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Fluorescent proteins in dominant mesophotic reef-building corals

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ABSTRACT: Reef-building corals inhabiting the mesophotic zone (30–150 m) not only survive but thrive in light-limiting environments. Similar to shallow corals, mesophotic corals also exhibit coral fluorescence. Because fluorescent proteins (FPs) absorb high-energy light and emit lower-energy light, FPs could play an important role in mesophotic coral physiology and ecology. For 4 species of the Hawaiian mesophotic reef-building coral *Leptoseris* (65–125 m), we investigated the abundance of fluorescent morphs, types of FPs, fluorescence emission phenotypes, and the physiological relationship between coral fluorescence and endosymbiotic *Symbiodinium* (dinoflagellate; Dinophyta). Cyan/green coral fluorescence emission was widespread in mesophotic *Leptoseris* spp.; more than 70% of corals fluoresced, yet fluorescent and nonfluorescent corals co-occurred at all depths investigated. Coral fluorescence was attributed to 2 proteins, a cyan fluorescent protein (CFP, λ_{ex} = 424 nm, λ_{em} = 490 nm) and a green fluorescent protein (GFP, λ_{ex} = 478 nm, λ_{em} = 502 nm). The type of FP in *Leptoseris* colonies was correlated with depth; CFP was dominant in corals from shallower depths (65–85 m), GFP was dominant in corals from deeper depths (96–125 m), and CFP and GFP were present in corals from middle depths (86–95 m). Coral FP emission was primarily localized in the coenosarc and/or the oral disc. *Symbiodinium* from corals with and without fluorescence emission had similar genotypes, abundances, photosynthetic pigments, photosynthetic efficiencies, photosynthetic rates, and chlorophyll excitation spectra. As such, it is unlikely that these FPs play a significant role in enhancing symbiont photosynthesis. The high abundance of fluorescent morphs (>70%) dominating this energetically limited environment may suggest that FPs play an integral and conserved physiological role in corals.

KEY WORDS: Photobiology · Ecophysiology · Fluorescent proteins · Green fluorescent proteins · GFP · Coral ecology · *Leptoseris* spp. · Deep water · Mesophotic coral ecosystems · MCE

INTRODUCTION

Mesophotic coral ecosystems (MCEs), which inhabit deep fore-reef zones, are similar to shallow reefs in that hermatypic corals build a complex structure that becomes the foundation of the community. In contrast to shallow reefs, the physical environment of the mesophotic zone is characterized by low solar irradiance with blue spectral enrichment, low wave disturbance, reduced temperatures, and often a higher abundance of upwelled nutrients (Lesser et al. 2009, Kahng et al. 2010). Furthermore, mesophotic

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reefs are less vulnerable to stressors such as overfishing, climate change, and pollution than shallow reefs (Bak et al. 2005, Lesser et al. 2009). Because shallow and deep reefs are linked physically and biologically, there is the potential for mesophotic ecosystems to serve as refugia and a source/sink for shallow species (Lesser et al. 2009, Kahng et al. 2010). The difficulties accessing this zone limit knowledge of the productivity, physiology, and ecology of mesophotic communities (Bak et al. 2005, Lesser et al. 2009, Kahng et al. 2010). While mesophotic coral reefs are found from 30 m to over 150 m depths, this study focuses on dominant reef-building corals at depths below 60 m. Many mesophotic corals such as *Leptoseris* spp. are restricted to these extreme low-irradiance environments (Rooney et al. 2010). Understanding what drives the distribution, biological interactions, or physiological traits of mesophotic corals may provide unique insights to clarify the paradox of reef-building corals flourishing under extreme low-irradiance conditions.

Reef-building scleractinian corals host endosymbiotic dinoflagellates from the genus *Symbiodinium*. In shallow corals, much of the energy needed for calcification of coral reefs is provided by photosynthetic products produced by *Symbiodinium* (Muscatine 1990). While obligate symbiotic corals have been observed as deep as 165 m (Maragos & Jokiel 1986), the extent to which mesophotic corals rely on photosynthetic products from *Symbiodinium* remains poorly understood. The downwelling irradiance in the mesophotic zone (~65–115 m) is about 3–10% of surface irradiance (Kahng et al. 2012). The dramatic decline in the quantity of photosynthetically active radiation (PAR) is compounded by a remarkable change in quality (spectral composition) at depth. Blue wavelengths penetrate most deeply in clear oceanic waters and are the most abundant wavelength at depth, while red wavelengths and ultraviolet radiation are significantly reduced at depth (Kirk 1994). The deep mesophotic zone in the present study (65–125 m) is an extreme low-irradiance habitat akin to deeply shaded terrestrial systems, but with blue rather than red spectral enrichment to the benefit of deep water algae with chlorophyll *a* (chl *a*)-based light harvesting systems. The irradiance spectral composition and intensity are key regulating components of corals and *Symbiodinium* on multiple temporal and spatial scales (Roth 2014). In mesophotic photosynthetic communities, irradiance is a determining factor that is likely to have profound effects on coral and symbiont physiology and ecology.

Mesophotic reef-building corals may use a variety of strategies to live in an extreme low-irradiance environment, including reduced energetic requirements, increased rates of heterotrophy, and flattened skeletal morphology to optimize light collection (Kühlmann 1983, Lesser et al. 2009, Kahng et al. 2010). While *Symbiodinium* in shallow-water corals can vary concentrations of photosynthetic pigments, thereby affecting light-harvesting capabilities, photosynthetic efficiency, and rates of photosynthesis with depth (see Falkowski et al. 1990), *Symbiodinium* spp. in mesophotic-restricted corals surprisingly appear to lack these conspicuous physiological acclimatizations (see Kahng et al. 2014). Investigations into mesophotic-restricted corals and their symbionts, which are successful in these extreme low-irradiance environments, may provide novel insights into coral and symbiont biology.

Coral photobiology, fluorescent proteins (FPs), and mesophotic habitats pose intriguing research opportunities. FPs have the potential to play important roles in the physiology of corals and their symbionts because they absorb higher-energy irradiance (primarily blue wavelengths) and emit lower-energy irradiance. Coral fluorescence emission is ubiquitous in shallow corals; 97% of reef flat corals at a site on the Great Barrier Reef exhibit medium to high levels of fluorescence (Salih et al. 2000). While coral fluorescence has been observed from mesophotic-restricted corals (Schlichter & Fricke 1990, Kahng et al. 2012), the distribution and abundance of fluorescent coral morphs in the deep mesophotic zone is unknown. Additionally, shallow corals exhibit different patterns of fluorescence emission including uniform (fluorescence over the whole coral), highlighted (varying fluorescence with concentrations in particular anatomical regions such as the oral disc), and complementary (different FPs expressed in specific anatomical regions) (Gruber et al. 2008). Cnidarians produce a rainbow of FPs, including cyan fluorescent proteins (CFPs), green fluorescent proteins (GFPs), yellow fluorescent proteins (YFPs), red fluorescent proteins (RFPs), and purple-blue fluorescent proteins that do not fluoresce (also known as pocilloporins or chromoproteins) (Alieva et al. 2008). Regardless of the color of emission or lack of emission, FPs all have a similar protein sequence and structure (Alieva et al. 2008). The dominant fluorescence in shallow corals is cyan/green emission (Vermeij et al. 2002, Gruber et al. 2008). In *Leptoseris* corals, cyan/green coral fluorescence has been observed from both the shallow Caribbean coral *L. cucullata* (Mazel et al.

2003) and the mesophotic-restricted corals *L. fragilis* (Red Sea; Schlichter & Fricke 1990) and *Leptoseris* spp. (Hawai'i; Kahng et al. 2012). However, the fluorescent pigment of *L. fragilis* has not been identified and it is unlikely to be a FP (Matz et al. 2006a). Additionally, mesophotic corals that have been studied for coral FPs include *Favia* sp. from the Red Sea in which green and red fluorescence was observed and 11 potential FP homologs were identified (Pooyaei Mehr et al. 2013) and the ahermatypic coral *Carijoa riisei* from Hawai'i in which green fluorescence was observed (Kahng & Salih 2005). While reef-building corals have the capacity to produce significant quantities of FPs (Leutenegger et al. 2007), the function of FPs is unknown. As a result of ambiguous data which may be caused by the diversity of FPs and corals, hypothesized roles for the function of FPs include photoprotection (Salih et al. 2000, Roth et al. 2010, Smith et al. 2013), antioxidant activity (Mazel et al. 2003, Bou-Abdallah et al. 2006, Palmer et al. 2009a), photosynthesis enhancement (Salih et al. 2000), *Symbiodinium* regulation (Field et al. 2006), part of an immune response (Palmer et al. 2009b, D'Angelo et al. 2012), camouflage (Matz et al. 2006b), maintenance of color diversity (Dove et al. 2001), and attraction of free-living *Symbiodinium* (Hollingsworth et al. 2004). It is possible that different FPs could have different roles, particularly in distinct environments such as shallow or mesophotic habitats. Thus research on coral fluorescence in mesophotic corals may provide unique insights into the function of FPs and coral–algal physiology.

