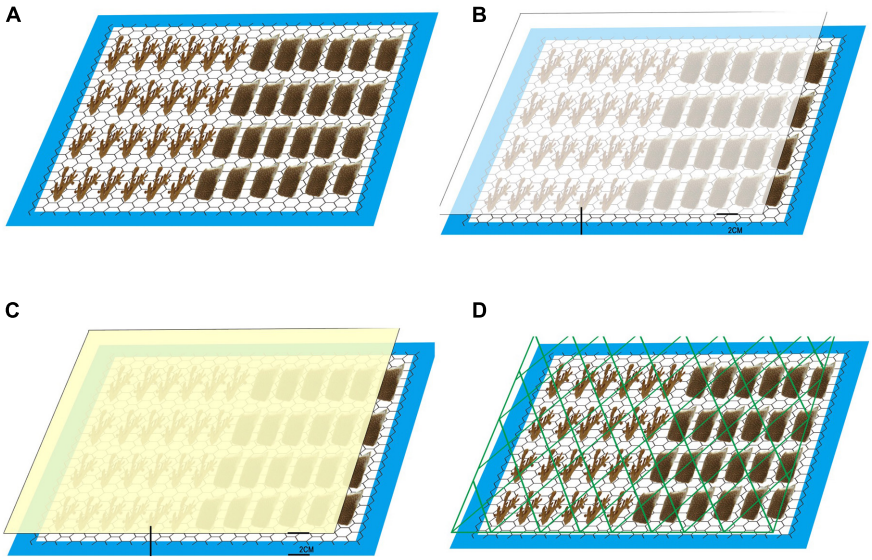

FIGURE 1 | Images of the experiment tank design. **(A)** Control, **(B)** Transparent bag, **(C)** Opaque bag, and **(D)** Fishing net.

Symbiotic Cell Density

Samples were collected every 2 weeks for zooxanthellae density analysis. The samples were water-picked into 50 mL of 0.2-μm-filtered seawater to remove the tissue from the skeleton. The slurry was centrifuged at 1,000 rpm for 10 min to produce a well-mixed sample (Hill and Ralph, 2007). Six replicates for each species and each experiment were counted using a haemocytometer under a light microscope. The cell density was determined per cm^2 following coral surface area determinations used the paraffin wax technique (Stimson and Kinzie, 1991).

Photosynthetic Pigment Concentration

The slurry from determination of symbiotic cell density was also used in pigment concentration determinations. For photosynthetic pigment [chlorophyll (Chl) *a* and c_2] analyses, the slurry was centrifuged for 5 min at 5,000 rpm. After that, the supernatant was discarded and algal pellets were re-suspended in 3 ml of 90% acetone and stored in darkness for 24 h at 4°C. After centrifugation, chlorophyll *a* and c_2 ($\mu g\ cm^{-2}$) concentrations were determined using the standard spectrophotometric method of Ritchie (2006) with absorbance measured at 630, 664, and 750 nm (Winters et al., 2009).

Calcification Rate

Buoyant weight technique was used for calculating calcification rate of the coral skeleton (Jokiel et al., 1978; Spencer Davies, 1989). In theory each sample is weighed in seawater of known density, and the dry weight of the sample in air is estimated by using Archimedes' principle (Sinutok et al., 2011). Each replicate coral nubbin was weighted at the start of the experiment and the end of experiment (week 4) using a 4-digit precision balance (OHAUS, United States). Skeleton bulk densities used for *P. lutea* and *P. acuta* were 1.19 and 2.01 $g\ cm^{-3}$, respectively (Tanzil et al., 2009; Ng et al., 2019). The temperature and salinity of seawater were measured first. Then the glass stopper was weighted both in air and in water for calculating the density of seawater (Spencer Davies, 1989). The skeleton growth rate was calculated from equation:

$$G = \sqrt[c]{\frac{a}{b}} - 1$$

in which G = growth, a = weight at the end of experiment, b = weight at the start of experiment, and c = number of days between measuring a and b.

Statistical Analysis

For zooxanthellae density, pigment contents and calcification rate, one-way ANOVA was used to test for significant differences between the marine debris treatments. For chlorophyll fluorescence parameters, mixed repeated ANOVA was used to determine statistical differences among times of sampling, treatments and species. All tests used 95% confidence level threshold. *Post hoc* LSD test was used to determine statistical differences among the distinct groups. If the data distribution was not normal (Kolmogorov–Smirnov test) or not equal in variances (Levene's test), the data were transformed with square-root or log10 before testing. If the transformed data did not meet the assumptions, non-parametric tests were used.

RESULTS

Visual Appearances and Physicochemical Properties of Seawater

In transparent plastic bag treatment, the plastic gradually turned opaque due to biofouling from algae. The light reduction increased from 3% at the start to 76% at the end of experiment. However, with the opaque plastic bag treatment, light reduction

increased from 15 to 89% during the same 4 weeks treatment. A few *P. acuta* fragments showed a slight color decrease during the experiment. Dissolved oxygen under opaque and transparent plastic covered area did not significantly decline when compared with the area above plastic, indicating no suffocation effects on the corals (**Table 1**). In the fishing net treatment, we observed the production of mucus. *P. acuta* showed significant bleaching after 2 weeks and death at the end of experiment (**Figure 2D**). *P. lutea* had partial tissue necrosis (**Figure 2H**) and swelling of the tissues as a result of the fishing net treatment (**Figure 2I**).

TABLE 1 | Statistical analysis of dissolved oxygen above and below marine debris in each treatment tank.

