

Published in final edited form as:

Front Mar Sci. ; 7: . doi:10.3389/fmars.2020.00013.

## Physiological characteristics of *Stylophora pistillata* larvae across a depth gradient

Federica Scucchia<sup>1,2</sup>, Hagai Nativ<sup>1</sup>, Maayan Neder<sup>1,2</sup>, Gretchen Goodbody-Gringley<sup>3,\*</sup>, Tali Mass<sup>1,\*</sup>

<sup>1</sup>Department of Marine Biology, Leon H. Charney School of Marine Sciences University of Haifa, Israel

<sup>2</sup>The Interuniversity Institute of Marine Sciences, Eilat 88103, Israel

<sup>3</sup>Bermuda Institute of Ocean Sciences, St. George, Bermuda

### Abstract

Depth related parameters, specifically light, affect different aspects of corals physiology, including fluorescence. GFP-like pigments found in many coral species have been suggested to serve a variety of functions, including photo-protection and photo-enhancement. Using fluorescence imaging and molecular analysis, we further investigated the role of these proteins on the physiology of the coral *Stylophora pistillata* and its algal partners. Fluorescence was found to differ significantly between depths for larvae and adult colonies. Larvae from the shallow reef presented a higher GFP expression and a greater fluorescence intensity compared to the larvae from the mesophotic reef, reflecting the elevated need for photo-protection against high light

\*Corresponding authors: Gretchen.Goodbody-Gringley@bios.edu; tmass@univ.haifa.ac.il.

#### Author contribution statement

FS, MN, HN, GGG and TM carried out the experiments and participated in the design of the study. FS, GGG and TM participated in the data analysis. FS carried out the molecular analysis; FS, MN and GGG carried out the microscopy imaging and the image analysis. HN carried out the in-situ imaging. FS, TM and GGG wrote the manuscript. All authors contributed to the writing and improving of the manuscript and approved its final version.

#### Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

#### Contribution to the field

In this paper we characterized the GFP expression patterns and its fluorescence intensity between *Stylophora pistillata* larvae from the shallow and mesophotic reef. The higher GFP expression and the greater fluorescence intensity of the shallow larvae reflects the elevated need for photo-protection against the high light levels of the shallow reef, corroborating the notion that GFPs act as UVR screeners. Furthermore, the lower but still occurring expression under non-damaging low light conditions suggests that GFP proteins might regulate the amount of photosynthetically available light for the benefit of the symbiotic algae. Additionally, we propose that the observed differences in GFP expression and fluorescence between shallow and deep larvae indicate that these proteins might serve to attract different symbiont clades. This paper would significantly contribute to the coral reef research field, as it contains valuable insights into the still controversial role of GFP proteins and into the functional significance of the association between coral GFPs fluorescence and the symbiont assemblages. The resilience of coral reefs to increasing threats such as climate change hinges on successful recruitment. Thus, it is crucial to comprehend coral larvae physiological adaptation to environmental characteristics, since their survival provides the basis for the future of the reef.

#### Ethics statements

##### Studies involving animal subjects

Generated Statement: No animal studies are presented in this manuscript.

##### Studies involving human subjects

Generated Statement: No human studies are presented in this manuscript.

##### Inclusion of identifiable human data

Generated Statement: No potentially identifiable human images or data is presented in this study.

levels characteristic of the shallow reef, thus supporting the “sunscreen” hypothesis. Additionally, given the lower but still occurring protein expression under non-damaging low light conditions, our results suggest that GFP-like proteins might act to regulate the amount of photosynthetically usable light for the benefit of the symbiotic algae. Moreover, we propose that the differences in GFP expression and green fluorescence between shallow and deep larvae indicate that the GFPs within coral larvae might serve to attract and retain different symbiont clades, increasing the chances of survival when encountering new environments.

## Keywords

Fluorescence; corals; GFP; Horizontal acquisition; Mesophotic reef; *Symbiodinium*

---

## Introduction

Scleractinian corals are the foundation organisms of tropical coral reefs, providing the physical structure and habitat that supports the world most diverse and productive biological communities on Earth. They owe their success, in part, to their symbiotic relationship with photosynthetic endosymbiotic dinoflagellate algae in the family Symbiodinaceae, that supply the coral host with sufficient photosynthetic products, in particular lipids and carbohydrates, to support the hosts’ metabolism, growth, reproduction, and survival (Allemand and Furla, 2018; Davy et al., 2012; Hoegh-Guldberg, 1999). During photosynthesis Symbiodinaceae rely on light as the energy source, thereby restricting the distribution of many coral species to the photic zone. However, corals are able to persist and form reef structures in deeper waters despite limited light availability (Ezzat et al., 2017). Referred to as mesophotic reefs, these light-dependent coral ecosystems (Armstrong et al., 2006; Hinderstein et al., 2010) range in depth from 30 meters to almost 150 meters in some areas with particularly clear waters (Bongaerts, 2010). Given the documented influence of light on the photosynthetic features of *Symbiodinium sp.*, light characteristics are one of the main factors influencing the depth-zonation of symbiotic corals (Nir et al., 2011). Across their vertical distribution, from high-light-dominated shallow areas to low-light-dominated mesophotic reefs, variations in light intensity induce both the coral and the dinoflagellate partner to present adaptive traits and photo-physiological modifications (Ezzat et al., 2017; Bongaerts et al., 2011; Smith et al., 2017).

Corals also possess pigments related to the green fluorescence proteins (GFP) of *Aequorea victoria* (Tsien, 1998) that have been suggested as important players in corals acclimatization and adaptation to changing light conditions with depth (Dove et al., 2001; Salih et al., 2000). In shallow-water corals these pigments are suggested to serve as sunscreens (Ben-Zvi et al., 2015; Salih et al., 2000), reducing light stress of the algal symbiont through the absorption of high energy and potentially harmful photons and re-emitting light with lower energy (Roth and Deheyn, 2013). However, intensely fluorescent corals have been observed in low-light, deep-water environments, suggesting a different role for these GFP-like proteins in mesophotic reefs (Smith et al., 2017). In particular, these pigments have been hypothesized to have a photo-enhancement function through the transformation of the incident light into wavelengths of peak absorption by the algae

photosynthetic pigments (Kawaguti, 1969; Schlichter and Fricke, 1990). To date, the functions of GFP-like proteins in corals remains controversial and ambiguous, with other proposed functions for these proteins including attraction of free-living *Symbiodinium sp.* (Hollingsworth et al., 2005), oxidative stress response (Bou-Abdallah et al., 2006), camouflage (Matz et al., 2006) and immune response (Palmer et al., 2009a). Regardless, the connection between coral fluorescence and abiotic factors indicates that these proteins may play a major role in coral interactions with the physical environment (Roth et al., 2013).

Abiotic factors that vary with depth also affect patterns of coral recruitment, with light representing one of the main elements influencing pre- and post-settlement survival and behavior (Turner et al., 2018). The resilience of coral reefs to increasing threats such as climate change hinges on successful recruitment, where phenotypic diversity and plasticity of larvae may enable a wide range of tolerances and the variation necessary for natural selection to act (Roth et al., 2013). It is critical, therefore, to obtain a comprehensive understanding of coral larval physiological adaptation to environmental variation, since their effective settlement provides the basis for the future of the reef (Aranda et al., 2011).

In the present study, we examined the characteristics of early life stages of the depth generalist coral *Stylophora pistillata*, Esper, 1797, along a depth gradient in the Gulf of Eilat, Red Sea. *S. pistillata* is one of the most abundant hermatypic coral species in the Gulf of Eilat (Loya, 1972). This hermaphroditic brooder typically releases planula larvae between December and July (Grinblat et al., 2018). Here, we examined key physiological features of *S. pistillata* larvae, analyzing differential photo-physiology and GFP-like protein characteristics between shallow and mesophotic planulae, to explore the potential connection between these proteins and different light environments, and to discuss their possible role in the physiology of corals and of their algal partners.