The Indo-Pacific mesophotic zone offers a remarkable opportunity to study vibrant coral reef ecosystems that are dominated by several species of reef-building coral *Leptoseris* below 60 m (Kahng et al. 2010, Luck et al. 2013). With the deepest *in situ* observation of *L. hawaiiensis* from 165 m at Johnston Atoll, an isolated reef in the Central Pacific (Maragos & Jokiel 1986), these reef-building corals exemplify the paradox of photosynthetic organisms thriving in low-irradiance environments. This study focuses on coral fluorescence in dominant mesophotic-restricted corals and is part of a 'Deep Reef' research collective characterizing the biodiversity, productivity, physiology, and ecology of mesophotic *Leptoseris* reefs and their associated communities in Hawai'i. Because the extent of coral fluorescence is uncharacterized among species of the deep mesophotic zone, this study investigated the abundance of fluorescent morphs, the types of FPs, and the FP emission phenotypes in 4 species of mesophotic-restricted *Leptoseris* from 65 to 125 m in Hawai'i. Additionally, we explored the physiological relationship between coral fluorescence and a variety of *Symbiodinium* characteristics, including genotype, abundance, photosynthetic parameters, and pigment quantities, to determine if mesophotic FPs could have a role in photosynthesis. This research is the first to describe the ecology of coral fluorescence in dominant reef-building corals of the mesophotic zone and combines multiple data sets on corals and *Symbiodinium* from a distinctive, rarely accessible environment. Overall, these findings have significant implications for the function of mesophotic FPs in corals.

MATERIALS AND METHODS

Irradiance measurements

Irradiance attenuation profiles were obtained using a spherical underwater irradiance sensor (LICOR LI-193) corrected for underwater use connected to a data logger (LICOR LI-1400) enclosed within a PVC housing integrated with a pressure transducer to calculate depth. Measurements were taken directly over mesophotic coral reefs (20° 45.510' N, 156° 34.503' W) in close vicinity to the corals used in this study. Measurements of PAR (400–700 nm) were taken at approximately 1 m intervals in the water column at midday (11:00–12:30 h) to a depth of ~90 m during 3 clear, calm, cloudless days in the summers of 2008 and 2010. These data were used to calculate the light attenuation according to Beer's Law, which was then used to model the irradiance profile for each day. Irradiance profiles were averaged between all 6 days and the standard error at each depth was calculated.

Sample collection

Leptoseris spp. (n = 48) were collected from reefs at 65 to 125 m depths in the 'Au'au Channel offshore of Olowalu, west Maui (20° 46.851' N, 156° 40.391' W), in February and March 2011 using the 'Pisces V' submersible (www.soest.hawaii.edu/HURL). The 'Au'au Channel separates the islands of Maui and Lāna'i, and has a bottom topography consisting of a gently sloping, continuous limestone bridge.

At each site, representative corals ~20 to 30 cm in diameter were haphazardly selected from the middle

of a *Leptoseris* reef, with each collected sample separated by at least 10 m in distance. The entire coral or a small, triangular piece of coral (spanning from the middle to the outer edge of the coral head) was gently removed using a Schilling Titan 4 manipulator arm, and placed in individual sample containers in the sampling basket. Collected samples were kept in a darkened container at ambient *in situ* seawater temperatures, and processed in a darkened airconditioned laboratory (using red light headlamps with an intensity of ~1 µmol quanta m^{-2} s^{-1} from a distance of 40 to 50 cm) onboard the R/V 'Ka'imikai-O-Kanaloa' (www.soest.hawaii.edu/UMC/cms/kaimikai-o-kanaloa/) within 3 to 9 h of ascent. Photosynthetic characteristics were assessed with chlorophyll fluorescence measurements taken with a pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz). Subsequently, corals were assessed for FPs by visual examination of emission phenotypes (see below) and fragmented for further subsampling. Fragments of *Leptoseris* spp. destined for analyses of spectral properties were maintained alive in individual aquaria at ambient *in situ* seawater temperatures under low irradiance levels (~10 mol quanta m^{-2} s^{-1}) and the water was changed daily, for ≤5 d. At the end of the cruise, corals were packed in damp, light-proof containers and transported to the Hawai'i Institute of Marine Biology, O'ahu, for spectral measurements (see below). Fragments for host genetics, symbiont genetics, *Symbiodinium* abundances, and photosynthetic pigments analyses were frozen at −80°C on the cruise, transported on dry ice to the University of Hawai'i at Mānoa, O'ahu, and maintained in a −80°C freezer until analyzed. Data analyses were conducted with actual collection depth measurements, while in graphs, corals were grouped in roughly 10 m depth increments, based on sites of collection: 70 m (65–75 m, n = 11), 80 m (76–85 m, n = 10), 90 m (86–95 m, n = 9), 100 m (96–105 m, n = 6), and 120 m (120–125 m, n = 12).

Coral and symbiont genetics

The 48 *Leptoseris* spp. samples selected in this study were a subset of a more detailed host/symbiont genetic study (Pochon et al. 2015). To address the high cryptic diversity in the genus *Leptoseris* (Luck et al. 2013), we used the fast-evolving mitochondrial marker *cox1*-1-rRNA intron to assign host species following the protocol of Luck et al. (2013). Briefly, primers ZFCOXIF (forward; 5′-TCT GGT GAG CTC TTT GGG CTC T-3′) and ZFtrnar (reverse; 5′-CGA ACC CGC TTC TTC GGG GC-3′) and the thermocycling conditions described in Luck et al. (2013) were used to generate an approximately 800 bp fragment of the *cox1*-1-rRNA intron. For each coral sample, direct bi-directional sequences were generated using the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit and an ABI 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystems). All sequences were compared to the sequence dataset of Luck et al. (2013) for species-level identification.

To determine symbiont genotype in each coral sample, the 1057 bp fragment of *Symbiodinium* spp. *COI* mtDNA was PCR-amplified using primers COX1_FOR2 (forward; 5′-CCA CCA TTA TCC ACT TCT TTT A-3′) and COX1_REV1 (reverse; 5′-GGC ATA ACA TTA AAT CCT AAG AA-3′), using the thermocycling conditions described in Pochon et al. (2012). To test if *Symbiodinium* diversity was correlated with corals with different fluorescence patterns, a subset of corals (n = 12) were sampled in the calyx and coenosarc for additional genotyping. PCR products were purified and sequenced directly in both directions as described above.

Abundance of fluorescent morphs and characterization of fluorescence emission phenotypes

Coral fluorescence was examined by using a blue light source (6 mW cm^{-2}, ~450 nm, NightSea) and yellow barrier filter (≥500 nm longpass, NightSea) onboard the research vessel following PAM measurements. Presence and absence of green/cyan fluorescence was observed in the coenosarc and/or oral disc. Usual fluorescence phenotypes were defined as coenosarc (includes coenosarc and oral disc fluorescence), oral disc only, or nonfluorescent.

Spectral properties of coral fluorescent pigments and *Symbiodinium* pigments

Live coral tissue was removed using 0.2 µm filtered seawater and a Waterpik®. A portion of the whole coral homogenate was centrifuged to obtain coral and *Symbiodinium* enriched fractions. Excitation and emission spectra of the whole coral homogenate (coral and symbiont), the coral fraction, and the *Symbiodinium* fraction were measured using a

fluorescence spectrofluorometer (SpectraMax M2, Molecular Devices). Samples (300 µl) were measured in a black 96-well microtiter plate. To characterize FPs, emission scans were conducted with an excitation beam of 450 ± 9 nm and emission was followed from 475 to 700 nm (3 nm increments) on the coral fraction and coral homogenate. FP excitation was scanned from 400 to 480 nm (3 nm increments) with an emission of 501 ± 9 nm. Excitation and emission spectra were normalized to the highest peak in each scan. FPs were classified according to Alieva et al. (2008): fluorescence with emission peaks from 485 to 490 nm and excitation and emission peaks with wide (~55 nm) full width at half maximum (FWHM) were classified as CFP, and fluorescence with emission peaks ≥500 nm and excitation and emission peaks with narrow FWHM (≤35 nm) were classified as GFP.

To characterize photosynthetic pigments, an excitation scan was conducted from 400 to 650 nm (3 nm increments) at an emission of 680 ± 9 nm (chl *a*) on whole-cell *Symbiodinium* in the *Symbiodinium* enriched fraction and in the coral homogenate. Spectra were normalized to the highest peak of each scan. One photosynthetic pigment excitation spectrum was excessively noisy and was smoothed with a moving average prior to analyses (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m521p063_supp.pdf).