Treatments	Df	*F*	*P*-value
Control	1	0.072	0.791
Opaque plastic bag	1	1.419	0.245
Transparent plastic bag	1	0.039	0.845
Fishing net	1	0.487	0.493

Chlorophyll *a* Fluorescence

Mixed repeated ANOVA showed that F_v/F_m of *P. acuta* had stabilized and remained mostly constant over the 4-week experiment ($p = 0.115$; **Figure 3A**), and there were no significant differences among treatments ($p = 0.370$; **Figure 3A**). F_v/F_m of *P. lutea* was more sensitive to marine debris stress than *P. acuta* ($p < 0.05$). F_v/F_m of *P. lutea* that was exposed to opaque bag, transparent bag or net treatment significantly declined from T7 ($p < 0.05$; LSD's *post hoc* comparisons). At the end of the exposure period (week 4), F_v/F_m of *P. lutea* with opaque bag, transparent bag and net treatments were significantly lower than for the control ($p = 0.001$; **Figure 3B**). F_v/F_0 had similar trend as F_v/F_m in both of the two species. *P. lutea* responded more to marine debris than *P. acuta* in all three treatments ($p < 0.05$; **Figures 3C,D**). There was no significant difference

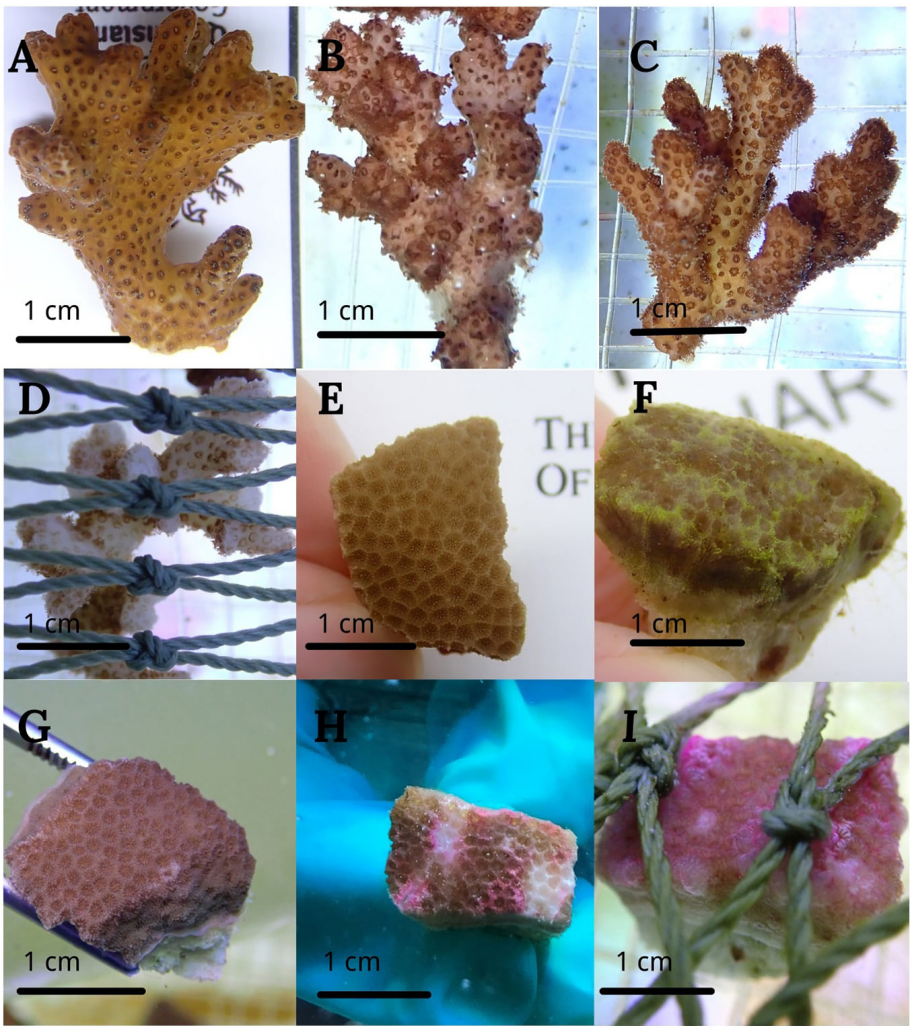

FIGURE 2 | Images of the different corals subjected to treatments for 4 weeks. *P. acuta* and *P. lutea* in control **(A,E)**, transparent bag **(B,F)**, opaque bag **(C,G)**, and fishing net treatments **(D,H,I)**.

