



Genomic, morphological, and physiological insights into coral acclimation along the depth gradient following an *in situ* reciprocal transplantation of planulae

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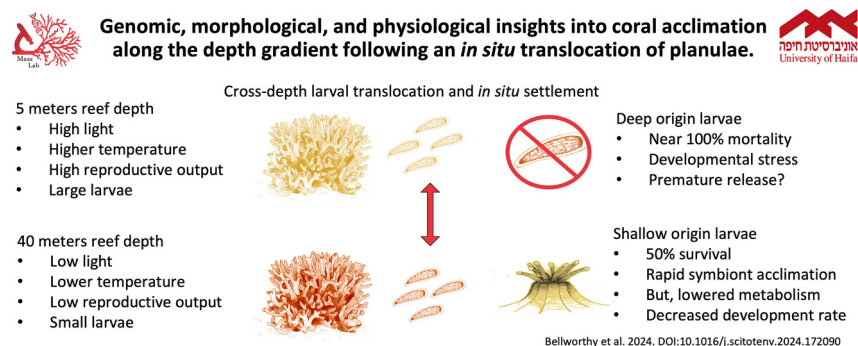
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HIGHLIGHTS

- Deep origin planulae had higher mortality rates than shallow planulae.
- Gene expression suggested that deep planulae lack settlement competency and experience increased developmental stress.
- Symbiont photochemical acclimation to depth occurred within 8 days, but no algal symbiont shuffling was detected.
- Coral juvenile acclimation to the deep phenotype was incomplete after 60 days and may limit the fitness of such individuals.

GRAPHICAL ABSTRACT



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ABSTRACT

Mesophotic coral reefs have been proposed as refugia for corals, providing shelter and larval propagules for shallow water reefs that are disproportionately challenged by global climate change and local anthropogenic stressors. For mesophotic reefs to be a viable refuge, firstly, deep origin larvae must survive on shallow reefs and, secondly, the two environments must be physically connected. This study tested the first condition. Planulae of the reef-building coral *Stylophora pistillata* from 5–8 and 40–44 m depth in the Gulf of Aqaba were tested in a long-term reciprocal transplantation experiment for their ability to settle and acclimate to depth *in situ*. We assessed survival rates, photochemical, physiological, and morphological characteristics in juveniles grown at either their parental origin or transplantation depth. Differences in gene expression patterns were compared between mesophotic and shallow corals at the adult, juvenile, and planula life stages. We found high mortality rates among all mesophotic-origin planulae, irrespective of translocation depth. Gene expression patterns suggested that deep planulae lacked settlement competency and experienced increased developmental stress upon release. For surviving shallow origin juveniles, symbiont photochemical acclimation to depth occurred within 8 days, with symbiont communities showing changes in photochemical traits without algal symbiont shuffling. However, coral host physiological and morphological acclimation towards the typical deep phenotype was

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incomplete within 60 days. Gene expression was influenced by both life stage and depth. A set of differentially expressed genes (DEGs) associated with initial stress responses following transplantation, latent stress response, and environmental effects of depth was identified. This study therefore refutes the Deep Reef Refugia Hypothesis, as the potential for mesophotic-origin *S. pistillata* planulae to recruit to the shallow reef is low. The potential remains for shallow planulae to survive at mesophotic depths.

1. Introduction

Mesophotic coral ecosystems (MCEs, 30–150 m depth) are deep reef environments that function as important habitats, supporting diverse communities of low light-adapted scleractinian corals, macroalgae, sponges, and many other organisms (Kahng et al., 2016; Lesser et al., 2009, 2018). Depth-generalist coral species can be found from shallow to mesophotic depths and tend to exhibit wide phenotypic variation to adjust to the changing abiotic factors with depth that include light intensity and spectral changes, temperature, nutrient fluxes, and wave action (Hoogenboom and Connolly, 2009; Kahng et al., 2019; Lesser et al., 2018). Genetic variation (Malik et al., 2020; Scucchia et al., 2021a, 2023; Studivan and Voss, 2020) drives morphological (Einbinder et al., 2009; Goodbody-Gringley and Waletich, 2018; Malik et al., 2020; Scucchia et al., 2023), physiological (Martinez et al., 2020; Mass et al., 2007) and symbiont-related differences between depths. Some coral species are known to host distinct Symbiodiniaceae communities that are specialised to live either in shallow or mesophotic depths (Bongaerts et al., 2011, 2013; Eckert et al., 2020; Einbinder et al., 2016; Martinez et al., 2020; Studivan et al., 2019).