PAM fluorometry

Upon collection, corals were maintained in seawater in the dark and at ambient *in situ* temperatures for 3 to 9 h prior to measurements. Bio-optical measurements were collected using a Diving-PAM fluorometer (Walz). A 2 cm long piece of black tubing (1 cm diameter) was attached to the PAM fiber optic sensor to standardize the area measured and to ease placement of the sensor onto the coral surface. Actinic PAR values from the PAM with the fiber optic sensor tubing were calibrated with a cosine underwater quantum sensor (LICOR LI-192SA) and data logger (LICOR LI-1400). To account for potential spatial variation in coral physiology, ~10 measurements of maximum quantum yield of photosystem II (F_v/F_m; F_v = variable fluorescence and F_m = maximum fluorescence; Ralph & Gademann 2005) were taken from haphazardly selected, spatially separated points on the coral tissue surface, and averaged for each sample. Rapid light response curves (RLCs) were used to measure photosynthetic performance under different light levels according to Ralph & Gademann (2005). RLC irradiances were set to match the local environment, and increased from 0 to 75 µmol photons m^{-2} s^{-1} in 8 steps. The average electron transport rates by actinic irradiance intensities (ETRs) for each algal sample were fit to a 3-parameter nonlinear model as described by Frenette et al. (1993). RLC data were used to estimate the relative maximum electron transport rate ($rETR_{max}$), α (initial slope of the RLC), and the minimum saturating irradiance for photosynthesis (E_k). We used the relative measure of rETR because the exact absorbance of the coral is unknown, and likely varies by species and depth-related skeletal phenotypic differences. Curves were fitted using the Regression Wizard in Sigmaplot (v. 12.0, SPSS), and estimates of $rETR_{max}$, α, and E_k were used for analyses. In all samples, the model fit the data well with an R^2 of 0.97 ± 0.01 (mean ± SEM).

Symbiodinium abundances and pigments

Flow cytometry was used to determine symbiont cell counts according to Apprill et al. (2007). Briefly, the coral tissue was removed from the coral skeleton using a Waterpik® and filtered seawater, then blended, centrifuged, and washed repeatedly until free of host material, and resuspended in filtered seawater with 1% paraformaldehyde. Samples were analyzed using a Beckman-Coulter XL flow cytometer with a 15 mW argon ion laser providing excitation at 488 nm. The flow cytometer was interfaced with an Orion syringe pump for quantitative sample analysis using a 3 ml syringe delivering 100 µl of suspended cells at a flow rate of 50 µl min^{-1} for measurement of fluorescence emission of chlorophyll (630 nm dichroic filter, 680 nm bandpass filter), as well as forward and side scatter signals. *Symbiodinium* abundances were standardized to the coral surface area measured with the aluminum foil method (Marsh 1970) and to grams of ash-free dry weight (AFDW).

Photosynthetic pigments were analyzed using HPLC. Sample preparation was carried out following Padilla-Gamiño et al. (2013). Briefly, coral homogenate containing symbionts were filtered and extracted in 100% acetone. Pigments were analyzed by a Varian 9012 HPLC system equipped with a Varian 9300 autosampler, a Timberline column heater (26°C), and a Waters Spherisorb® 5 µm ODS-2 analytical column (4.6 × 250 mm) and corresponding guard cartridge (7.5 × 4.6 mm) following Bidigare et al. (2005). Pigments were detected with a ThermoSeparation Prod-

ucts UV2000 detector (λ_1 = 436, λ_2 = 450). Peak identity was determined by comparing retention times of pure standards with those of extracts prepared from algal cultures of known pigment composition. Pigments were standardized per cell and to coral surface area measured as above (Marsh 1970).

Data analyses

Statistical analyses were conducted using JMP version 8.0 (SAS Institute). The correlation between depth (actual depth) and FPs was assessed using the nonparametric Spearman's rho (ρ). Two-tailed *t*-tests were used to compare the *Symbiodinium* physiology and abundance data from nonfluorescent and fluorescent corals. Because *Symbiodinium* data sets were collected primarily from symbionts in the coenosarc, fluorescence emission was classified as: fluorescent corals (coenosarc and oral disc fluorescent) and nonfluorescent corals (oral disc only fluorescent and nonfluorescent). Two-way ANOVAs were conducted for the 2 factors depth (actual depth) and coral fluorescence for each parameter, and none had significant interaction effects ($p > 0.05$); therefore, depths were combined for *t*-tests of the effects of fluorescence on symbiont physiology and abundance. Data were tested for assumptions of normality and transformed prior to analyses if appropriate. Data were also tested for equal variances (Levene's test) and when necessary, unequal *t*-tests were used. Statistical differences were significant at the $\alpha < 0.05$ level. Data are represented as mean ± SEM.

RESULTS

Genetic diversity of *Leptoseris* spp. and *Symbiodinium*

The detailed phylogenetic analysis of the 48 *cox1*-1-rRNA mtDNA coral sequences indicated unambiguous correspondence for 4 species of *Leptoseris*: *L. scabra* (clade VII, n = 11), *L. tubulifera* (clade Ia, n = 11), *L.* sp. 1 (clade Ia, n = 14), and *L. hawaiiensis* (clade Ib, n = 11) (clades as indicated in Luck et al. 2013 and Pochon et al. 2015). Host genetics were not conducted on 1 sample (L76). GenBank accession numbers for *cox1*-1-rRNA mtDNA sequences can be found in Table S1 in the Supplement at www.int-res.com/articles/suppl/m521p063_supp.pdf.

Symbiodinium COI sequences grouped into 3 unambiguous mitochondrial haplotypes (COI-1, n = 11; COI-2, n = 27; and COI-3, n = 10), all belonging to *Symbiodinium* clade C but different from the previously published *COI* sequences produced in Pochon et al. (2012) for *Symbiodinium* C1 (4–6 bp differences), C15 (3–7 bp), C90 (13–14 bp), and C91 (14–17 bp) (data not shown). Haplotypes COI-1, COI-2, and COI-3 differed from each other by 3 to 7 bp changes. No mixed *Symbiodinium* genotypes were found within individual coral colonies, regardless of location on the coral (Table S2 in the Supplement). GenBank accession numbers for COI-1, COI-2, and COI-3 are HG942426, HG942427, and HG942428, respectively.

Ecology of FPs in *Leptoseris* spp.

Over the habitat of the *Leptoseris* spp. mesophotic reef ecosystem, irradiance ranged from 68.7 ± 6.3 µmol quanta m^{-2} s^{-1} at 65 m to 6 ± 0.9 µmol quanta m^{-2} s^{-1} at 125 m (Fig. 1a), which represented 2.69% and 0.23% of surface PAR irradiance respectively. Most corals from the entire depth range (65–125 m) exhibited cyan/green fluorescence emission (64–89%; Fig. 1a); fluorescent and nonfluorescent corals were found at every depth sampled. Two FPs were responsible for the cyan/green fluorescence: the higher-energy CFP had peak excitation at 424 nm (FWHM > 54 nm) and peak emission at 490 nm (FWHM > 54nm) (n = 16; Fig. 1b), and the lower-energy GFP had peak excitation at 478 nm (FWHM > 18 nm) and peak emission at 502 nm (FWHM = ~30 nm) (n = 9; Fig. 1c). Two corals (collected from 86–95 m) produced both CFP and GFP.

Technical limitations arose in the precise estimation of the excitation peak of GFP; the measurements did not extend above 480 nm. However, we consider that it was likely that the measured GFP excitation peak (478 nm) was very close to the true peak because the Stokes shift of GFP in corals is ~9–24 nm (Alieva et al. 2008). Additionally, some of the FWHM were also not precisely measured because the FWHM was out of the range of the measurement. However, the data clearly showed that CFP had much wider excitation and emission spectra compared to GFP, which is typical of these types of FPs. As a negative control, nonfluorescent corals lacked any FP peaks in their spectra (n = 13). For some corals with low fluorescence, such as visual fluorescence in the oral disc only (n = 8) and weak coenosarc fluorescence (n = 1), it was not possible to obtain FP spectra, perhaps due to low instrument sensitivity. Additionally, one coral died prior to spectral analysis.