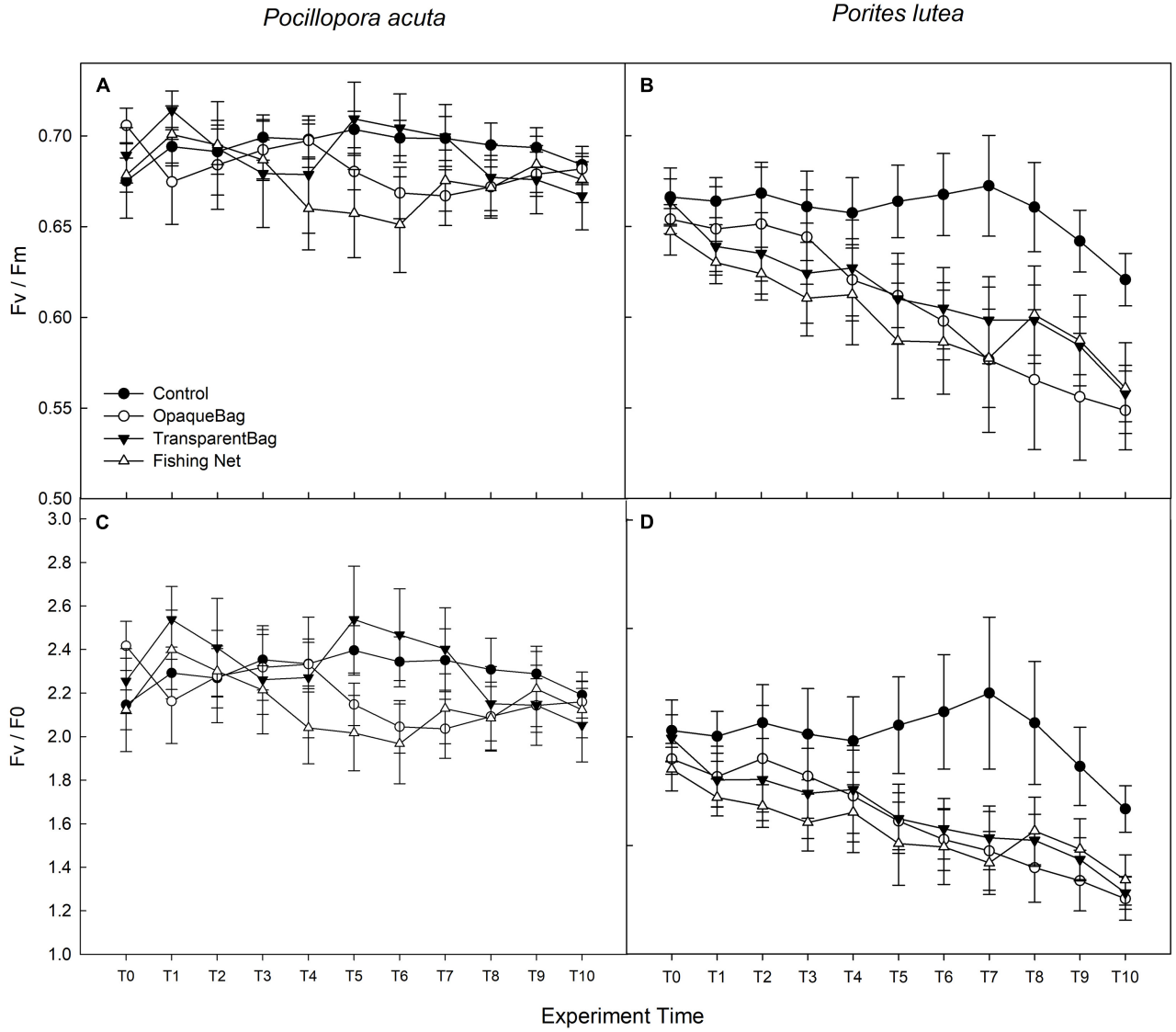

FIGURE 3 | Maximum quantum yield of PSII (F_v/F_m; **A,B**) and F_v/F_0 **(C,D)** in *P. acuta* (left column), and *P. lutea* (right column) from T0 to T10 (Graph treated with moving average, T1 in week 1, T4 in week 2, T7 in week 3, and T10 in week 4). Data are presented as Mean ± SE ($n = 6$).

among treatments in *P. acuta* ($p = 0.271$; **Figure 3C**), but F_v/F_0 in opaque bag, transparent bag and fishing net treatments were significantly lower than in control treatment of *P. lutea* ($p < 0.001$; **Figure 3D**) after 2 weeks (LSD *post hoc* comparisons) (**Table 2**).

Measurements of RLC were used to calculate alpha, $rETR_{max}$ and E_k. Alpha of *P. acuta* had slightly changed but without significant differences over time ($p = 0.672$; **Figure 4A**) or among treatments ($p = 0.927$; **Figure 4A**). In *P. lutea*, alpha significantly decreased over the exposure period ($p = 0.005$; **Figure 4B**), but had no significant differences amongst treatments ($p = 0.132$; **Figure 4B** and **Table 2**).

Regarding E_k and $rETR_{max}$ in *P. acuta* and *P. lutea*, the impact of marine debris was strong in the fishing net treatment. Both E_k and $rETR_{max}$ of *P. acuta* ($p < 0.001$ and $p < 0.001$, respectively; **Figures 4C,E**) and *P. lutea* ($p < 0.001$ and $p < 0.001$, respectively; **Figures 4D,F**) in fishing net treatments were significantly lower than in the control, opaque bag or transparent bag treatments (**Table 2**).

Chlorophyll Concentration

After 2 weeks, there were no significant differences in chlorophyll *a* and c_2 concentrations per surface area (μg cm^{-2}) in *P. acuta* ($p = 0.101$ and $p = 0.140$, respectively; **Figures 5A,C**) or in *P. lutea* ($p = 0.400$ and $p = 0.363$, respectively; **Figures 5B,D**) among the treatments. However, cellular chlorophyll *a* concentration (pg $cell^{-1}$) of *P. acuta* and *P. lutea* in opaque bag treatment were significantly higher than in control ($p = 0.001$ and $p = 0.002$, respectively; **Figures 5E,F**).

TABLE 2 | Statistical analysis of PAM between treatments (Mixed Two-way Repeated ANOVA).

	Pocillopora acuta			*Porites lutea*		
Parameter	**Df**	***F***	***P*-value**	**Df**	***F***	***P*-value**
F_v/F_m	3	1.106	0.370	3	8.772	0.001*
$rETR_{max}$	3	68.666	<0.001*	3	17.914	<0.001*
F_v/F_0	3	1.403	0.271	3	10.921	<0.001*
Alpha	3	0.153	0.927	3	2.100	0.132
E_k	3	26.911	<0.001*	3	15.003	<0.001*

*Significant difference.

When measured after 4 weeks, the cellular chlorophyll *a* concentration in both species had returned to the same level as the control ($p = 0.680$ and $p = 0.230$, respectively; **Figures 5E,F**).