## Methods

### Sample collection

Larvae traps were made using a 160µm plankton net with a plastic collection container attached to the top. Larvae of the stony coral *S. pistillata* were collected from randomly selected colonies on the reef adjacent to the Interuniversity Institute of Marine Sciences (IUI, 29°30'06.0"N 34°54'58.3"E) in the Gulf of Eilat (Israel), both from the shallow reef (depth of 3-6 m) and from the mesophotic reef (depth of 40-45 m) under a special permit from the Israeli Natural Parks Authority (Fig. 1).

Collection traps were placed on random 15 colonies for each depth shortly before sunset and retrieved the following morning; this process was repeated for three nights near the full moon of April 2019, following peak releases (Shlesinger et al., 1998). Light intensity and seawater temperature of the sampling sites were measured using HOBO Water Temp Pro data loggers (Onset Computers). For each sample, half of the planulae collected were immediately placed in Petri dishes with seawater for larval size and fluorescence imaging analysis, the remaining larvae were immediately snap-frozen in liquid nitrogen and stored with TRI reagent (Life Technologies) at -80°C for DNA and RNA extraction.

### Larval size image analyses

Larvae from samples pooled by depth were randomly sub-sampled (n=36, shallow; n=32 mesophotic) and digitally photographed from a side view (elliptical in shape) using a Dino-Lite digital microscope under a 3x magnification. Larval volume was determined by measuring the length and width of the longitudinal and transverse axes in ImageJ and applying the equation for volume of an elliptical sphere,  $V = \frac{4}{3}\pi ab^2$ , where a is 1/2 width and b is 1/2 length (Isomura and Nishihira, 2001). Larval volume was square-root transformed in order to meet the assumptions of normality (Shapiro-Wilk's test) and homogeneity (Levene's test) and compared by depth using an Analysis of Variance (ANOVA) followed by a Tukey's HSD post-hoc test performed in RStudio v1.1.423 (R Development Team) using the above function in the package car (Fox et al., 2019).

### RNA extraction and cDNA synthesis

Total RNA was extracted from pools of 10-15 larvae per sample using the Invitrogen PureLink RNA micro kit (ThermoFisher Scientific, USA) according to the manufacturers' protocol. DNAase treatment was performed within the RNA extraction procedures according to the manufacturers' instructions (ThermoFisher Scientific, USA). RNA concentration and quality were confirmed using a NanoDrop 2000 (ThermoFisher Scientific, USA) and electrophoresis using a 1% agarose gel under denaturing conditions. The RNA integrity was assessed based on clear 28S and 18S ribosomal RNA bands on a 1% agarose gel using electrophoresis. First strand cDNA for each sample was synthesized from 150ng total RNA using the Thermo Scientific RevertAid First Strand cDNA Synthesis kit following the manufacturers' protocol (ThermoFisher Scientific, USA).

### Quantification of GFP expression

Expression levels of GFP proteins were assessed in coral larvae using quantitative real-time polymerase chain reaction (qRT-PCR). GFP primers (5'-GAAATGAGCCTCAAAGGCAAC-3' forward and 5'-GTTGGTTCAGCCAAGA-3' reverse) were selected according to Grinblat et al. (2018), as well as the two internal control genes primers, b-actin and adenosylhomocysteinase (AdoHcyase).

Each reaction contained 2X SYBR Green PCR Master Mix (ThermoFisher Scientific, USA), 0.2  $\mu$ M specific primers and cDNA of each sample. Amplification reactions were carried out with an initial denaturation step of 95 °C, followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s, a dissociation step of 95 °C for 15 s, annealing step at 60 °C for 1 min and then melting back to 95 °C over 20 min. (StepOnePlus™ Real-Time PCR System, Applied Biosystems, USA).

### *Symbiodinium* ITS2 analyses

Eight larvae from the shallow and eight larvae from the mesophotic reef were pooled together in four different subsamples (two per depth range). Genomic DNA was extracted from the four subsamples using the Wizard genomic DNA purification kit (Promega Corporation, USA) according to the manufacturers' protocol. The internal transcribed spacer (ITS2) region of *Symbiodinium* rDNA was amplified using *Symbiodinium*-specific primers

taken from Arif et al. (2014). The amplified ITS2 fragments were separated with electrophoresis using a 1% agarose gel under denaturing conditions. Ten  $\mu$ L of the above PCR products were sent to HyLab (Hy Laboratories Ltd., Israel) where they were subjected to a second PCR using the Access Array tag for Illumina primers (Fluidigm Corporation, USA). This second PCR added the Index and adaptor sequences required for sequencing on the Illumina system. The samples were then purified using AMPure XP beads (Beckman Coulter Inc., USA) and the concentration was determined by Qubit (ThermoFisher Scientific, USA). The samples were pooled together and sequenced on the Illumina Miseq using a v2-500 cycle kit to generate 250x2, paired-end reads. The data was de-multiplexed by the Illumina software, and the de-multiplexed FASTQ files were further analyzed. The resulting OTUs sequences were aligned with ClustalX (Larkin et al., 2007) and blasted on GenBank (<http://www.ncbi.nlm.nih.gov/BLAST>).

### In-situ imaging system

In-situ adult corals were imaged during the day when the polyps were opened by modifying the wide band fluorescence imaging system, FluorIS (Treibitz et al., 2015) for fluorescence imaging in-situ. The systems consist of a Nikon D850 camera with a Nikon 35 mm 1.8 lens, housed within a Nauticam housing. In the FluorIS system, the camera was modified by replacing the IR filter over its sensor with a clear filter that transmits the entire light spectrum; the sensor is sensitive thoroughly 300–1200 nm. A custom-made 25\*25 cm quadrat was used to enable rapid imaging of the same area. Using the above methodology, the fluorescence images were captured using the FluorIS as previously described (Treibitz et al., 2015; Zweifler et al., 2017). Ambient light ( $I_{\text{ambient}}$ ) was subtracted from the daytime fluorescence ( $I_{\text{day}}$ ) images in MATLAB in order to receive a fluorescence image similar to nighttime fluorescence image ( $F_{\text{strokes}}$ ) using the equation  $F_{\text{strokes}} = I_{\text{day}} - I_{\text{ambient}}$ . Mean GFP and chlorophyll fluorescence intensities were determined in ImageJ (Schneider et al., 2012) delimiting and selecting the area of the images covered by the colonies and calculating the mean gray value, which was estimated separately for the green and the red channel. The average intensities ( $N = 3$ ) were calculated and expressed as percentage change  $\pm$  relative standard error.

### Fluorescence microscopy and image analyses

Coral planulae were imaged while not being retracted using an Inverted Phase Contrast Fluorescent Microscope (Nikon Eclipse Ti, Melville, NY, USA). Each sample was observed with a DS-Ri2 camera using two single fluorescence channels, red (emission 590 nm) and green (emission 515 nm), that were additionally merged together. Exposure and gain settings per each channel were kept constant between shallow and deep larvae (150ms exposure and 1.1 gain for the red channel, 20ms exposure and 1 gain for the green channel). All images were acquired with the Nikon Nis-Elements software (Nikon Instruments, Melville, NY, USA). Mean GFP and chlorophyll fluorescence intensities were determined using the mean gray value in ImageJ (Schneider et al., 2012), delimiting and selecting the area within the larvae edges. The average intensities ( $N = 3$ ) were calculated and expressed as percentage change  $\pm$  relative standard error.

## Data analysis

Univariate comparisons of qPCR data relative to the GFP expression were performed using the non-parametric one-way ANOVA (Kruskal-Wallis test), followed by the Mann-Whitney U-test ( $P < 0.05$ ), after verifying the deviations from parametric ANOVA assumptions (Normality: Shapiro-Wilk's test; equal variance: Bartlett's test and Brown-Forsythe test). The GraphPad Prism 8 software (GraphPad Inc.) was used to perform all the statistical tests and to create the graphs. Data are presented as means (fold changes)  $\pm$  standard errors.