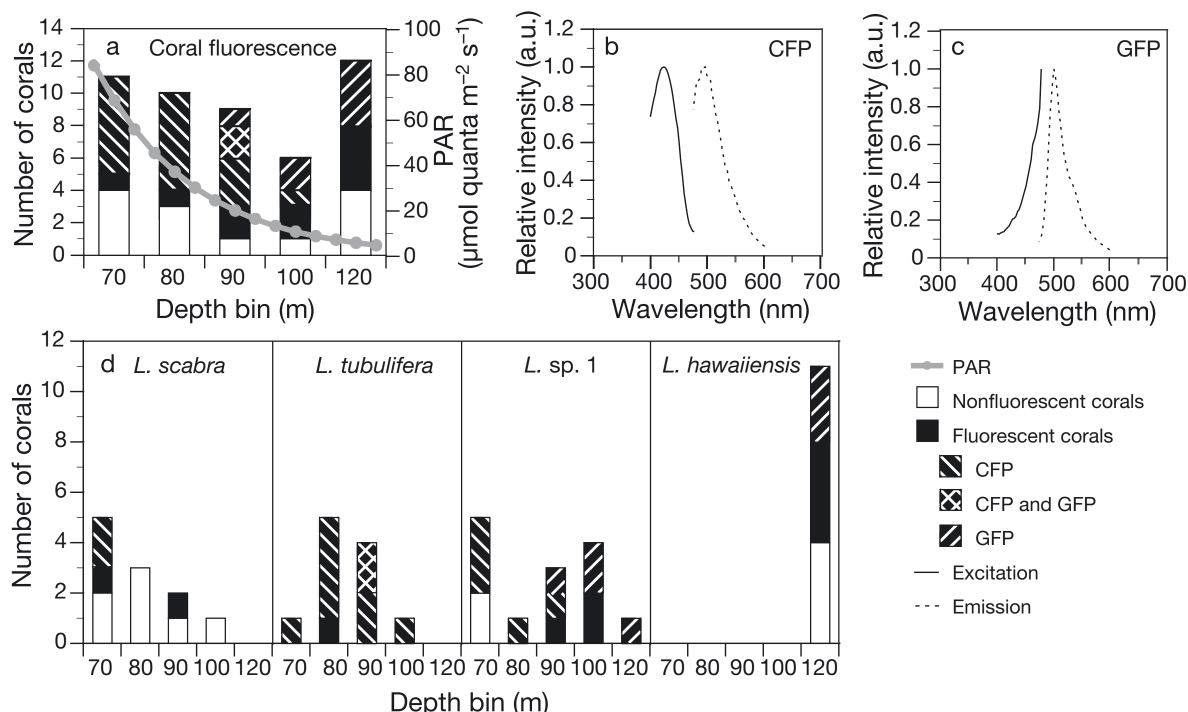

Fig. 1. Ecology of coral fluorescent proteins (FPs) in *Leptoseris* spp. collected at 65 to 125 m. (a) Fluorescent and nonfluorescent corals by depth plotted with photosynthetically active radiation (PAR) levels. A cyan fluorescent protein (CFP) and a green fluorescent protein (GFP) were identified from *Leptoseris* spp. (b,c) FP spectral characteristics showing excitation spectra with emission peak at 501 nm and emission spectra with 450 nm excitation. The excitation peaks were 424 and 478 nm for CFP and GFP, respectively, and the emission peaks were 490 and 502 nm, respectively. (d) Fluorescent corals and nonfluorescent corals by depth and *Leptoseris* spp. Solid black bars represent fluorescent corals with unidentified FP type

FP type was significantly correlated with depth among all the species ($\rho = 0.71$, $p < 0.0001$, $n = 27$; Fig. 1a) and within the species *L.* sp. 1 ($\rho = 0.80$, $p < 0.05$, $n = 9$; Fig. 1d). CFP was prevalent in shallower corals (65–85 m), the GFP was dominant in deeper corals (96–125 m), and the FPs overlapped at middle depths (86–95 m). The 4 species of *Leptoseris* all contained fluorescent and nonfluorescent individuals (Fig. 1d). *L. scabra* had the lowest proportion of fluorescent morphs (36%; n = 11), while 100% of *L. tubulifera* were fluorescent (n = 11). *L.* sp. 1 (n = 14) and *L. hawaiiensis* (n = 11) had 86% and 64% of corals with fluorescence, respectively (Fig. 1d). *L. scabra* only produced the CFP; *L. tubulifera* mostly produced the CFP, with 2 individuals exhibiting both the CFP and the GFP; *L.* sp. 1 produced both the CFP and the GFP; and *L. hawaiiensis* only produced the GFP (Fig. 1d). *L. scabra* was collected mostly from the shallower sites (65–85 m) and less with increasing depth; *L. tubulifera* was collected primarily from mid-range depths (76–95 m); *L.* sp. 1 was collected over the entire depth range (65–125 m); and *L. hawaiiensis* was only collected from the deepest range (120–125 m) (Fig. 1d).

FP emission phenotypes in *Leptoseris* spp.

Mesophotic corals were observed with FP emission present in the coenosarc (includes oral disc) (Fig. 2a), in the oral disc only (Fig. 2b), or without coral fluorescence (Fig. 2c). In areas of the corals without cyan/green FP emission, the red fluorescence from *Symbiodinium* photosystem II was visible (Fig. 2b,c). *L. tubulifera* was only observed with coenosarc fluorescence emission phenotype, while *L. scabra*, *L.* sp. 1, and *L. hawaiiensis* contained both coenosarc and oral disc only fluorescence emission phenotypes (Fig. 2d). The coenosarc fluorescence emission phenotype resulted from the CFP in *L. scabra*, GFP in *L. hawaiiensis*, and both CFP and GFP in *L. tubulifera* and *L.* sp. 1. In contrast, the oral disc fluorescence only phenotype in *L. scabra* and *L.* sp. 1 resulted from only CFP. However, the number of samples with fluorescence emission only from the oral disc was low (n = 3), resulting in unclassified FP type(s) for most oral disc only samples (n = 8). Rare coral fluorescence emission phenotypes included a bright localization of fluorescence spots (n = 7) in either random placement (Fig. S2a in the Supplement) or striations (Fig. S2b in

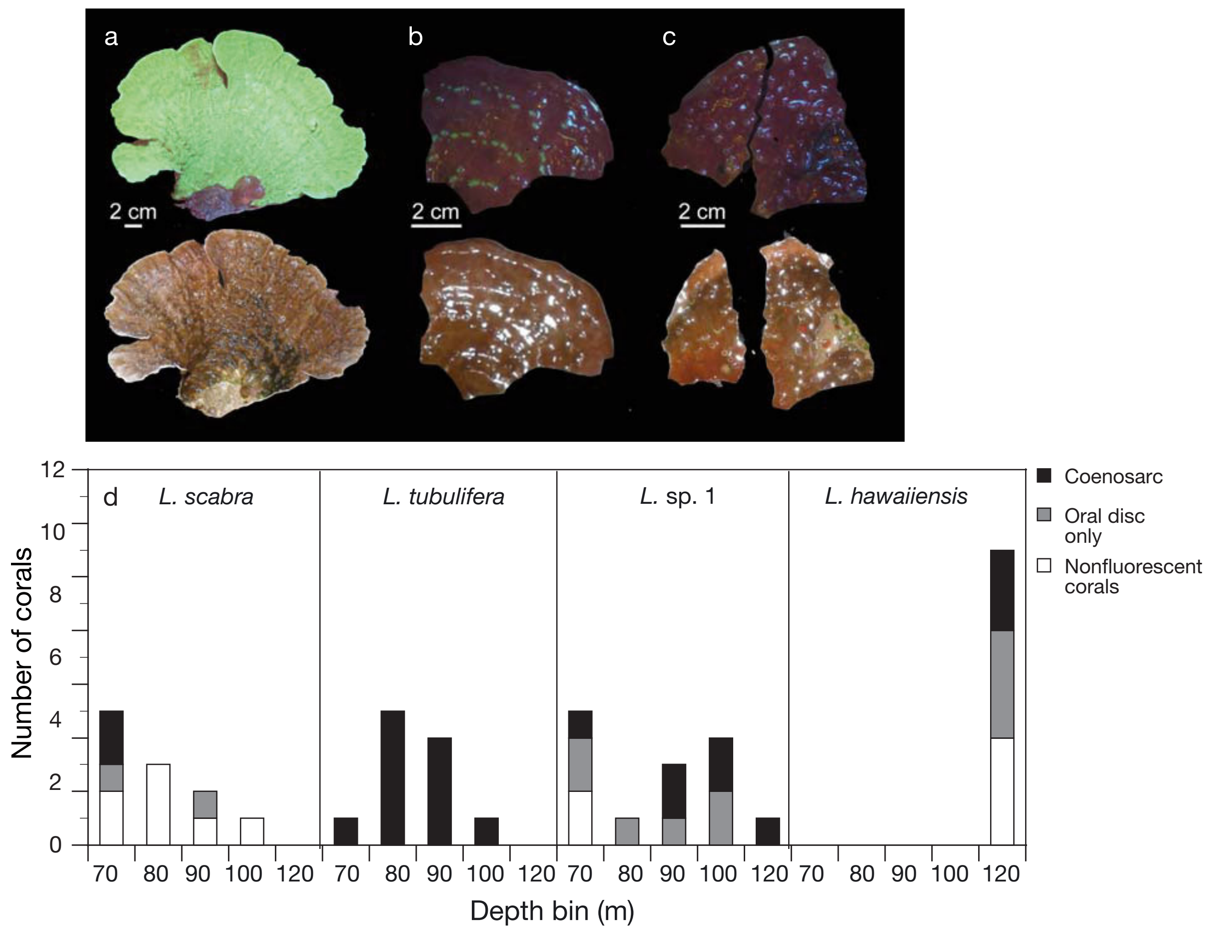

Fig. 2. Coral fluorescent protein localization in *Leptoseris* spp. Common patterns observed: (a) coral fluorescence in the coenosarc and oral disc, (b) coral fluorescence in the oral disc only, and (c) no coral fluorescence. *Leptoseris* spp. were imaged using a camera (Olympus C-5050) with a blue excitation light (Inon Z-240 strobe with NightSea blue filter, ~450 nm) and barrier filter (≥500 nm longpass, NightSea) on the lens of the camera for fluorescence (top row) and under white light (Inon Z-240 strobe) (bottom row). (d) Common fluorescence localization patterns by species and depth