After 2 weeks, there were no changes in chlorophyll *a* and c_2 concentrations in fishing net treated *P. acuta* ($p > 0.05$), but surface chlorophyll *a* and c_2 concentrations decreased from control in week 4 ($p = 0.021$ and $p = 0.021$, respectively; **Figures 5A,C,G**). In *P. lutea* chlorophyll *a* and c_2 concentrations per surface area declined in week 4 ($p = 0.026$ and $p = 0.013$, respectively; **Figures 5B,D**), but interestingly in week 2 chlorophyll *a* and c_2 concentrations per cell had increased ($p = 0.002$ and $p = 0.001$, respectively; **Figures 5F,H**). At the end of treatment, the chlorophyll *a* and c_2 concentrations per cell had the same levels as in control ($p = 0.230$ and $p = 0.244$, respectively; **Figures 5F,H** and **Table 3**).

Zooxanthellae Density

In *P. acuta*, there was no significant difference in symbiont density after 2 weeks of exposure to marine debris ($p = 0.201$; **Figure 6A**), while there was significantly lower symbiont density in the net treatment, compared to other treatments after 4 weeks of exposure ($p = 0.014$; **Figure 6A**). In *P. lutea*, significantly lower zooxanthellae densities in opaque bag and fishing net treatments were observed on weeks 2 and 4 of exposure ($p < 0.001$ and $p < 0.001$, respectively; **Figure 6B** and **Table 3**).

Calcification Rate

Average calcification rate (% day^{-1}) of both species in opaque bag, transparent bag and fishing net treatments was lower than in control treatment. Calcification rates of both species in fishing net treatment were significantly lower than in the other treatments ($p < 0.001$ and $p = 0.026$, respectively; **Figures 7A,B** and **Table 3**).

DISCUSSION

Opaque Plastic Bag and Transparent Plastic Bag

Plastic bag debris can cause coral shading and suffocation resulting in bleaching as well as physical damage by abrasion, necrosis and ultimately mortality (Ryan, 2015; Valderrama Ballesteros et al., 2018). Indeed, shading directly decreases light availability for photosynthesis used by zooxanthellae, and we measured that response with opaque plastic bag and transparent plastic bag which, respectively, reduced light by 15 and 3% at the start of treatment. Although corals in close contact with plastic bags can suffer suffocation from oxygen depletion and lack of metabolite exchange (Ryan, 2015; Mouchi et al., 2019), in our experiments the plastic bag was located 2 cm above the coral, and the dissolved oxygen level under plastic covered area did not significantly decline relative to the area above the plastic bag, and so no suffocation was observed (**Table 3**). After 4 weeks of experimental treatment, the shading had increased to 89 and 76% for opaque plastic bag and transparent plastic bag cases, which was caused by algae covering the plastic bags. *P. acuta* showed no significant change in any indicator of chlorophyll fluorescence from opaque or transparent plastic bag stress. In contrast, photochemical efficiency (F_v/F_m) of *P. lutea* significantly declined in transparent and opaque plastic bag treatments, while efficiency of photosynthesis (alpha), saturating irradiance (E_k), and maximum $rETR_{max}$ were not significantly different. This may indicate that *P. lutea* has less resilience to shading than *P. acuta*. Species-specific resilience to marine plastic debris has been found in scleractinian corals (Mouchi et al., 2019; Mueller and Schupp, 2020). A F_v/F_m decline can be caused by limited light availability, low density of symbionts and D1 protein degradation in PS II (Keren et al., 1997; Warner et al., 1999; Muller-Parker et al., 2015). As a result, the symbiont density decreased in the opaque plastic bag treatment in *P. lutea*, confirming the decline in maximum quantum yield. The study by Mueller and Schupp (2020) observed that *Porites rus* slightly paled in the first 21 days, then rapidly decreased when exposed to a transparent plastic bag, which might explain the fact that zooxanthellae density did not significantly decrease in our 4 weeks of experiment. Keshavmurthy et al. (2014) put forward the resistance mechanisms of hosts or the sustainable relationships with the clades or types of *Symbiodiniaceae* corals that can contend natural and anthropogenic stresses. Different coral species can associate with different symbiont compositions and the variety of components of *Symbiodiniaceae* provide unequal tolerances (Rouzé et al., 2016). Most of *P. acuta* in the sampling area associated with symbionts in *Durusdinium* spp. (D1) and *Durusdinium* sp. (D1-6; unpublicized report from Yucharoen, 2020), while *P. lutea* is commonly dominated by C15 (*Cladocopium* sp.; Chankong et al., 2018, 2020; Tan et al., 2020). However, there is no direct evidence to demonstrate that coral reactions to marine debris were affected by genus of