For some coral species, planula larvae show depth-dependent phenotypes that closely resemble those of the adults (Scucchia et al., 2020; Shlesinger and Loya, 2021; Goodbody-Gringley et al., 2021). Such similarity may confer higher fitness to planulae settling in their parental environment. Concomitantly it could lead to higher mortality when planulae disperse to environmental conditions that contrast with their parental environment and to which they are less fit (Shlesinger and Loya, 2021). In fact, evidence points to higher success in larval settlement in conditions that mimic those at the depths of the parental colonies (Baird et al., 2003; Prasetya et al., 2022; Shlesinger and Loya, 2021; Strader et al., 2015; Wellington and Fitt, 2003; Bellworthy et al., 2023). However, connectivity across depth has been observed for several species in different locations (Bongaerts et al., 2017; Serrano et al., 2014; Serrano et al., 2016; Studivan and Voss, 2018), suggesting that coral planulae might still be phenotypically flexible enough to adjust and colonise a range of environments. These observations raise questions about the role of deeper reefs in replenishing degraded shallower reefs. MCEs have, in fact, been suggested as regions that may serve as refugia from environmental stressors, such as increasing seawater temperature and storm damage, that to date have typically had a greater impact on shallower reef zones (Bongaerts et al., 2010; Bongaerts and Smith, 2019). A few studies have shown the refuge potential of mesophotic depths, by either continuing to host coral species extinct in shallow waters following bleaching (Sinniger et al., 2013; Prasetya et al., 2017), or reporting putative species migration from increasingly warmer shallow water to cooler deeper regions (Martinez et al., 2021). However, open questions remain, such as whether MCEs can act as larval sources and, if so, what is the time frame required for juvenile acclimation. Can deep to shallow larval recruitment occur on a timescale that matches current rates of reef degradation?

Previous laboratory-based studies have attempted to examine acclimation potential and how depth-dependent phenotypic differences influence planulae survival and settlement when exposed to contrasting conditions relative to their parental origins (Bellworthy et al., 2023; Goodbody-Gringley et al., 2021; Prasetya et al., 2022; Shlesinger and Loya, 2021). However, such studies have mostly been based on light intensity manipulations, thus they did not fully mimic the environmental variability found in the field (water flow, nutrient flux variation,

presence of other organisms, etc.). In this study, we performed a long-term *in situ* reciprocal transplantation experiment to investigate the potential for planulae settlement across the vertical cline, exploring the biological processes involved in the survival and growth of early coral life stages at different depths. We collected newly released planulae of the depth-generalist coral *Stylophora pistillata*, one of the most abundant species on Eilat's reefs in the Gulf of Aqaba. This species releases internally brooded planula larvae and vertically transmits Symbiodiniaceae communities. Planulae were translocated *in situ* to either the shallow (5–8 m) or the mesophotic reef (40–44 m) in a reciprocal transplantation experiment. After 8- and 60-days following transplantation, we assessed the degree of acclimation of juveniles to the reciprocal depth. By examining survival and settlement rates, morphological, physiological, and photochemical characteristics, as well as gene expression changes, we evaluated if depth-dependent differences in planulae phenotype and parental origin are a barrier to successful recruitment in a different depth environment. Such an evaluation allows us to determine if and at what rate acclimation can occur for planulae dispersing along the vertical gradient, ultimately aiding the modelling of potential shifts in community structure post disturbance.

2. Methods

2.1. Sampling and experimental design

Sample collection and *in situ* experiments were conducted on the reef directly adjacent to Interuniversity Institute for Marine Sciences in Eilat, Israel (IUI). This is an area of narrow fore reef to 3–8 m depth after which a steep reef slope reaches 40–45 m depth within approximately 75 m from shore. This steep reef topography, close to shore, facilitates access to mesophotic depths as well as minimising the physical distance between experimental deep and shallow sites and consequently differences in abiotic parameters other than the experimental factor of depth itself.

Data presented within this manuscript are a combination of planulae collections and repeat runs of *in situ* experiments conducted between late April to June 2021 and March to July 2022. Data were acquired over this extended period due to the large logistical effort required, the number of technical dives required, permit restrictions, COVID-19 restrictions, and limited planulae output from deep colonies, particularly in 2021. A complete log of planulae collections, sampling dates, and analyses conducted is presented Supplementary Table 1.