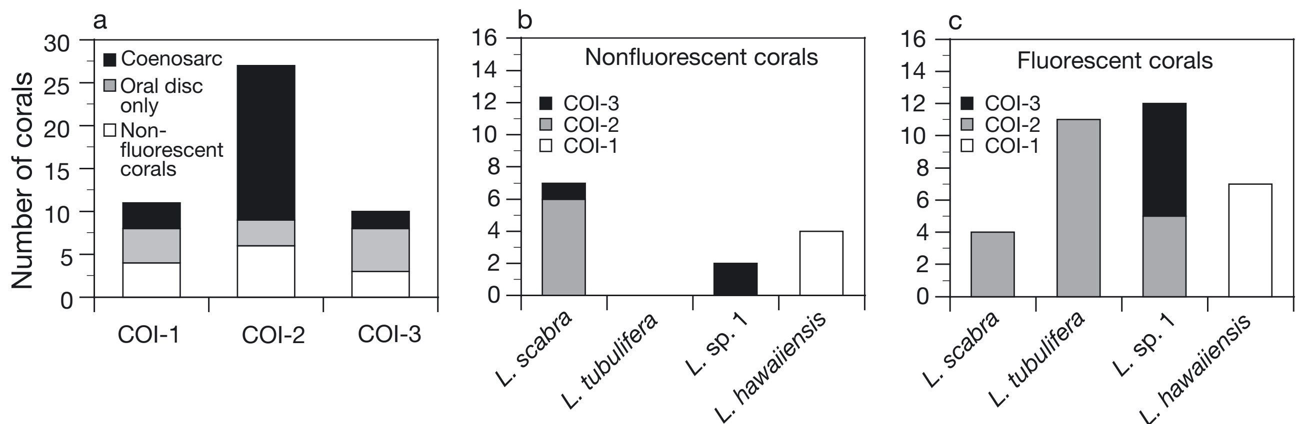

Fig. 3. Fluorescence patterns and clade C *Symbiodinium* spp. in *Leptoseris* spp. (a) Corals with all 3 *Symbiodinium* haplotypes COI-1, COI-2, and COI-3 and with either coenosarc fluorescence, oral disc only fluorescence, or no fluorescence. (b,c) *Symbiodinium* haplotypes COI-1, COI-2, and COI-3 in nonfluorescent and fluorescent corals

Table 1. Comparison of *Symbiodinium* characteristics between fluorescent (coenosarc fluorescence) vs. nonfluorescent *Leptoseris* spp. (includes nonfluorescent and oral disc only fluorescent corals), including means and *t*-tests (2-tailed). $rETR_{max}$: relative maximum electron transport rate, α: initial slope of the rapid light response curve, E_k: minimum saturating irradiance for photosynthesis, F_v: variable fluorescence, F_m: maximum fluorescence, AFDW: ash-free dry weight

Parameter	Mean ± SEM		*t*-test		
	Fluorescent	Nonfluorescent	*t*-ratio	p	df
$rETR_{max}$ (μmol electrons m^{-2} s^{-1})	3.70 ± 0.33	3.67 ± 0.38	0.1	0.95	33
α (μmol electrons)	0.25 ± 0.01	0.27 ± 0.02	–0.8	0.41	33
E_k (μmol photons m^{-2} s^{-1})	14.19 ± 0.73	13.40 ± 0.82	0.7	0.47	33
Maximum quantum yield (F_v/F_m)	0.66 ± 0.01	0.67 ± 0.01	–0.5	0.65	35
Symbiodinium density (cells cm^{-2})	$5.6 \times 10^5 \pm 0.4 \times 10^5$	$5.5 \times 10^5 \pm 0.5 \times 10^5$	0.3	0.75	45
Symbiodinium density (cells g^{-1} AFDW)	$1.2 \times 10^{10} \pm 0.1 \times 10^{10}$	$1.2 \times 10^{10} \pm 0.1 \times 10^{10}$	–0.3	0.77	45
Chl *a* (pg $cell^{-1}$)	19.5 ± 1.5	16.9 ± 1.1	1.4	0.17	45
Chl c_2 (pg $cell^{-1}$)	2.7 ± 0.2	2.2 ± 0.1	1.8	0.09	45
Peridinin (pg $cell^{-1}$)	8.7 ± 0.6	7.7 ± 0.5	1.3	0.20	45
Diadinoxanthin + diatoxanthin (pg $cell^{-1}$)	2.5 ± 0.2	2.1 ± 0.1	1.7	0.10	45
β-carotene (pg $cell^{-1}$)	0.46 ± 0.04	0.38 ± 0.02	1.8	0.07	45
Chl *a* (ng cm^{-2})	355 ± 36	293 ± 24	–0.7	0.48	45
Chl c_2 (ng cm^{-2})	49 ± 5	39 ± 3	1.7	0.10	45
Peridinin (ng cm^{-2})	160 ± 17	132 ± 11	1.4	0.17	45
Diadinoxanthin + diatoxanthin (ng cm^{-2})	46 ± 4	37 ± 3	1.7	0.10	45
β-carotene (ng cm^{-2})	8 ± 1	8 ± 1	1.9	0.06	45
Chl c_2:chl *a*	0.14 ± 0.01	0.13 ± 0.01	1.1	0.27	45
Peridinin:chl *a*	0.45 ± 0.01	0.45 ± 0.01	–0.9	0.40	45

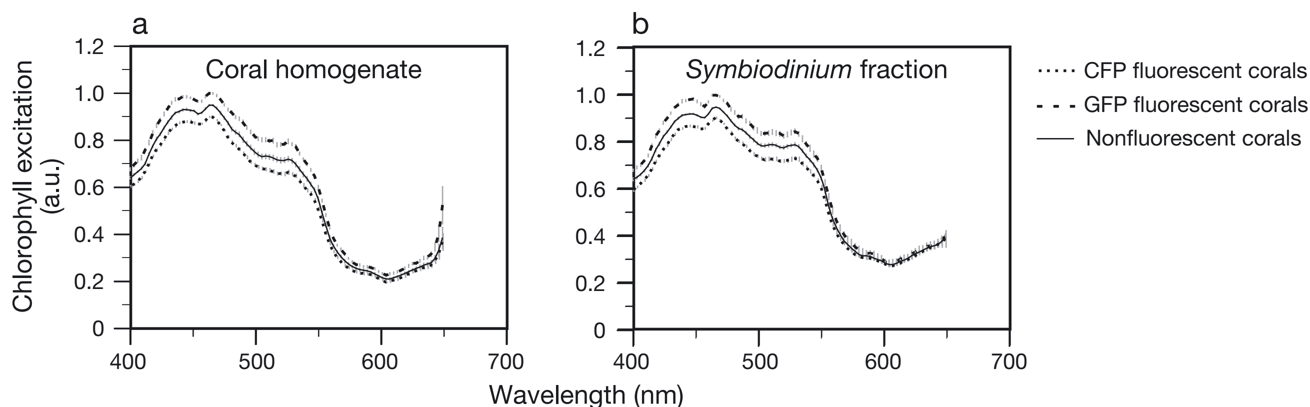

Fig. 4. *Leptoseris* spp. and *Symbiodinium* spp. Normalized photosynthetic pigment excitation spectra from *Leptoseris* spp. (a) The whole coral homogenate and (b) the *Symbiodinium* fraction (mean ± SEM). For better visualization of the spectra, samples from corals with the cyan fluorescent protein (CFP) were normalized to 90% (n = 12), samples from corals with the green fluorescent protein (GFP) normalized to100% (n = 7), and samples from corals with no coral fluorescence normalized to 95% (n = 13)

the Supplement) in corals with coenosarc fluorescence in *L. tubulifera* and *L.* sp. 1.