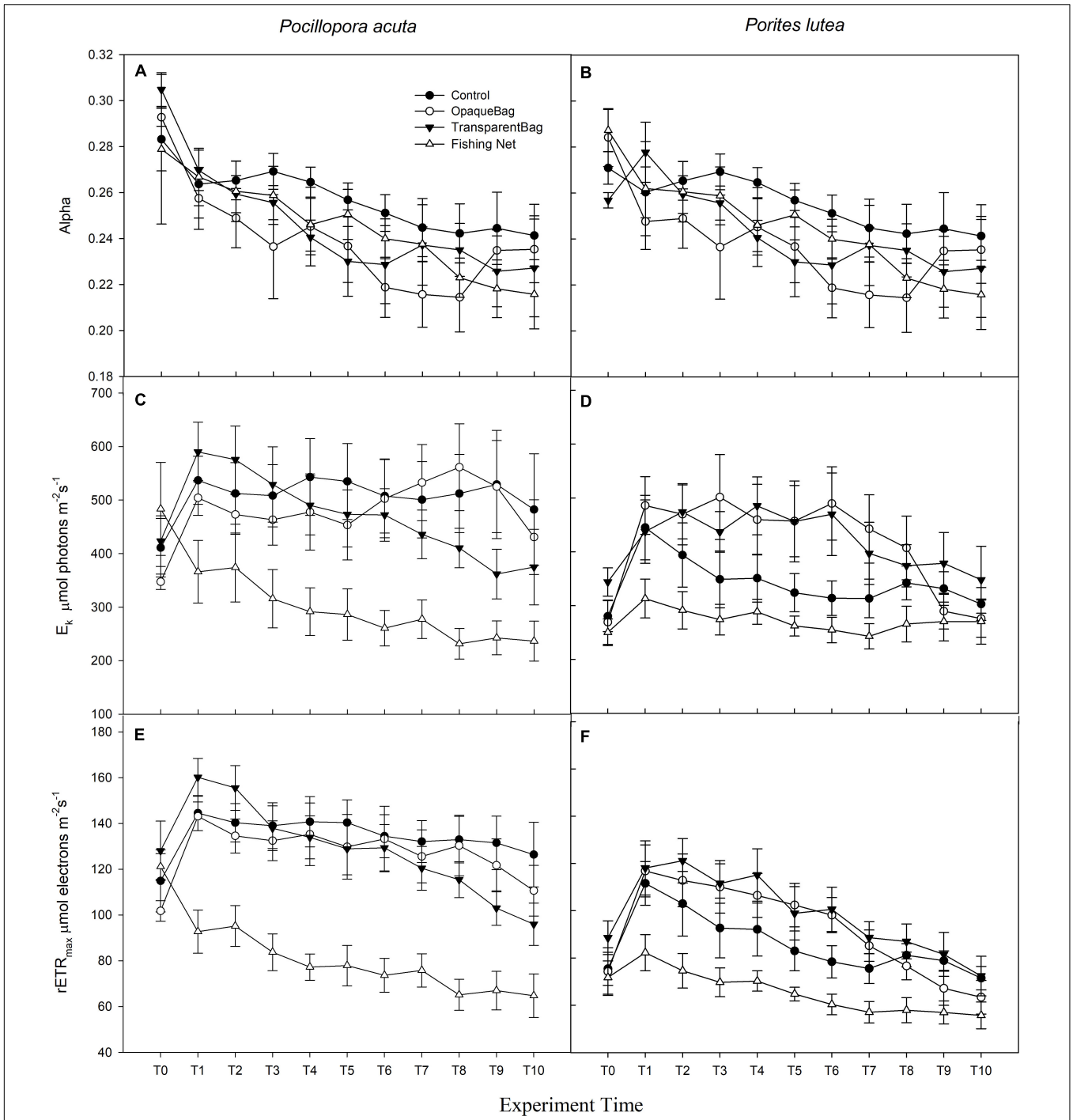

FIGURE 4 | Alpha **(A,B)**, E_k (**C,D**; µmol photons m^{-2} s^{-1}), and $rETR_{max}$ (**E,F**; µmol electrons m^{-2} s^{-1}) of *P. acuta* (left column charts), and *P. lutea* (right column charts) from T0 to T10 (Graph treated with moving average, T1 in week 1, T4 in week 2, T7 in week 3, and T10 in week 4). Data are presented as Mean ± SE ($n = 6$).

zooxanthellae, while from the changed environmental factors, we hypothesized that types of *Symbiodiniaceae* might drive the corals to perform differently under marine debris stress.

Physella acuta in the opaque plastic bag treatment had an effect on its calcification rate. The skeleton growth of scleractinian corals is mainly influenced by light and water flow (Scoffin et al., 1992; Houlbrèque et al., 2003; Schutter et al., 2011; Browne, 2012; Wijgerde et al., 2012). Light motivates coral tissue extension and skeleton growth (Mass et al., 2007; Schutter et al., 2011; Cohen and Dubinsky, 2015; Eyal et al., 2019). During the decreased light condition in opaque plastic bag treatment, we considered that calcification rate got affected by declined