Similarities between GFPs gene sequences were determined using the percent identity matrix calculated after the alignment with ClustalX. The alignment figure was created using Jalview 2.10.5 (Waterhouse et al., 2009).

*Symbiodinium* OTUs resulted from the ITS2 analyses were aligned using ClustalX and the percent identity matrix was used to determine similarities between OTUs of the same clade.

## Results

### Larval size image analyses

Larvae were collected from 45 different colonies at each depth during 3 nights near the full moon of April 2019. Forty colonies spawned at the shallow water while only nine colonies spawned at the deep water during these nights, suggesting a delayed spawning peak at the mesophotic water. A total of 600 and 100 larvae were collected from the shallow and deep-water colonies respectively.

The mean larval volume of the shallow larvae was found to be significantly higher compared to the deep larvae by approximately 2-fold (Fig. 2,  $p < 0.0001$ ). Light intensity, measured with the HOBO Water Temp Pro data loggers, ranged from 6200 to 228 lux at 6 m and 45 m respectively.

### *Symbiodinium* clade analyses

A total of 119,399 ITS2 high quality sequence reads (mean length = 320 bp) were obtained from 4 total samples (two samples per depth range), each containing 4 coral larvae. A large proportion of the filtered OTUs were non -*Symbiodinium* organisms that were prevalent mostly in the water and as part of the coral holobiome. After the removal of non -*Symbiodinium* sequences and of singletons (OUTs with 1 sequence read) only *Symbiodinium* OTUs remained and were clustered into clade A and clade C, at 97% similarity (sequences were blasted on GenBank, <http://www.ncbi.nlm.nih.gov/BLAST>).

The deep larvae contained symbiont consortia dominated by *Symbiodinium* clade C (99.8 %) with a small percentage representing *Symbiodinium* clade A (0.2 %). In contrast, the symbiont consortia present in shallow larvae was dominated by *Symbiodinium* clade A (99.9 %) with a small percentage of *Symbiodinium* clade C (0.1 %) present.

### Fluorescence in-situ and microscopy analysis

Coral fluorescence of both GFP and chlorophyll-a were visibly different between adult *S. pistillata* colonies from 6 m (Fig. 3 A-C) and those from 45 m (Fig. 3 D-E). GFP



fluorescence intensity was on average  $65.4 \% \pm 6.6$  (N = 3) lower for the mesophotic adult corals compared to the shallow adult corals (Fig. 3 B, E), while *Symbiodinium* chlorophyll intensity was on average  $54.6 \% \pm 8.4$  (N = 3) lower for the shallow corals compared to the deep corals (Fig. 3 C, F). In addition, the GFP fluorescence appeared evenly distributed along the length of all the branches in the coral at 6 m (Fig. 3 B inset), but it was more intense at the branch tips of the coral at 45 m (Fig. 3 E inset). A similar pattern of GFP and chlorophyll fluorescence was also found for shallow and mesophotic larvae as to the adult colonies at the same depth, with an average  $54.5 \% \pm 7.9$  (N = 3) lower GFP fluorescence in the deep larvae compared to the shallow larvae (Fig. 4 B, E), and an average  $27.7 \% \pm 2.3$  (N = 3) lower chlorophyll intensity in the shallow larvae compared to the deep larvae (Fig. 4, F). Furthermore, the distribution of GFP within the larvae was also similar to the adults, where shallow larvae displayed a uniform fluorescence pattern and mesophotic larvae had higher GFP intensity around the mouth and at the aboral epidermis (Fig. 4 A, D).

### Quantification of GFP expression

Specific expression of the *S. pistillata* GFP-like chromoprotein (Accession number: DQ206398.1; Grinblat et al., 2018) was evaluated in *S. pistillata* larvae from the shallow and mesophotic reef through comparisons of mRNA expression and was found to be significantly lower for mesophotic larvae (0.8-fold down-regulation) compared to shallow larvae (Fig. 5,  $p < 0,005$ ).

The expression pattern of the GFP-like chromoprotein observed in this study corresponds with the expression patterns analyzed for several GFP transcripts in adult colonies of *S. pistillata* from different depths (5 m, 10 m, 20 m, 30 m, 50 m and 60 m) in the Gulf of Eilat (Malik et al., under review) (Fig. S1, Supplemental material). In particular, a correspondence was found between the GFP-like chromoprotein gene analyzed for the larvae and the GFP2 gene examined in the adults (95 % identity) (Fig. 6).

### Discussion

Resilience of coral reefs under rapid environmental change heavily relies on larval survival and effective settlement, thus it is crucial to thoroughly comprehend coral larval physiological adaptation to environmental features. Many coral species have been shown to differ in morphology and physiology along a broad depth gradient, and these modifications have often been indicated as the result of phenotypic plasticity in response to the variation of environmental conditions with depth (Bruno and Edmunds, 1997; Einbinder et al., 2009; Goodbody-Gringley and Waletich, 2018). In this study, we observed clear patterns of depth-related physiological characteristics in *S. pistillata* larvae from the shallow and the mesophotic reef, particularly looking at the differences in larval size, in the association with different symbiont assemblages and in the GFP expression and fluorescence.

We found that the shallow larvae are significantly larger than the deep ones (Fig. 2), and we attributed this difference to the constraints of the light environment in the parental habitat. In fact, it has been previously shown that coral larvae deriving from high light adapted parents were larger than larvae belonging to low light adapted adults (Roth et al., 2013). Specifically, higher light availability in shallow reef conditions may result in higher

energetic reserves, enabling larvae from the shallow reef to obtain a larger size compared to deep larvae, which might not be able to support a similar size range given the limited light energy available on the mesophotic reef (Kahng et al., 2019; Lesser et al., 2018).

Although energetic content of the larvae was not addressed in this study, comparisons between parental depth and the amount of maternally derived lipids within the resulting larvae that are used as endogenous source of energy may further elucidate the role of light availability on larval size (Rivest et al., 2017; Woodley et al., 2015). Differences in the density of Symbiodinaceae cells and chlorophyll concentrations within larvae can also affect the amount of translocated metabolites from the symbiotic algae, which can act as a significant sources of energy for coral larvae (Hariri et al., 2010; Richmond, 1987).

Previous studies have shown that deep and low light adapted corals contain more chlorophyll per *Symbiodinium* sp. than those adapted to high light environments (Cohen and Dubinsky, 2015; Falkowski and Dubinsky, 1981; Smith et al., 2017, Mass et al., 2007). Thus, the greater concentration of photosynthetic pigments observed in this study in the adults (Fig. 3 F) and in the deep larvae via differential fluorescence (Fig. 4 F), may be attributed to the maximization of the algae light harvesting capacity through increased concentration of photosynthetic pigments within the symbionts, as formerly reported in *S. pistillata* by Titlyanov et al. (2001), representing a habitat-specific adaptation to light-limited mesophotic depths (Winters et al., 2009).

The differential patterns of representation by the two *Symbiodinium* clades detected in this study in the larvae are similar to the ones found in adult *S. pistillata* colonies by Malik et al. (under review), where shallow colonies harbored predominantly clade A, and deep colonies harbored predominantly clade C.

Roughly 90% of brooding scleractinian species vertically transmit *Symbiodinium*, one of these being *S. pistillata* (Baird et al., 2009) and, within these species, if the same parent or different parents host different symbionts, the progeny can inherit all or none of the parental symbionts (Byler et al., 2013; Reich et al., 2017). Given the similarity in *Symbiodinium* consortia between *S. pistillata* adult colonies (Malik et al., under review) and the larvae, this study provides evidence to further support vertical acquisition of both primary and secondary symbiont clades, as previously hypothesized for *S. pistillata* (Byler et al., 2013) and for other scleractinian species (Van Oppen, 2004; Reich et al., 2017). However, horizontal acquisition during development may also occur, where *S. pistillata* might be able to attract and acquire specific symbiont clades from the environment during larval and juvenile stages (Reich et al., 2017).