Leptoseris spp. fluorescence and *Symbiodinium*

Three clade C haplotypes of *Symbiodinium* were identified from all corals both with and without fluorescence emission (Fig. 3a). The proportion of corals with any type of visual fluorescence and haplotype COI-1 (n = 11), COI-2 (n = 27), and COI-3 (n = 10) was 64%, 78%, and 70% respectively. Corals that produced the CFP hosted COI-2 and COI-3 haplotypes, while corals that produced the GFP hosted all 3 haplotypes. The corals that produced both the CFP and GFP hosted the COI-2 haplotype. *L. scabra* and *L.* sp. 1 hosted 2 types of *Symbiodinium* (COI-2 and COI-3), while *L. tubulifera* only hosted COI-2, and *L. hawaiiensis* only hosted COI-1 (Fig. 3b,c). Nonfluorescent *L. scabra* hosted both COI-2 and COI-3, while fluorescent *L. scabra* only hosted COI-2. In contrast, nonfluorescent *L.* sp. 1 hosted COI-3 and

fluorescent *L.* sp. 1 hosted both COI-2 and COI-3. The subset of corals with additional *Symbiodinium* genotyping from the oral disc and coenosarc had the same haplotype in all locations regardless of haplotype type, coral species, fluorescence emission patterns, or type of FP (Table S2 in the Supplement).

Corals with and without fluorescence emission had *Symbiodinium* with similar physiological characteristics (Table 1). There were no significant differences among *Symbiodinium* abundance, $rETR_{max}$, α, E_k, F_v/F_m, chl *a* per cell or per cm^2, chlorophyll c_2 (chl c_2) per cell or per cm^2, peridinin per cell or per cm^2, xanthophyll pool (diadinoxanthin + diatoxanthin) per cell or per cm^2, β-carotene per cell or per cm^2, chl c_2:chl *a* ratio, and peridinin:chl *a* ratio (Table 1). The excitation spectrum of photosynthetic pigments peaked at ~465 nm, with a secondary peak at ~450 nm (chl *a* and c_2), and there was a shoulder from 500–550 nm (peridinin) (Fig. 4; cf. Bricaud et al. 2004). The excitation spectra of photosynthetic pigments in both the whole coral homogenate (includes *Symbiodinium*) and *Symbiodinium* fraction were similar, and the spectra were similar among corals with CFP (n = 12), GFP (n = 7), and no fluorescence (n = 13) (Fig. 4).

DISCUSSION

This study combined data on visual and spectral coral fluorescence, host genetics, symbiont genetics, symbiont abundances, photosynthetic characteristics, and photosynthetic pigment concentrations to provide a thorough analysis on a unique understudied environment, and has implications for the function of FPs in corals. Specifically, this study focused on coral FPs in several species of mesophotic-restricted *Leptoseris*, the dominant reef-building coral in the extreme low-irradiance ecosystem in the Hawaiian Archipelago.

Coral fluorescence in the mesophotic zone

Coral fluorescence was ubiquitous in the Hawaiian mesophotic zone; it was observed in all 4 investigated species of *Leptoseris* and over the entire sampled depth range (65–125 m). Overall, 73% of corals sampled were fluorescent, which is slightly lower than reports for shallow coral reef flats on the Great Barrier Reef, Australia (Salih et al. 2000). To our knowledge, the prevalence of coral fluorescence from deep (>60 m) mesophotic reefs has not previously been explored. Furthermore, we demonstrate that fluorescent and nonfluorescent morphs co-occurred at mesophotic depths, as reported for shallow corals in the Great Barrier Reef (Salih et al. 2000). The mesophotic cyan/green fluorescence emission of *Leptoseris* spp. is consistent with the principal fluorescence emission of shallower corals in the Caribbean (10–60 m in Vermeij et al. 2002, 3–30 m in Mazel et al. 2003) and the Great Barrier Reef (1–30 m in Gruber et al. 2008), suggesting that CFP and GFP may play a highly conserved role in the physiology of this coral–algal association. Fluorescence emission phenotypes found in this study including fluorescence over the entire coral or isolated to the oral disc is also consistent with what has been observed in shallow corals (Gruber et al. 2008). It is important to acknowledge that this study may have been biased towards CFP and GFP and methodologically unable to detect RFPs. However, because of the broad spectrum of excitation light and longpass characteristics of the filters used in this study, RFPs could have been observed as they had in other species of corals (M. S. Roth pers. obs.), but were not. Additionally, RFPs are much less common than CFP and GFP in shallow corals (Gruber et al. 2008). In sum, this study provides the first ecological characterization of FPs in a deep mesophotic zone.

Deep mesophotic zones are low-irradiance environments that are enriched in blue wavelengths due to spectral attenuation (Kirk 1994). At 70 m in the nearby Kalohi Channel, the blue spectral region (~410–480 nm) is nearly 20% of surface irradiance (Kahng et al. 2012). The present study found that mesophotic *Leptoseris* spp. express 2 FPs with different spectral characteristics: CFP (λ_{ex} = 424 nm, λ_{em} = 490 nm) and GFP ($\lambda_{ex} \approx$ 478 nm, λ_{em} = 502 nm). Strikingly, CFP was dominant in corals at shallower depths (65–85 m), while the GFP prevailed in corals at deeper depths from 96–125 m. In the middle region (86–95 m), both CFP and GFP were equally present in corals. CFP and GFP are correlated with depth among all the coral species as well as within species, and the FPs appear to absorb the predominant wavelengths of PAR present at the sampled depths. The wavelengths of PAR that excite GFP are enriched in deeper waters as compared to the spectral regions that excite CFP (Mass et al. 2010). There is some precedence for changes in types of FPs based on habitat. In species of the Caribbean coral *Madracis*, numbers of fluorescent color morphs decrease from 10 to 60 m depth, yet green fluorescence emission is observed over the sampled depth range (Vermeij et al. 2002). In the Indo-Pacific coral *Seriatopora hystrix,* swimming larvae synthesize 2

GFPs, while the benthic adults produce a CFP, and these 2 life history stages occupy different habitats with distinct irradiance environments (Roth et al. 2013). Overall, our finding of 2 types of FPs along the depth gradient among congeners and conspecifics in the mesophotic zone fits well with other studies in shallow systems, but has never been observed over these depth zones of 65 to 125 m.

Previous studies have also shown that corals can also vary the amount of FPs within their tissues in response to changes in environmental and physiological conditions (D'Angelo et al. 2008, Roth et al. 2010, Roth & Deheyn 2013). However, in shallow Caribbean corals (3–30 m), there is no correlation between depth and GFP abundance in *Montastrea cavernosa* and *M. faveolata* (Mazel et al. 2003). Gene expression of *M. cavernosa* (8–24 m) suggests that 1 or possibly 2 GFPs are inversely related with depth, but 5 other GFPs are not correlated with depth (Kao et al. 2007). FP gene expression can change dynamically in response to environmental conditions in shallow corals and FP transcription is most regulated by blue light rather than red or green light (D'Angelo et al. 2008). Additionally, the abundance of FPs within corals rapidly decreases in response to both cold and heat stress (Roth & Deheyn 2013). Future studies should quantify the FP abundance and gene expression in mesophotic corals from a variety of species and depths, as well as compare CFP and GFP genes from shallow and mesophotic corals to determine possible differences or similarities.

The similarity in gross morphology of *Leptoseris* spp. and the high morphological plasticity within species makes it difficult to separate species both while viewing corals from the submersible as well as when handling the samples in the laboratory. In this study, genetics revealed 4 species of *Leptoseris* (*L. scabra*, *L. tubulifera*, *L.* sp. 1, and *L. hawaiiensis*), each with distinct fluorescence patterns. The cyan/green fluorescence was localized in the coenosarc in all species, but only *L. scabra*, *L.* sp. 1, and *L. hawaiiensis* exhibited the oral disc only phenotype. The most divergent species in our study, *L. scabra* (Luck et al. 2013), had the lowest abundance of fluorescent corals (36%) and only produced CFP. It is intriguing to ask why *L. scabra* has a much lower abundance of fluorescence, and it may suggest that their photophysiology is different from the other species with less selective pressure for fluorescence. *L. tubulifera* and *L.* sp. 1, which were the most closely related (Luck et al. 2013), had the highest abundances of fluorescent corals (100% and 86% respectively) and produced CFP and GFP. All colonies of *L. tubulifera* were fluorescent over their entire surface, suggesting that fluorescence may have an adaptive value in this species. Colonies of *L. hawaiiensis*, which were only located at the deepest sampled depths (120–125 m), were commonly fluorescent (64%) and only produced GFP. Corals can quickly regulate FP transcription and concentration on short time scales in response to changes in their environment and stress (D'Angelo et al. 2008, Roth et al. 2010, Roth & Deheyn 2013). Thus, in species where fluorescent morphs are highly abundant, weakly fluorescence or nonfluorescent morphs could indicate a different physiological state. Coral fluorescence has also been noted in species of shallow *Leptoseris* and mesophotic GFP emission has similar emission to GFP from the Caribbean coral *Leptoseris cucullata* collected from 25 m and often shallower (Mazel et al. 2003, C. H. Mazel pers. comm.).