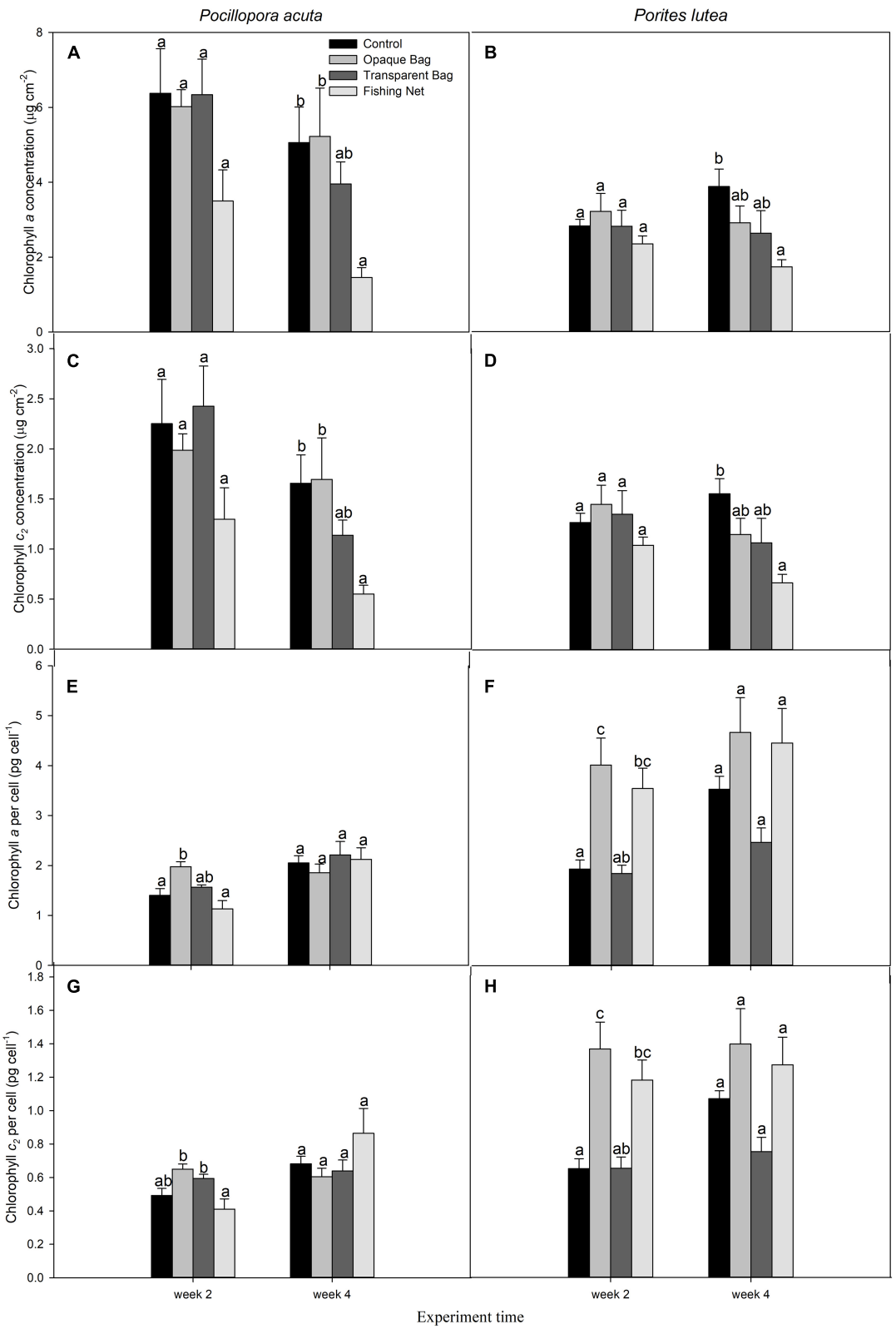

FIGURE 5 | Chlorophyll *a* concentration (μg cm^{-2}; **A,B**), chlorophyll c_2 concentration (μg cm^{-2}; **C,D**), cellular chlorophyll *a* concentration (pg $cell^{-1}$; **E, F)**, and cellular chlorophyll c_2 concentration (pg $cell^{-1}$; **G,H**) of *P. acuta* (left column chart) and *P. lutea* (right column chart) subjected to treatments, at week 2 and week 4. Data are presented as Mean ± SE ($n = 6$).). a, b, and c indicate significant difference.

advisability light. Furthermore, symbiotic zooxanthellae mainly affect hermatypic host calcification with photosynthesis, which supplies the inorganic carbon (Erez et al., 2011). Under lower light conditions, corals will reduce the growth rate due to less photosynthesis and calcification (Anthony and Hoegh-Guldberg, 2003b) Furthermore, decreased calcification may represent the photosynthetic performance of symbionts. In our study, the photosynthetic activity in *P. acuta* had no effect throughout, but the cellular chlorophyll *a* and c_2 concentrations temporarily increased in week 2, which confirms the photoacclimation mechanisms of endosymbiotic dinoflagellates that adapt to reduced light conditions (Falkowski and Dubinsky, 1981; Jones and Yellowlees, 1997; Anthony and Hoegh-Guldberg, 2003a; Stambler and Dubinsky, 2005; Jones et al., 2020). *P. lutea* also increased cellular chlorophyll *a* and c_2 concentrations in week 2, but continuous symbiont cell degradation was observed throughout. In some extreme low light conditions, decreased symbiont density also serves as photoacclimation of coral physiological responses to environmental conditions (Warner et al., 2002; Ulstrup et al., 2008).

In general, plastic shading affected more the response indicators for *P. lutea* than for *P. acuta*. This may be linked to the complex architecture of *P. acuta*, which provides light microhabitat diversification, thereby improving light utilization by the branching coral (Helmuth et al., 1997; Anthony and Hoegh-Guldberg, 2003b). Flow morphology like that of a massive coral has more work required for adaption to light-limited conditions, whereas species that have complex architectures, they are able to occupy extremely low or high-light regimes and consequently may have reduced competition in those habitats. Also, a greater physiological tolerance of light-habitat dynamics (e.g., after physical disturbances) will increase the physiological potential for colonization of newly formed light gaps.

The amount of biofouling by algae on plastic bags, which increased as the experiment proceeded, might also be a factor. Algal competition with coral has been proven, and it can affect corals via allelochemicals, abrasion, shading and overgrowth (McCook et al., 2001; Jompa and McCook, 2003; Rasher et al., 2011; Vieira et al., 2016). Swierts and Vermeij (2016) assumed that fast-growing corals (e.g., branching coral) suffer negative effects on growth from competition with epiphytic algae.

TABLE 3 | Statistical analysis of chlorophyll *a* and c_2, zooxanthellae density and calcification rate (One-way ANOVA).