The ability of individual juveniles to gain and maintain novel symbionts through horizontal acquisition might provide a crucial mechanism to increase the chances of survival in a new environment during dispersal at the larval stage (Byler et al., 2013) and, at the same time, the inheritance of symbionts from the parents would assure the supply of photosynthetic products during the delicate initial larval stages. Previous studies have shown that different clades of *Symbiodinium* have differential preferences for the light intensity required for photosynthesis and that they are differently affected by the host internal light environment



variation, which can be achieved through the modulation of the GFPs fluorescence intensity (Aihara et al., 2018; Ezzat et al., 2017; Quigley et al., 2018). Since the light intensity necessary to attract specific symbionts likely differs with light spectra and depth (Aihara et al., 2018), coral larvae from shallow and deep reefs potentially generate diverse internal light environments, displaying different GFP fluorescence depending on the preferred association with different *Symbiodinium* clades (Yuyama and Higuchi, 2014). Thus, the different intensities of GFP fluorescence detected in this study between shallow and mesophotic larvae (Fig. 4 B, E) suggests that host-modulated fluorescence may represent a mechanism to attract specific symbiont types with unique physiologies. Moreover, the concentrated GFPs fluorescence around the mouth could further denote the ability of the larvae to attract and capture free-living *Symbiodinium sp.* via consumption, as suggested by Hollingsworth et al. (2005).

The depth ranges chosen for this study (6 and 45 m) represent two distinct habitats where corals endure either high intensity or limited light levels. Corals possess specific physiological characteristics to cope with such varying light environments, one of these being the expression of the GFP proteins, which has been indicated as a photoprotective mechanism in shallow water corals (Salih et al., 2000, 2006) and as a way to increase light availability for the symbiotic algae in deep water corals (Dove et al., 2001). In this study the higher GFP expression levels (Fig. 5) and the higher intensity of GFP fluorescence found in the shallow larvae (Fig. 4 B) is consistent with the role of these proteins as UVR screeners. In fact, coral larvae are particularly susceptible to UVR (Aranda et al., 2011) and they receive a higher amount of solar radiation in shallow-water areas compared to the mesophotic reef, therefore mechanisms to dissipate high-energy light wavelengths are crucial to provide photoprotection to both the coral and the symbiotic algae.

In the adult colonies, we observed a higher fluorescence intensity in the shallow reef (Fig. 3 B) compared to the mesophotic reef (Fig. 3 E); furthermore, the majority of the examined GFP transcripts showed a significant down-regulation with depth, with a marked differential expression between 10 meters and 20-60 meters depth (Fig. S1), in accordance with the photoprotective role of the GFP proteins (Salih et al., 2000) and the upregulation of oxidative stress response and DNA-repair genes, which correlate with higher photosynthesis activity of the symbiont in shallow water (Malik et al., under review).

Previous studies have shown that GFPs have a robust photo-acclimation response, modulating the coral internal light environment and potentially influencing the physiology of the symbiotic algae and of the coral itself (Lyndby et al., 2016; Roth et al., 2010). Results of the present study support the light screening model whereby GFPs in shallow-water corals protect against the damages caused by elevated UVR levels, but it can also disclose the possible role of these proteins in deep-water corals. For example, this study found that GFPs are still being expressed under non-damaging low light conditions (Fig. 5) and that certain GFPs are more highly expressed in the deep reef compared to the shallow (GFP5, Fig. S1). Additionally, some GFPs were found to be upregulated at deeper depths (60 meters, Fig. S1) relative to shallower areas (20 and 30 meters, Fig. S1). Taken together, these results suggest that these proteins have different roles depending on the depth (Ben-Zvi et al., 2015). In particular, in deeper waters GFPs might act to regulate the amount of

photosynthetically available light, creating an optimal environment for photosynthesis (Dove et al., 2001; Quigley et al., 2018). Specifically, GFPs in the mesophotic corals could be actively regulating of the amount of photosynthetically available light through transformation of blue-green wavelengths into longer wavelengths via fluorescence in the coral tissue, thereby enhancing photosynthetic performance of the symbionts (Quigley et al., 2018; Salih et al., 2006; Smith et al., 2017). Such modifications of internal wavelengths would alter the light spectrum and increase internal light intensity surrounding the symbiotic dinoflagellate cells in order to overcome limited light availability on mesophotic reefs. This mechanism, together with the differential preferences for light intensity required for photosynthesis of different *Symbiodinium* clades, might explain the relative differences in GFP expression and in the hosted *Symbiodinium* clades observed in this study between the shallow and the deep reef in *S. pistillata* larvae and adults.

## Conclusions

The data presented here highlight that depth-related parameters, specifically light, play a major role in the photo-physiology of corals and their associated *Symbiodinium*, and corroborate the notion that GFPs act as UVR screeners in high light environments, as supported by the higher GFP expression and the greater fluorescence intensity observed in *S. pistillata* shallow larvae, compared to the deep ones. Moreover, the lower but still occurring GFP expression under non-damaging low light conditions underlines another possible function of these proteins, such that they might act to regulate the amount of photosynthetically available light in order to optimize the internal light field for the benefit of the symbiotic algae.

Additionally, the observed differences in the GFP expression and the green fluorescence between the shallow and deep larvae, suggests that GFPs within coral larvae may act to attract and retain different symbionts clades with specific physiologies, as well as enhance photosynthesis through the creation of divergent internal light environments, increasing the chances of survival when encountering new environments. This work potentially provides the bases for future studies investigating the functional significance of the association between coral GFPs fluorescence and the symbiont assemblages, especially focusing on a crucial life stage for corals, such as the larval phase.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We thank the technical staff of the Moris Kahn Marine Research Station for their invaluable help. We thank Alex Chequer, Shai Eindinder, and Stephan Martinez for their assistance with technical diving field work. We thank the technical staff of the Interuniversity Institute of Marine Sciences for the invaluable help with the field study. The study was performed in accordance with regulations and guidelines set by the Israel Nature and National Park Protection Authority. This work was supported by the Israel Science Foundation (Grant 312/15), United States-Israel Binational Science Foundation (BSF; Grant # 2016321), the European Research Council (ERC; Grant # 755876) and the ASSEMBLE Plus consortium for an access grant (ref. SR16022018108e1) to the Inter-University Institute for Marine Sciences in Eilat.

### Funding statement

*Front Mar. Sci.* Author manuscript; available in PMC 2020 January 28.

This work was supported by the Israel Science Foundation (Grant 312/15), United States-Israel Binational Science Foundation (BSF; Grant # 2016321), the European Research Council (ERC; Grant # 755876) and the ASSEMBLE Plus consortium for an access grant (ref. SR16022018108e1) to the Inter-University Institute for Marine Sciences in Eilat.

## Data availability statement

Generated Statement: This manuscript contains previously unpublished data. The name of the repository and accession number(s) are not available.