Because FPs are part of the photophysiology of the coral–algal symbiosis, this study also investigated a large number of *Symbiodinium* characteristics. To our knowledge, this is the first study to show that *Symbiodinium* genotype does not have any relationship with coral fluorescence. Additionally, if the coral is fluorescent, the genotype of *Symbiodinium* does not affect the fluorescence emission phenotype. *Symbiodinium* density was highly variable, and no significant differences were observed with corals that were fluorescent or nonfluorescent. Similarly, a study of shallow corals showed no overlap between the distribution of FPs and *Symbiodinium* (Gruber et al. 2008). Photosynthetic pigments absorb different wavelengths, with the primary peak of chl *a* absorbing higher-energy light than chl c_2, which is then absorbing higher-energy light than peridinin (Bricaud et al. 2004). Peridinin is an important light-harvesting pigment in *Symbiodinium* that extends the spectral range of light harvested for photosynthesis into green wavelengths (Bricaud et al. 2004). The peak absorbance of peridinin most closely aligns with peak emissions of CFP and GFP. In this study, the concentrations of chl *a*, chl c_2, and peridinin were similar between fluorescent and nonfluorescent corals, as were the ratios of chl c_2:chl *a* and peridinin:chl *a*. The chlorophyll excitation spectra from corals with GFP, CFP, and no fluorescence were similar in the coral homogenate, the *Symbiodinium* fraction, and between the two fractions. The FP peak emissions (490 and 502 nm) do not overlap with the absorbance peaks of chlorophylls (440–465 nm), but rather the shoulder of the photosynthetic pigment excitation and the peak absorbance of peridinin (490–510 nm). It may be possible that the relationship between FPs and photosynthesis was dis-

rupted when the coral tissue was removed from the skeleton; unfortunately, measurements with the skeleton provided unreliable excitation spectra and therefore the coral tissue needed to be removed for measurements.

It is interesting to note that the excitation peaks of CFP and GFP fall on either side of the chlorophyll absorbance peaks, which may reduce competition for particular wavelengths of PAR and may be evidence of energy partitioning. Having FPs and antennae complexes with distinct maximum absorbance peaks may signal an evolutionary change to avoid competition for irradiance. However, the excitation peak, and in particular that of CFP as well as intact light-harvesting complexes, are broad and therefore there may be some overlap between wavelengths that can be absorbed by FPs or photosynthetic pigments. But, because of the distance between FPs and antenna complexes, energy cannot be directly transferred from coral FPs to *Symbiodinium* in shallow corals (Gilmore et al. 2003). Nonetheless, it may be possible for antenna complexes to absorb re-emitted wavelengths from FPs. However, the data in this study show that CFP and GFP absorption have limited impact on symbiont photosynthesis in mesophotic corals, and this would be particularly true under the extremely low-irradiance regimes in winter months. This finding is similar to that from shallow Caribbean corals, where a study also concluded that FP absorption, emission, and reflection has an insignificant effect on *Symbiodinium* photosynthesis (Mazel et al. 2003).

Additional perspective is gained in examining the photosynthetic parameters $rETR_{max}$, α, E_k, and F_v/F_m in both fluorescent and nonfluorescent corals. There were no detectable differences between these corals, suggesting that *Symbiodinium* photophysiology is not influenced by coral fluorescence. Because spectral features influence photosynthetic measurements (Mass et al. 2010), it is possible that using an irradiance source with a spectrum similar to the native habitat of these corals rather than the white light that was available would have produced a different result. For example, in *Stylophora pistillata* from 40 m, the ratio of gross photosynthesis to respiration doubled when measured under blue light as opposed to full spectrum light (Mass et al. 2010). In the Red Sea, the mesophotic-restricted coral *Leptoseris fragilis* has an unidentified coral fluorescent pigment that is hypothesized to be involved in improving the photosynthesis of *Symbiodinium* because higher photosynthetic rates were obtained with a narrow spectrum of light matching the excitation of the fluorescent pigment at some light intensities (Schlichter & Fricke 1990). The fluorescent pigment of *L. fragilis* has a peak emission (~440 nm) that overlaps with the peak absorbance of chl *a* (Schlichter & Fricke 1990). It is not possible to measure the emission of a FP in chloroform because FPs degrade in chloroform, and therefore it seems unlikely that the fluorescent pigment in *L. fragilis* is a FP (Matz et al. 2006a). Unfortunately, the fluorescent pigment for *L. fragilis* was not characterized further and remains unidentified. In contrast, the FPs of Hawaiian mesophotic *Leptoseris* did not have any influence over the photophysiology of *Symbiodinium* in mesophotic corals, with the methods used here.

This study provides a first characterization of the prevalence and types of coral FPs and fluorescence emission phenotypes in dominant mesophotic reef-building corals and suggests that FPs are important for coral physiology. Because of the difficulty of obtaining samples from the mesophotic zone, this study does have limitations of modest sample sizes, particularly when trying to compare among different depths and species. However, this study offers a foundation for coral fluorescence in the mesophotic zone and hopefully will stimulate more research in this direction. Future research could investigate the relationship between FP gene expression, FP abundance within coral tissues, and localization of FPs over depth. Microscopy measurements on the spatial separation between FPs and *Symbiodinium* will confirm or refute the possibility of energy transfer. While the focus of this study was broad in including 4 closely related species over a large depth range (65–125 m), future studies targeting a specific *Leptoseris* species, perhaps with only 2 depth ranges (e.g. a shallow versus deeper site) and including reciprocal transplant experiments and additional physiological measurements, such as oxygen evolution, under a variety of irradiance intensities and spectral qualities, may improve the understanding of the role of FPs in corals. While this study was focused on the ecological characterization among dominant corals of the mesophotic zone and does not determine the function of FPs in mesophotic corals, this study can provide evidence to support or negate various hypotheses.

On the function of FPs

Despite early observations of coral fluorescence and the widespread use of FPs as a tool in research, the function of FPs in shallow and deep corals

remains unknown. The inability to conduct laboratory work to knockdown/knockout genes in corals or *in situ* manipulations at depth does not allow for conclusive studies on the function of FPs. Moreover, recent studies have showed conflicting results, leading to multiple hypotheses on the functions of FPs. FPs from distinct corals from disparate environments are likely to have unique functions. The present study on coral fluorescence of dominant mesophotic reef-building corals has implications for the prevailing hypotheses of FPs.

In irradiance-limiting environments, it is hypothesized that coral FPs could enhance photosynthesis of their endosymbiotic algae by converting non-harvested PAR wavelengths into wavelengths of irradiance that can be absorbed by the light-harvesting pigment protein complexes of *Symbiodinium* (Salih et al. 2000). The position of FPs within the host tissue can vary; low-light corals have FPs localized below or within *Symbiodinium* populations, whereas in high-light corals, FPs are located above *Symbiodinium* (Salih et al. 2000). However, fluorescence kinetics and mapping of excitation and emission in shallow corals show that FP emission can only play a minor role in chlorophyll excitation (Gilmore et al. 2003). Additionally, measurements on reflectance and chlorophyll excitation spectra indicate that FPs have negligible impact on coral photosynthesis under high-irradiance regimes (Mazel et al. 2003). In the present study, the similarity in photosynthetic pigment excitation of the *Symbiodinium* fraction from corals with CFP, GFP, and no fluorescence emission suggests that it appears unlikely that FPs and photosynthesis are linked. Additionally, the lack of relationship between the abundances and relative abundances of different photosynthetic pigments and coral fluorescence also does not support a role of FP in enhancing photosynthesis in mesophotic corals. Furthermore, the photosynthetic parameters were similar between corals with and without fluorescence, suggesting little or no relationship between FPs and the symbiont photosynthesis. Ultimately, the lack of increase in proportion of corals with fluorescence at greater depths, combined with the data on the concentrations of photosynthetic pigments, photosynthetic pigment excitation, and photosynthetic parameters, suggest that these mesophotic FPs are not involved in augmenting photosynthesis of *Symbiodinium*, but rather may serve another function in mesophotic corals.