Parameter	*Pocillopora acuta*			*Porites lutea*		
	Df	*F*	*P*-value	Df	*F*	*P*-value
Chl *a* (μg cm^{-2}) week 2	3	2.374	0.101	3	1.031	0.400
Chl c_2 (μg cm^{-2}) week 2	3	2.046	0.140	3	1.123	0.363
Chl *a* (pg cell^{-1}) week 2	3	8.621	0.001*	3	6.875	0.002*
Chl c_2 (pg cell^{-1}) week 2	3	6.356	0.003*	3	7.841	0.001*
Chl *a* (μg cm^{-2}) week 4	3	4.041	0.021*	3	3.802	0.026*
Chl c_2 (μg cm^{-2}) week 4	3	4.068	0.021*	3	4.594	0.013*
Chl *a* (pg cell^{-1}) week 4	3	0.510	0.680	3	1.559	0.230
Chl c_2 (pg cell^{-1}) week 4	3	1.710	0.197	3	1.502	0.244
Zooxanthellae ($\times 10^6$ cell cm^{-2}) week 2	3	1.693	0.201	3	10.057	0.001*
Zooxanthellae ($\times 10^6$ cell cm^{-2}) week 4	3	4.529	0.014*	3	11.319	0.001*
Calcification rate (% D^{-1})	3	9.749	0.001*	3	3.812	0.026*

**Significant difference.*

Fishing Net

In our experimental plan, the fishing net directly contacted coral fragments creating the potential for abrasion. Both species showed strong effects from fishing net stress, but different coral species respond to fishing nets differently. Heavy bleaching was observed in *P. acuta* after treatment with a fishing net for 2 weeks (**Figure 2D**). Bleaching is associated with coral health, because bleaching results from coral releasing symbiotic zooxanthellae (Douglas, 2003), making the coral more sensitive to diseases (Muller et al., 2008), and can negatively affect overall coral growth (Pratchett et al., 2015; McClanahan et al., 2018). Cell degradation occurred and surface chlorophyll concentration significantly declined after week 2, confirming this speculation. Tissue necrosis in *P. lutea* was observed under our treatments (**Figure 2H**), which can result in symbiont degradation and photosynthetic pigment decrease. Moreover, corals with small polyps have limited ability to physically clean up the foreign matter, leading to bleaching and tissue mortality (Philipp and Fabricius, 2003).

The significantly reduced $rETR_{max}$ indicates the maximum photosynthetic capacity and this might affect growth of symbionts (Gao and Zheng, 2010; Xu et al., 2020). From prior research, abrasion of coral tissue by marine debris can result in disease outbreaks (Lamb et al., 2018), leading to decreased chlorophyll pigments and zooxanthellae cell degradation (Cervino et al., 2004). Therefore, we hypothesize that after 4 weeks coverage with fishing net, the reduced symbiont density and lower chlorophyll are related to coral disease. Furthermore, physical connection of nets to the corals affected heterotrophic energy acquisition, and increased energy consumption to produce mucus (Philipp and Fabricius, 2003). Producing the mucus required 35% of energy from an endosymbiotic (Riegl and Branch, 1995). Mainly, this energy source is used for host calcification and reproduction via symbionts synthesizing surplus carbon (Castrillón-Cifuentes et al., 2017), but under sedimentation conditions, the respiration from mucus production reversed the dominance and took up 65% from symbionts. Long-term energy cost combined with limited photosynthetic energy gain severely affected coral calcification (Anthony and Fabricius, 2000).

Porites trematodiasis is the most common disease caused by trematode larvae and manifests as pink swollen nodules, which we observed when *P. lutea* tissue contacted fishing nets (**Figure 2I**). Both biotic

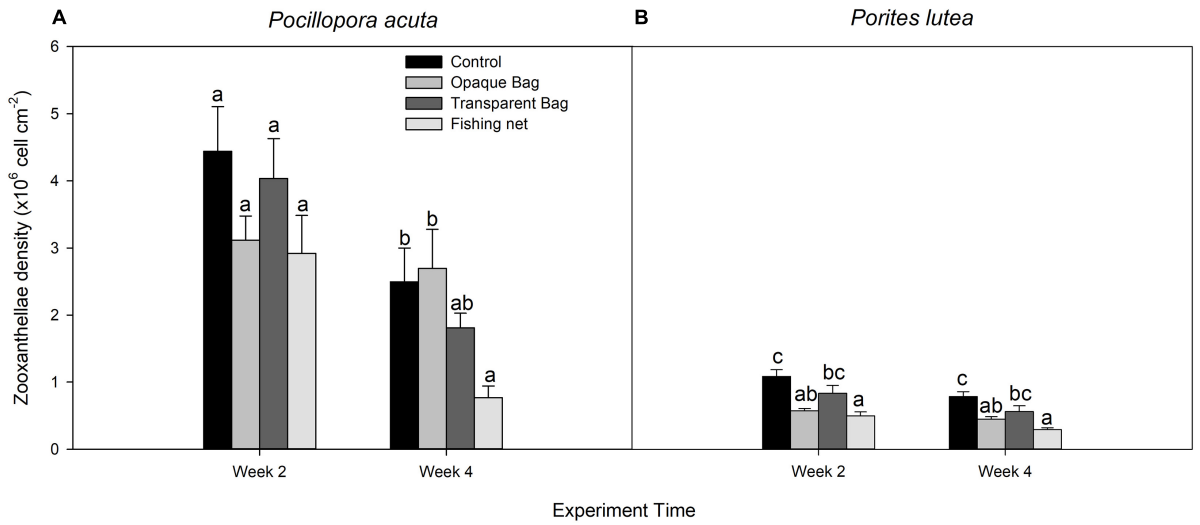

FIGURE 6 | Zooxanthellae density of *P. acuta* **(A)** and *P. lutea* **(B)** in control, opaque bag, transparent bag, and fishing net treatments at week 2 and week 4 of experiment. Data are presented as Mean ± SE ($n = 6$). a, b, and c indicate significant difference.