## References

- Aihara Y, Maruyama S, Baird AH, Iguchi A, Takahashi S, Minagawa J. Green fluorescence from cnidarian hosts attracts symbiotic algae. *Proceedings of the National Academy of Sciences*. 2019; 116(6):2118–2123. DOI: 10.1073/pnas.1812257116
- Allemant D, Furla P. How does an animal behave like a plant? Physiological and molecular adaptations of zooxanthellae and their hosts to symbiosis. *Comptes rendus biologiques*. 2018; 341(5):276–280. DOI: 10.1016/j.crv.2018.03.007 [PubMed: 29650460]
- Anders S. Differential gene expression analysis based on the negative binomial distribution. *Journal of Marine Technology & Environment*. 2009; 2
- Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2015; 31(2):166–169. DOI: 10.1093/bioinformatics/btu638 [PubMed: 25260700]
- Aranda M, Banaszak AT, Bayer T, Luyten JR, Medina M, Voolstra CR. Differential sensitivity of coral larvae to natural levels of ultraviolet radiation during the onset of larval competence. *Molecular ecology*. 2011; 20(14):2955–2972. DOI: 10.1111/j.1365-294x.2011.05153.x [PubMed: 21689186]
- Arif C, Daniels C, Bayer T, Banguera-Hinestroza E, Barbrook A, Howe CJ, Voolstra CR, et al. Assessing *Symbiodinium* diversity in scleractinian corals via next-generation sequencing-based genotyping of the ITS2 rDNA region. *Molecular ecology*. 2014; 23(17):4418–4433. DOI: 10.1111/mec.12869 [PubMed: 25052021]
- Armstrong RA, Singh H, Torres J, Nemeth RS, Can A, Roman C, Garcia-Moliner G, et al. Characterizing the deep insular shelf coral reef habitat of the Hind Bank marine conservation district (US Virgin Islands) using the Seabed autonomous underwater vehicle. *Continental Shelf Research*. 2006; 26(2):194–205. DOI: 10.1016/j.csr.2005.10.004
- Baird AH, Guest JR, Willis BL. Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. *Annual Review of Ecology, Evolution, and Systematics*. 2009; 40:551–571. DOI: 10.1146/annurev.ecolsys.110308.120220
- Ben-Zvi O, Eyal G, Loya Y. Light-dependent fluorescence in the coral *Galaxea fascicularis*. *Hydrobiologia*. 2015; 759(1):15–26. DOI: 10.1007/s10750-014-2063-6
- Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O. Assessing the ‘deep reef refugia’ hypothesis: focus on Caribbean reefs. *Coral reefs*. 2010; 29(2):309–327. DOI: 10.1007/s00338-009-0581-x
- Bongaerts P, Riginos C, Hay KB, van Oppen MJ, Hoegh-Guldberg O, Dove S. Adaptive divergence in a scleractinian coral: physiological adaptation of *Seriatopora hystrix* to shallow and deep reef habitats. *BMC evolutionary biology*. 2011; 11(1):303. doi: 10.1186/1471-2148-11-303 [PubMed: 22004364]
- Bou-Abdallah F, Chasteen ND, Lesser MP. Quenching of superoxide radicals by green fluorescent protein. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 2006; 1760(11):1690–1695. DOI: 10.1016/j.bbagen.2006.08.014 [PubMed: 17023114]
- Bruno JF, Edmunds PJ. Clonal variation for phenotypic plasticity in the coral *Madracis mirabilis*. *Ecology*. 1997; 78(7):2177–2190. DOI: 10.2307/2265954
- Byler KA, Carmi-Veal M, Fine M, Goulet TL. Multiple symbiont acquisition strategies as an adaptive mechanism in the coral *Stylophora pistillata*. *PLoS One*. 2013; 8(3):e59596. doi: 10.1371/journal.pone.0059596 [PubMed: 23555721]

- Cohen I, Dubinsky Z. Long term photoacclimation responses of the coral *Stylophora pistillata* to reciprocal deep to shallow transplantation: photosynthesis and calcification. *Frontiers in Marine Science*. 2015; 2:45.doi: 10.3389/fmars.2015.00045
- Davy SK, Allemand D, Weis VM. Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiology and Molecular Biology Reviews*. 2012; 76(2):229–261. DOI: 10.1128/mmbr.05014-11 [PubMed: 22688813]
- Dove SG, Hoegh-Guldberg O, Ranganathan S. Major colour patterns of reef-building corals are due to a family of GFP-like proteins. *Coral reefs*. 2001; 19(3):197–204. DOI: 10.1007/pl00006956
- Einbinder S, Mass T, Brokovich E, Dubinsky Z, Erez J, Tchernov D. Changes in morphology and diet of the coral *Stylophora pistillata* along a depth gradient. *Marine Ecology Progress Series*. 2009; 381:167–174. DOI: 10.3354/meps07908
- Ezzat L, Fine M, Maguer JF, Grover R, Ferrier-Pagès C. Carbon and nitrogen acquisition in shallow and deep holobionts of the scleractinian coral *S. pistillata*. *Frontiers in Marine Science*. 2017; 4:102.doi: 10.3389/fmars.2017.00102
- Falkowski PG, Dubinsky Z. Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. *Nature*. 1981; 289(5794):172.doi: 10.1038/289172a0
- Fox J, Weisberg S, Friendly M, Hong J, Andersen R, Firth D, Taylor S, Fox MJ. Package ‘effects’. 2019
- Goodbody-Gringley G, Waletich J. Morphological plasticity of the depth generalist coral, *Montastraea cavernosa*, on mesophotic reefs in Bermuda. *Ecology*. 2018; 99(7):1688–1690. DOI: 10.1002/ecy.2232 [PubMed: 29608800]
- Grinblat M, Fine M, Tikochinski Y, Loya Y. *Stylophora pistillata* in the Red Sea demonstrate higher GFP fluorescence under ocean acidification conditions. *Coral Reefs*. 2018; 37(1):309–320. DOI: 10.1007/s00338-018-1659-0
- Harii S, Yamamoto M, Hoegh-Guldberg O. The relative contribution of dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of the reef-building corals. *Marine Biology*. 2010; 157(6):1215–1224. DOI: 10.1007/s00227-010-1401-0
- Hinderstein LM, Marr JCA, Martinez FA, Dowgiallo MJ, Puglise KA, Pyle RL, Zawada DG, Appeldoorn R. Theme section on “Mesophotic coral ecosystems: characterization, ecology, and management”. 2010; doi: 10.1007/s00338-010-0614-5
- Hoegh-Guldberg O. Climate change, coral bleaching and the future of the world's coral reefs. *Marine and freshwater research*. 1999; 50(8):839–866. DOI: 10.1071/MF99078
- Hollingsworth LL, Kinzie RA, Lewis TD, Krupp DA, Leong JAC. Phototaxis of motile zooxanthellae to green light may facilitate symbiont capture by coral larvae. *Coral Reefs*. 2005; 24(4):523–523. DOI: 10.1007/s00338-005-0063-8
- Isomura N, Nishihira M. Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. *Coral Reefs*. 2001; 20(3):309–315. DOI: 10.1007/s003380100180
- Kahng, SE, Akkaynak, D, Shlesinger, T, Hochberg, EJ, Wiedenmann, J, Tamir, R, Tchernov, D. Light, temperature, photosynthesis, heterotrophy, and the lower depth limits of mesophotic coral ecosystems. *Mesophotic Coral Ecosystems*. Springer; Cham: 2019. 801–828.
- Kawaguti S. Effect of the green fluorescent pigment on the productivity of the reef corals. *Micronesica*. 1969; 5(313):121.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Thompson JD. Clustal W and Clustal X version 2.0. *bioinformatics*. 2007; 23(21):2947–2948. DOI: 10.1093/bioinformatics/btm404 [PubMed: 17846036]
- Lesser MP, Slattery M, Mobley CD. Biodiversity and functional ecology of mesophotic coral reefs. *Annual Review of Ecology, Evolution, and Systematics*. 2018; 49:49–71. DOI: 10.1146/annurev-ecolsys-110617-062423
- Loya Y. Community structure and species diversity of hermatypic corals at Eilat, Red Sea. *Marine Biology*. 1972; 13(2):100–123. DOI: 10.1007/bf00366561
- Lyndby NH, Kühl M, Wangpraseurt D. Heat generation and light scattering of green fluorescent protein-like pigments in coral tissue. *Scientific reports*. 2016; 6doi: 10.1038/srep26599