Another leading hypothesis for the function of FPs in shallow corals is for photoprotection (Salih et al. 2000, D'Angelo et al. 2008, Roth et al. 2010). Photoprotection, the processes and mechanisms to prevent damage from light, is important for both the coral as well as *Symbiodinium*. Photoprotection by FPs could include absorbing, screening, and scattering light as well as acting as an antioxidant (Salih et al. 2000, Gilmore et al. 2003, Bou-Abdallah et al. 2006, Palmer et al. 2009a). Ultraviolet radiation, PAR, and to a lesser extent blue light (~410–480 nm) are greatly reduced at mesophotic depths (Kirk 1994, Kahng et al. 2012, the present study). Mesophotic corals independent of their photosymbionts also express FPs. *Carijoa riisei*, an ahermatypic coral found from 0 to 125 m, has green-colored FPs on the anthocodia (distal part of a polyp) at all depths, which are hypothesized to serve a photoprotective role (Kahng & Salih 2005). In the present study, CFP absorbed the highest-energy wavelengths of light that would reach *Leptoseris* spp. and show a decline in abundance in corals with depth, which may suggest that CFPs could serve a photoprotective function, particularly for corals at <100 m depth. However, this selection pressure may only be present during the summer months, as winter may reduce irradiance from both lower intensities and shorter days. Shallow corals can use FPs to photoacclimate to different light intensities and spectral distributions (D'Angelo et al. 2008, Roth et al. 2010). The photosynthetic apparatus of *Symbiodinium* acclimates to both light intensity and spectral features of PAR (see Roth 2014), which could be influenced by FPs in corals. It has also been hypothesized that FPs could help regulate *Symbiodinium* through photosynthesis modulation and through regulatory photosensors (Field et al. 2006), but no differences in photosynthesis and photosynthetic pigments in fluorescent and nonfluorescent corals were observed in the present study.

While irradiance is much reduced at mesophotic depths, it is unclear if photosynthetic organisms in the mesophotic zone experience any excessive light. In reef-building shallow corals, multiple scattering by the coral skeleton increases the amount of light the coral cells and *Symbiodinium* are exposed to and offers many opportunities for photons of light to be absorbed or damage cells (Kühl et al. 1995, Enríquez et al. 2005). It has been hypothesized that the skeleton of mesophotic *Leptoseris* spp. may be specialized to increase the local light field within coral cells (Kahng et al. 2012), but detailed studies on the scatter and skeletal fractality of mesophotic *Leptoseris* spp. are lacking. Mesophotic corals also often have flattened skeleton morphologies to optimize light absorption (Kühlmann 1983). In shallow corals, blue light increases susceptibility to bleaching during thermal

stress and bleaching of cultured *Symbiodinium* (Fitt & Warner 1995), perhaps because blue light is known to damage photosystem II and inhibit its repair (Nishiyama et al. 2006). While the irradiance in the mesophotic zone is quite low, at noon in summer months, the PAR was measured to be higher than the photosynthetic saturating irradiance (E_k) of these corals. Once the saturation irradiance is exceeded, additional irradiance cannot be processed through photochemistry and the surplus light energy must be dissipated via other pathways such as non-photochemical quenching to prevent excessive production of reactive oxygen species and ultimately damage (see Roth 2014). Non-photochemical quenching, the dissipation of extra energy as heat, encompasses multiple processes on many timescales (see Roth 2014). Both hot and cold temperature anomalies increase the need for photoprotection under typical irradiances (Roth et al. 2012, Roth 2014). Mesophotic corals experience rapid reductions in temperatures from internal waves and upwelling (Bak et al. 2005, Lesser et al. 2009) and photoprotection may be necessary during those conditions. The extent to which mesophotic corals experience excess light and need and/or utilize these mechanisms is unknown.

FPs may also be able to act as antioxidants, which scavenge and neutralize reactive oxygen species (Mazel et al. 2003, Bou-Abdallah et al. 2006, Palmer et al. 2009a). Reactive oxygen species can damage proteins, lipids, and DNA (Lesser 2006). The production and accumulation of reactive oxygen species can be very dangerous for organisms and is termed oxidative stress (Lesser 2006). Reactive oxygen species are a common response to a variety of environmental insults such as temperature stress, light stress, and pollution (Lesser 2006). Additionally, the production of reactive oxygen species is inevitable during photosynthesis regardless of irradiance intensity (Foyer & Shigeoka 2011). Corals host *Symbiodinium* that are photosynthesizing and producing oxygen and reactive oxygen species within the cells of corals; therefore, shallow corals become hyperoxic during the daytime and are highly susceptible to oxidative stress (Kühl et al. 1995, Lesser 2006, Roth 2014). Mesophotic reef-building corals also host *Symbiodinium,* but it is unknown how vulnerable mesophotic coral–*Symbiodinium* symbioses are to oxidative stress at depth and how often the symbioses would encounter conditions to elicit oxidative stress. In this study, mesophotic *Symbiodinium* have similar concentrations of photoprotective pigments per cell, xanthophylls and β-carotene, as compared to *Symbiodinium* from shallow corals (Apprill et al. 2007). Because xanthophylls help dissipate excess energy as heat and xanthophylls and β-carotene serve as antioxidants (Lesser 2006), the equal presence of photosynthetic protective pigments in mesophotic and shallow corals may suggest a need to have defenses to neutralize reactive oxygen species even at depth. The high abundance of FPs throughout the mesophotic zone and in particular at the deepest range (120–125 m) may indicate that these proteins play an integral and conserved role in coral biology such as in scavenging reactive oxygen species.

Other roles proposed for the function of FPs include their visual properties. FPs are responsible for the vivid coloration of shallow corals (Dove et al. 2001). Because of the blue spectral enrichment at mesophotic depths, the cyan/green fluorescence is easily visible for the human eye at depth from the submersible. The coral fluorescence may be part of a countershading or camouflage from herbivorous fishes, which has been suggested for shallow corals (Matz et al. 2006b). Although mesophotic reefs can support significant fish biomass, they are dominated by planktivores (Kahng et al. 2014). Given the high abundance of corals and low abundance of herbivores, it seems unlikely that *Leptoseris* spp. would need camouflage as protection from fish in these ecosystems. Additionally, it has been hypothesized that coral fluorescence could be used to attract *Symbiodinium* (Hollingsworth et al. 2004). However, the present study showed similar densities of *Symbiodinium* regardless of fluorescence, suggesting that this is not a likely function of FPs in mesophotic corals. It also seems probable that free-living *Symbiodinium* would not be able to survive in the low-irradiance mesophotic habitat and that *Symbiodinium* within mesophotic corals may require products from the host to survive in such light-limiting conditions. It may be possible that coral fluorescence could attract prey, which could be beneficial to increase heterotrophy at depth, but this hypothesis has been largely untested.

Lastly, FPs may have a role in coral immunity (Palmer et al. 2009b, D'Angelo et al. 2012). It is common to observe varied fluorescence and in particular RFP near a coral parasite, borer, an erosion area, or other disturbance (Palmer et al. 2009b, D'Angelo et al. 2012). However, it is unlikely that the coral fluorescence observed in this study is from a disease-related scenario because the fluorescence phenotype patterns match with anatomical regions rather than a disturbance or variation in the tissue. However, we cannot exclude the possibility that mesophotic corals could express different FPs during wounds and healing because this was not tested in this study. In shal-

low corals, FPs are known to correlate with growth in addition to wound healing (Roth et al. 2010, D'Angelo et al. 2012, Roth & Deheyn 2013), which may suggest another basic physiological role. It is remarkable that FPs are pervasive and fluorescence is primarily cyan/green on mesophotic coral reefs, which is similar to shallow coral reefs.

CONCLUSION

This study encompassed the ecology of coral FPs in an extreme low-irradiance environment and considerable *Symbiodinium* physiological data to provide new insights into MCEs and coral fluorescence with implications on the function of FPs. While it appears unlikely that FPs in mesophotic *Leptoseris* spp. corals enhance photosynthesis, based on this study we cannot rule out the possibility that mesophotic FPs could play a photoprotective type role either through absorption, screening, or scattering of light or as an antioxidant. Many of these corals are exposed to light levels higher than their photosynthetic saturating irradiance and the broad excitation peaks of FPs have some overlap in wavelengths of light used in photosynthesis, which could provide some dissipation of light to wavelengths of light less used by photosynthesis. There is the potential for energy partitioning, as the main excitation peaks of the FPs fall on either side of the main photosynthetic peak, which may reduce competition and be important in an energy-limited environment. Additionally, we cannot rule out that fluorescence could be a remnant from a function no longer relevant for mesophotic corals. However, because mesophotic corals reduce their energetic requirements associated with the difficulties of living and thriving in such low-light environments (Lesser et al. 2009, Kahng et al. 2010), the widespread prevalence of coral fluorescence in 4 dominant reef-building corals throughout the mesophotic zone is unexpected. Furthermore, it is surprising that these corals appear to dedicate a significant amount of energy towards producing FPs, which are often present over the entire coral (coenosarc and oral disc), and suggests that the FPs have a significant role in coral and/or symbiont biology.

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