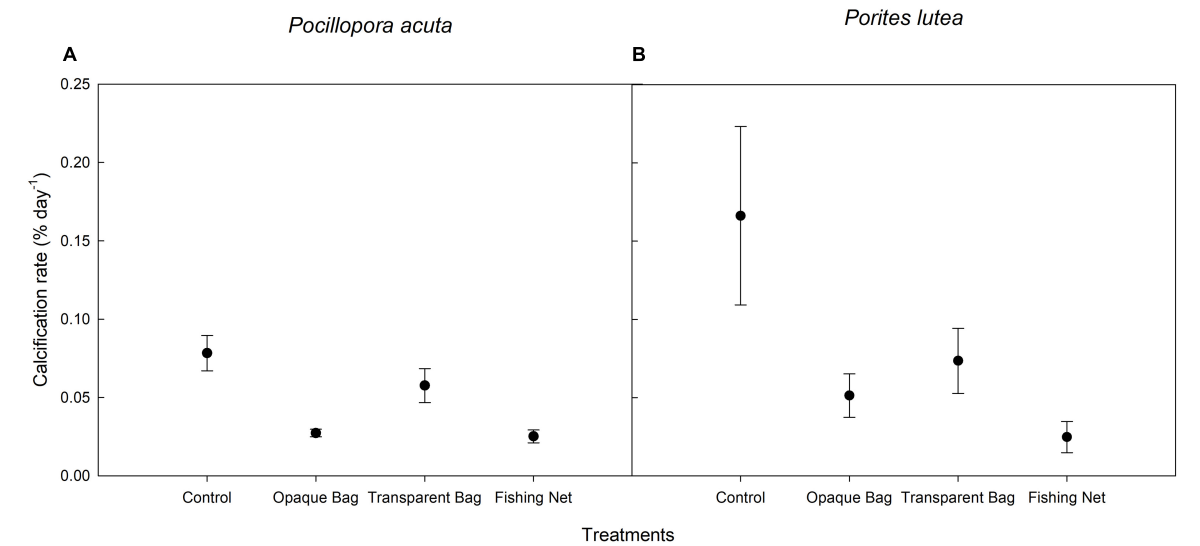

FIGURE 7 | Calcification rate of *P. acuta* **(A)** and *P. lutea* **(B)** in control, opaque bag, transparent bag, and fishing net treatments. Data are presented as Mean ± SE ($n = 6$).

and abiotic stresses can cause coral disease outbreaks, and changes in environmental conditions can promote physiological stresses that impair host immunity (Jackson and Tinsley, 2002; Lafferty and Holt, 2003). Similarly, the red algal *Corallophila huysmansii* overgrowth on *Porites cylindrica* caused severe stress and mortality (Jompa and McCook, 2003).

Fishing net not only severely affected maximum quantum yield of PSII, chlorophyll *a* and c_2 concentrations, symbiotic cell density and calcification rate, but also caused actual tissue necrosis in *P. lutea* under our laboratory conditions (**Figure 2H**). Instead, heavy bleaching was observed in *P. acuta* (**Figure 2D**). Corals with small polyps have limited ability to remove foreign matter and sediment particles, contributing to coral bleaching, and tissue mortality (Philipp and Fabricius, 2003). Significantly reduced $rETR_{max}$ as well as the maximum photosynthetic capacity indicated the poor growth status of symbionts (Gao and Zheng, 2010; Xu et al., 2020). From previous research, marine debris abrasion on coral tissue induces disease outbreaks (Lamb et al., 2018), and diseases have led to a decrease in chlorophyll pigment and to zooxanthellae cell degradation (Cervino et al., 2004). Therefore, we hypothesized that after 4 weeks of covering with fishing net, the reduced symbionts and decreased chlorophyll were related to coral

diseases. Interestingly, we also observed that cellular chlorophyll concentration increased in the second week, due to the photoacclimatization of living zooxanthellae that adapted to reduced light intensity and increased cellular chlorophyll to increase energy capture (Falkowski and Dubinsky, 1981; Jones and Yellowlees, 1997; Anthony and Hoegh-Guldberg, 2003a; Jones et al., 2020). Furthermore, exposure to fishing nets reduced energy acquired from photosynthesis and affected heterotrophic energy acquisition, increasing energy consumption to produce mucus (Philipp and Fabricius, 2003). Long-term negative energy costs combined with limited photosynthetic energy gains severely affected coral calcification (Anthony and Fabricius, 2000).

CONCLUSION

Different species of corals respond differently to various types of marine debris, differently. The responses may depend on the symbiont composition and density, coral morphology, and also on the resilience of the coral species. In this experiment, *P. acuta* showed less effects from each form of marine debris exposure than *P. lutea*, and a transparent plastic bag showed no effects on *P. acuta*. The different kinds of damage from different types of marine debris also make corals respond in different ways. Two types of plastic bag resulted in shading impacts on the corals in this experiment, whereas fishing net treatment affected corals with abrasion. The fishing net treatment had the strongest effects on both coral species tested. In conclusion, marine debris cover on coral causes stress responses in 2 weeks. We hope this work can help marine national parks and any related organizations to prioritize the clearing of marine debris. In aquarium experiments, we have separately assessed the shading and abrasion effects from marine debris, while in natural environment waves cause both shading and abrasion to happen simultaneously, which may accelerate the decline of coral health. The combined responses remain poorly understood and can be addressed in future work.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LY, SS, PA, PR, and PC contributed to conception and design of the study. LY, SS, PP, and PC processed the experiment. LY, SS, and PC organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work is financially supported by The Institute for the Promotion of Teaching Science and Technology (IPST; DPST Graduate with First Placement; Grant Number: 006/2559), the Coastal Oceanography and Climate change Research Center, Prince of Songkla University (PSU), the Faculty of Environmental Management, PSU, and the Graduate School of PSU.

ACKNOWLEDGMENTS

We thank the Coastal Oceanography and Climate Change Research Center (COCC), the Marine and Coastal Resources Institute (MACORIN), and the Faculty of Environmental Management, Prince of Songkla University for research facilities. The authors thank Mathinee Yucharoen and Phatcharee Roekngandee for assistances in the laboratory. The authors thank Seppo Karrila and the Research and Development Office, PSU for assistance with proofing the English.

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