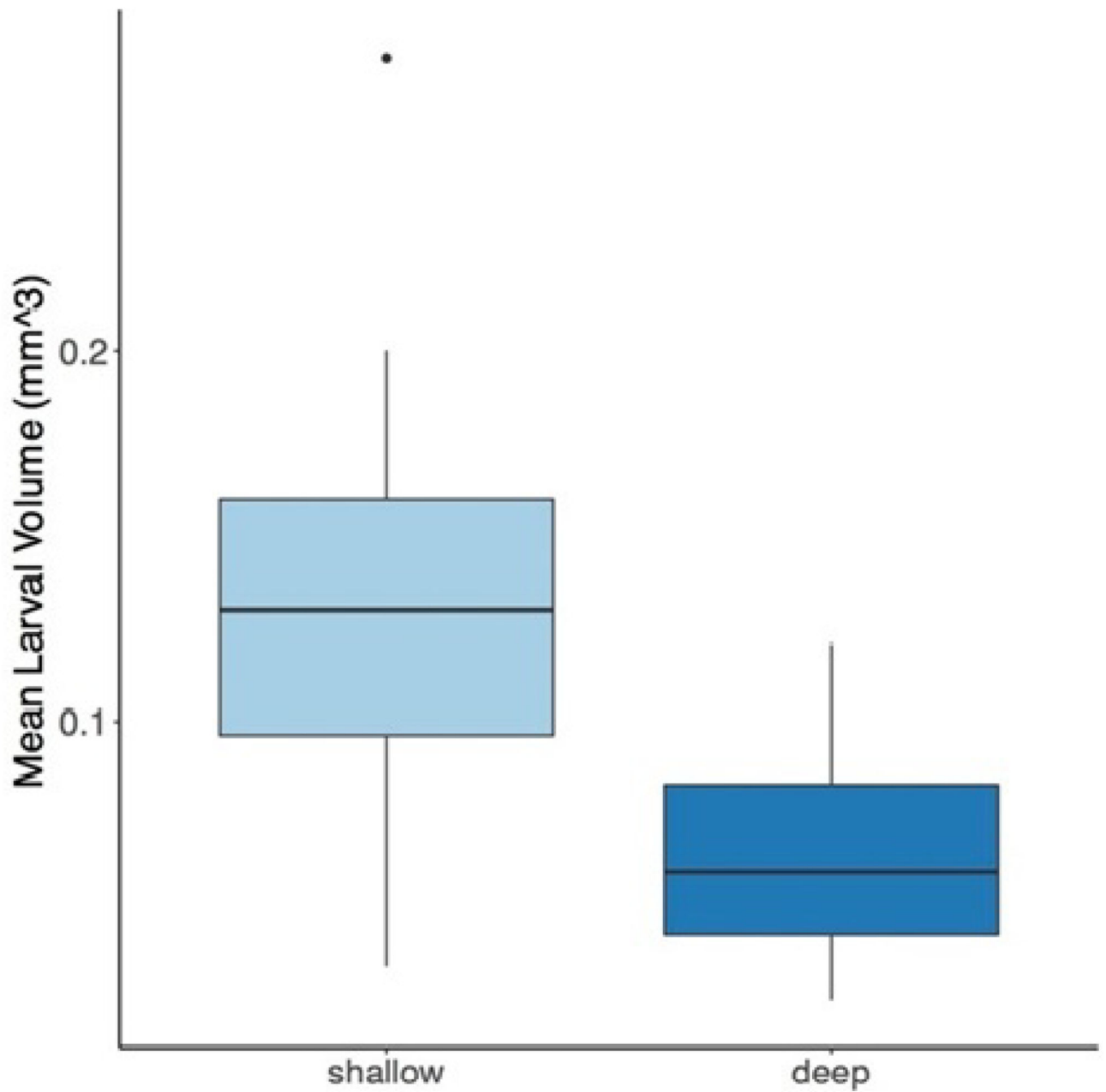
- Mass T, Einbinder S, Brokovich E, Shashar N, Vago R, Erez J, Dubinsky Z. Photoacclimation of *Stylophora pistillata* to light extremes: metabolism and calcification. *Marine Ecology Progress Series*. 2007; 334:93–102. DOI: 10.3354/meps334093
- Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet journal*. 2011; 17(1):10–12. DOI: 10.14806/ej.17.1.200
- Matz MV, Marshall NJ, Vorobyev M. Symposium-in-Print: Green fluorescent protein and homologs—Are corals colorful. *Photochem Photobiol*. 2006; 82:345–350. [PubMed: 16613484]
- Nir O, Gruber DF, Einbinder S, Kark S, Tchernov D. Changes in scleractinian coral *Seriatopora hystrix* morphology and its endocellular *Symbiodinium* characteristics along a bathymetric gradient from shallow to mesophotic reef. *Coral Reefs*. 2011; 30(4):1089. doi: 10.1007/s00338-011-0801-z
- Overmans S, Nordborg M, Díaz-Rúa R, Brinkman DL, Negri AP, Agustí S. Phototoxic effects of PAH and UVA exposure on molecular responses and developmental success in coral larvae. *Aquatic toxicology*. 2018; 198:165–174. DOI: 10.1016/j.aquatox.2018.03.008 [PubMed: 29550714]
- Palmer CV, Roth MS, Gates RD. Red fluorescent protein responsible for pigmentation in trematode-infected *Porites compressa* tissues. *The Biological Bulletin*. 2009; 216(1):68–74. DOI: 10.1086/bblv216n1p68 [PubMed: 19218493]
- Quigley KM, Strader ME, Matz MV. Relationship between *Acropora millepora* juvenile fluorescence and composition of newly established *Symbiodinium* assemblage. *PeerJ*. 2018; 6:e5022. doi: 10.1101/271155 [PubMed: 29922515]
- Reich HG, Robertson DL, Goodbody-Gringley G. Do the shuffle: changes in *Symbiodinium* consortia throughout juvenile coral development. *PLoS one*. 2017; 12(2):e0171768. doi: 10.1371/journal.pone.0171768 [PubMed: 28182684]
- Richmond RH. Energetics, competency, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. *Marine Biology*. 1987; 93(4):527–533. DOI: 10.1007/bf00392790
- Rivest EB, Chen CS, Fan TY, Li HH, Hofmann GE. Lipid consumption in coral larvae differs among sites: a consideration of environmental history in a global ocean change scenario. *Proceedings of the Royal Society B: Biological Sciences*. 2017; 284(1853)doi: 10.1098/rspb.2016.2825
- Roth MS, Deheyn DD. Effects of cold stress and heat stress on coral fluorescence in reef-building corals. *Scientific reports*. 2013; 3doi: 10.1038/srep01421
- Roth MS, Fan TY, Deheyn DD. Life history changes in coral fluorescence and the effects of light intensity on larval physiology and settlement in *Seriatopora hystrix*. *PLoS One*. 2013; 8(3):e59476. doi: 10.1371/journal.pone.0059476 [PubMed: 23544072]
- Salih, A; Cox, G; Szymczak, R; Coles, SL; Baird, AH; Dunstan, A; Cocco, G; Mills, J; Larkum, A. The role of host-based color and fluorescent pigments in photoprotection and in reducing bleaching stress in corals. *Proc 10th Int Coral Reef Symp*; 2006. 746–756.
- Salih A, Larkum A, Cox G, Kühl M, Hoegh-Guldberg O. Fluorescent pigments in corals are photoprotective. *Nature*. 2000; 408(6814):850. doi: 10.1038/35048564 [PubMed: 11130722]
- Schlichter D, Fricke HW. Coral host improves photosynthesis of endosymbiotic algae. *Naturwissenschaften*. 1990; 77(9):447–450. DOI: 10.1007/bf01135950
- Shlesinger Y, Goulet TL, Loya Y. Reproductive patterns of scleractinian corals in the northern Red Sea. *Marine Biology*. 1998; 132(4):691–701. DOI: 10.1007/s002270050433
- Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nature methods*. 2012; 9(7):671. doi: 10.1038/nmeth.2089 [PubMed: 22930834]
- Smith EG, D'angelo C, Sharon Y, Tchernov D, Wiedenmann J. Acclimatization of symbiotic corals to mesophotic light environments through wavelength transformation by fluorescent protein pigments. *Proceedings of the Royal Society B: Biological Sciences*. 2017; 284(1858)doi: 10.1098/rspb.2017.0320
- Titlyanov EA, Titlyanova TV, Yamazato K, Van Woesik R. Photo-acclimation dynamics of the coral *Stylophora pistillata* to low and extremely low light. *Journal of Experimental Marine Biology and Ecology*. 2001; 263(2):211–225. DOI: 10.1016/s0022-0981(01)00309-4
- Treibitz T, Neal BP, Kline DI, Beijbom O, Roberts PL, Mitchell BG, Kriegman D. Wide field-of-view fluorescence imaging of coral reefs. *Scientific reports*. 2015; 5doi: 10.1038/srep07694
- Tsien RY. The green fluorescent protein. *Annu Rev Biochem*. 1998; 67:509–544. [PubMed: 9759496]

- Turner JA, Thomson DP, Cresswell AK, Trapon M, Babcock RC. Depth-related patterns in coral recruitment across a shallow to mesophotic gradient. *Coral Reefs*. 2018; 37(3):711–722. DOI: 10.1007/s00338-018-1696-8
- Van Oppen MJ. Mode of zooxanthella transmission does not affect zooxanthella diversity in acroporid corals. *Marine Biology*. 2004; 144(1):1–7. DOI: 10.1007/s00227-003-1187-4
- Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics*. 2009; 25(9):1189–1191. DOI: 10.1093/bioinformatics/btp033 [PubMed: 19151095]
- Winters G, Beer S, Zvi BB, Brickner I, Loya Y. Spatial and temporal photoacclimation of *Stylophora pistillata*: zooxanthella size, pigmentation, location and clade. *Marine Ecology Progress Series*. 2009; 384:107–119. DOI: 10.3354/meps08036
- Woodley, CM, Downs, C, Bruckner, AW, Porter, JW, Galloway, SB, editors. *Diseases of coral*. Hoboken, NJ: John Wiley & Sons; 2015.
- Yuyama I, Higuchi T. Comparing the effects of symbiotic algae (*Symbiodinium*) clades C1 and D on early growth stages of *Acropora tenuis*. *PLoS One*. 2014; 9(6):e98999. doi: 10.1371/journal.pone.0098999 [PubMed: 24914677]
- Zweifler A, Akkaynak D, Mass T, Treibitz T. In situ Analysis of Coral Recruits Using Fluorescence Imaging. *Frontiers in Marine Science*. 2017; 4:273. doi: 10.3389/fmars.2017.00273

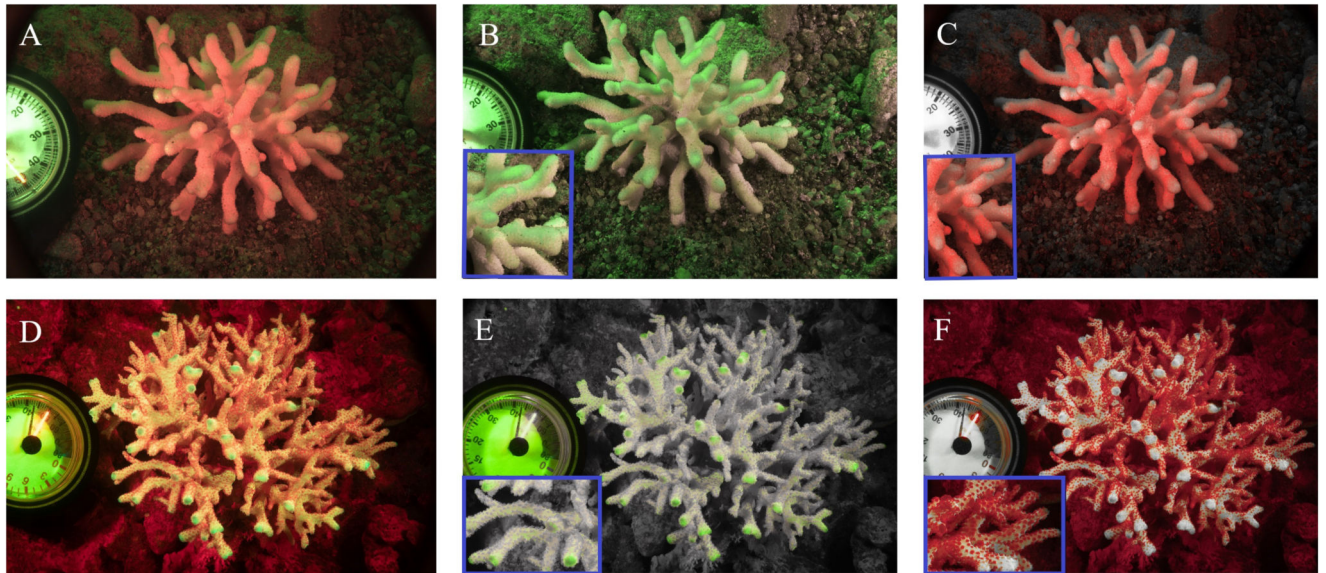




**Figure 1.**  
Images of larval collection traps on adult *Stylophora pistillata* colonies in the Red Sea at 3-6 (left) and 40-45 m (right) depths.

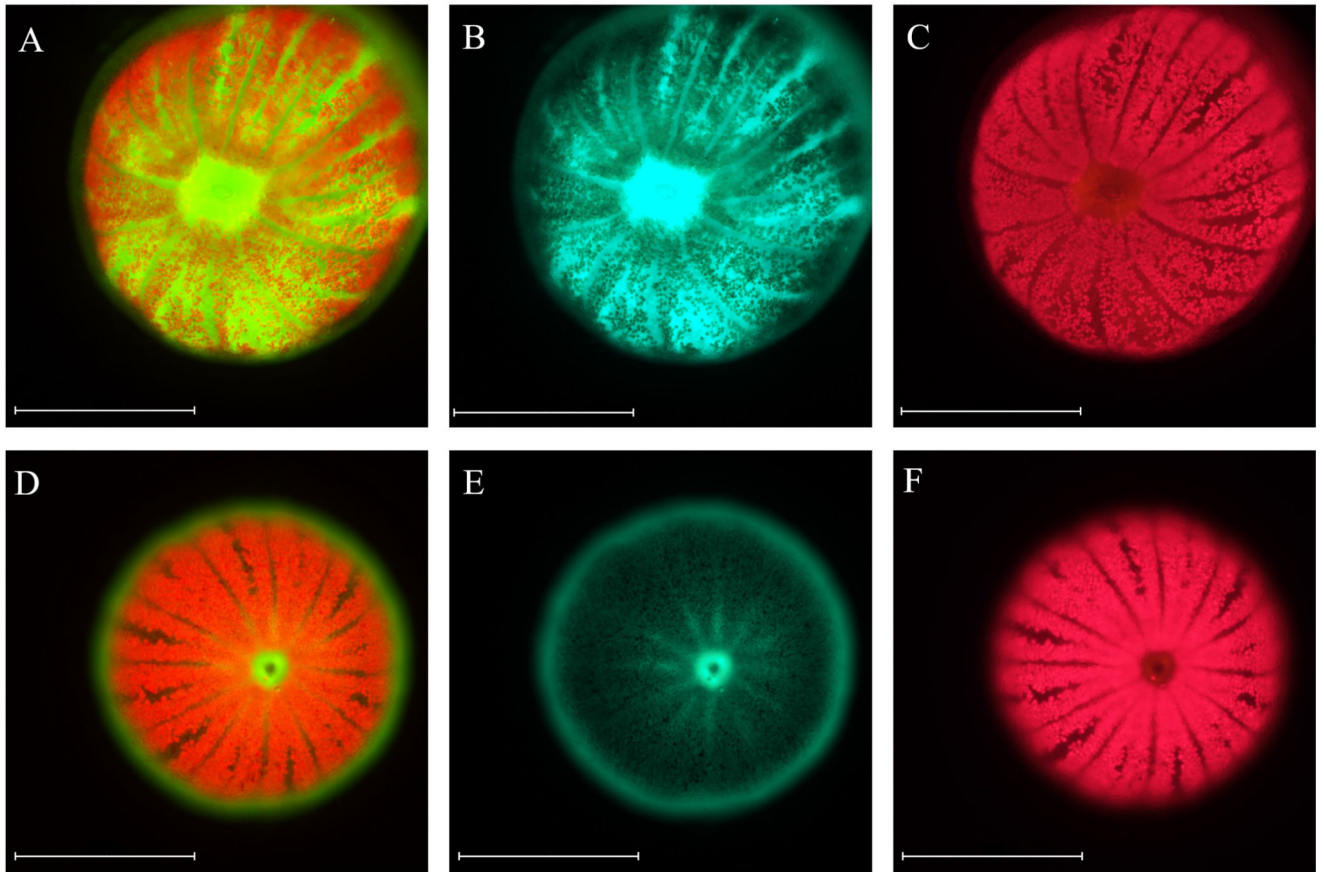


**Figure 2.** Mean larval volume of *Stylophora pistillata* larvae originating from shallow (3-6 m) and deep (40-45 m) colonies. Box-and-whisker plots represent median, upper and lower quartiles (n = 36 shallow; n = 32 deep) of the larval volume (mm<sup>3</sup>)(P < 0.0001, ANOVA).

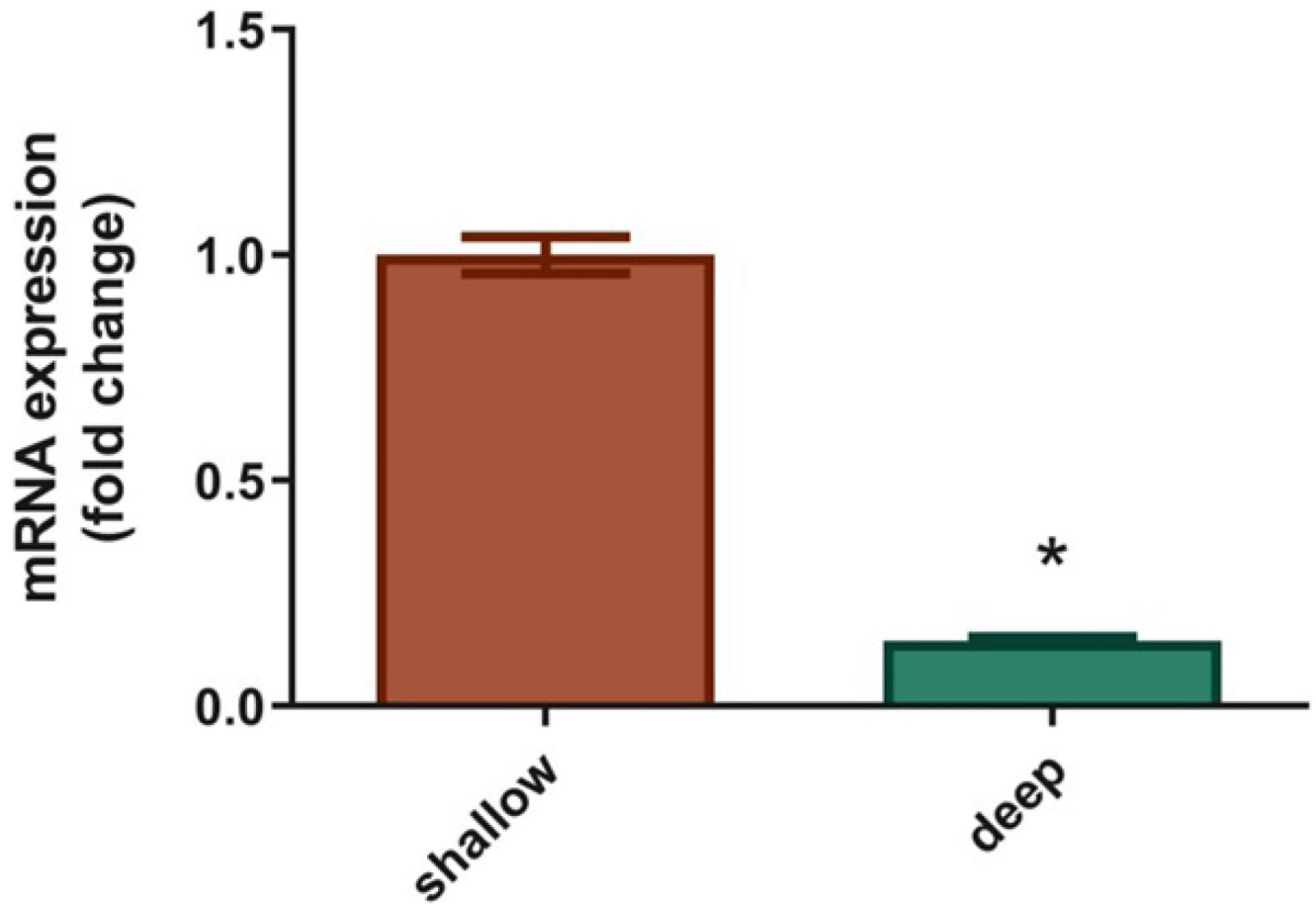


**Figure 3.**

Fluorescence camera images of *S. pistillata* adults from the shallow reef (A-C) and from the mesophotic reef (D-F). Green fluorescence indicates GFPs fluorescence (B, E), red fluorescence indicates the chlorophyll fluorescence of *Symbiodinium* (C, F), overlap of the 2 channels (A, D). Blue rectangular inserts represent a 4x magnification of the relative image (B, C, E, F).



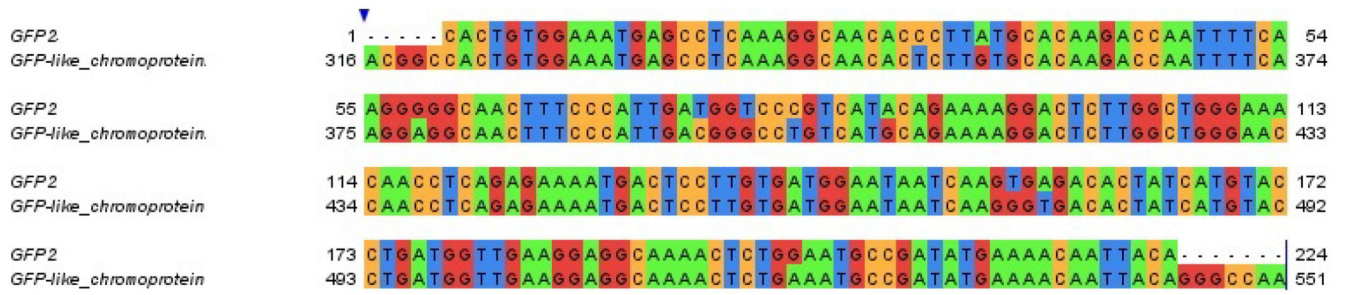
**Figure 4.** Microscopic images of *S. pistillata* larvae from the shallow reef (A-C) and from the mesophotic reef (D-F). Green fluorescence indicates GFPs fluorescence (B, E), red fluorescence indicates the chlorophyll fluorescence of *Symbiodinium* (C, F), overlap of the 2 channels (A, D). Microscope exposure and gain settings are identical in both shallow and mesophotic larvae; chlorophyll exposure is 20ms and gain 1, GFP exposure is 150ms and gain 1.1. Magnification: 10x. Scale bar: 500  $\mu$ m.



**Figure 5.**

Expression of GFP-like chromoprotein in *Stylophora pistillata* shallow and deep larvae. Levels of GFP are expressed as relative variation (fold change) of deep larvae GFP relative to shallow larvae GFP (means  $\pm$  SEMs; N = 6). \*P < 0.05 vs ctr (Mann-Whitney U-test).





**Figure 6.** Sequence alignment of the adult colonies GFP2 gene and of the larvae GFP-like chromoprotein gene. Gene sequences are clustered at 95% identity. Colors indicate different nucleotides.