Planulae were collected from the shallow reef (5–8 m depth) and mesophotic reef (40–44 m depth) by placing net mesh traps over adult colonies of *Stylophora pistillata* at sunset using open-circuit SCUBA diving and closed-circuit rebreathers (CCR TRIMIX, Megalodon, Innerspace Systems Corp.). All sampled colonies were larger than 20 cm in diameter with no visible signs of recent bleaching, breakage, or predation. *S. pistillata* is a common, internal brooding, reef building coral at this site with an extended planulation period (December – July) on the shallow reef (Shefy et al., 2018). Planulae traps were removed the following morning shortly after sunrise. All traps and planulae were placed in large opaque (dark) containers and remained immersed in seawater during sorting for analyses. On each collection date, planulae from all parent colonies were pooled by depth prior to analyses. Batches of 20 planulae from both shallow ($n = 6$) and deep colonies ($n = 5$) were immersed in 300 μ L RNA/DNA shield (Zymo Research), left at room temperature for 30 min, before storage at -20°C until analysis. In addition, 1 cm^2

branch fragments of three planulae-producing adult colonies from each depth were similarly preserved in 700 μ L RNA/DNA shield.

On the morning of collection, planulae from either the shallow or deep reef were placed into custom-made cylindrical polypropylene settlement chambers covered at both ends with 200 μ m mesh permitting gas and water exchange. In 2021, 40 planulae from the shallow reef were placed in each chamber (N chambers = 12, N planulae = 480). Not enough deep origin planulae were collected in 2021 to conduct settlement assays at this level of replication. In 2022, 10 planulae from each depth were placed in each chamber (shallow origin: N chambers = 24, N planulae = 240; deep origin: N chambers = 10, N planulae = 100). Each chamber contained two raised settlement plugs (polypropylene) pre-conditioned in reef seawater at the collection site for 5 weeks and had developed biofilm including visible crustose coralline algae. The settlement chambers were attached to plastic egg crates with cable ties and returned to the reef later the same day. Divers attached the crates to non-living structures at 5- and 40-m water depth. Half of the planulae (chambers) were returned to their collection depth, whilst the remaining half were placed at the alternate collection depth in a reciprocal design. This resulted in four *in situ* experimental 'treatments': shallow origin planulae at 5 m depth (shallow-shallow, SS); shallow origin planulae at 40 m depth (shallow-deep, SD); deep origin planulae at 5 m depth (deep-shallow, DS); deep origin planulae at 40 m depth (deep-deep, DD). Two HOBO light and temperature loggers were also attached to the crates at each depth and set to a continuous sampling interval of 5 min.

2.2. Survival, photochemistry, physiology, and morphology

After 8 days, half of the settlement chambers from each treatment were collected. The HOBO loggers and the remaining chambers were left *in situ* for a total of 60 days. On shore at IUI, chambers containing live juvenile corals were submerged in shaded flow through seawater tables and kept in the dark as much as possible to avoid light stress. The number of newly settled juveniles (spat) on all surfaces was counted for each chamber. Settlement was defined as the completion of metamorphosis and attachment to the substrate. The number of remaining swimming planulae was added to the number of settled spat to obtain the total number of survivors. The number of survivors subtracted from the initial number of planulae in each chamber gave the number of dead individuals (mortality). The number of surviving deep origin individuals (both DD and DS treatments) was too low at each sampling point to conduct any further analyses i.e., data for these treatments are limited to settlement and mortality counts.

Simultaneous to the opening of the chambers and counting the survivors, spat were placed immediately in 700 μ L RNA/DNA shield for nucleic acid extraction (see "Nucleic acid extraction, processing, and sequencing" for details). Remaining spats were dark acclimated for 20 min before photochemistry was assessed with an Imaging PAM (Walz GmbH, Germany) using increasing actinic light steps from 0 to 460 μ mol $\text{m}^{-2} \text{s}^{-1}$, 3 min per light step, as previously described (Bellworthy et al., 2023). Photosynthetic yield was typically zero above ca. 300 μ mol $\text{m}^{-2} \text{s}^{-1}$. R Studio was used to fit curves to rapid light curve data (Platt et al., 1980) using the fitPGH function from the phytotools package as previously adapted and described in detail (Lieberman et al., 2021). The parameters alpha (photosynthetic efficiency under light limiting irradiances), ETR_{MAX} (maximum relative electron transport rate), and E_K (saturation irradiance) were extracted. Y(II) following a saturation pulse where PAR = 0 is described as maximum quantum yield (F_V/F_M). All settled spats were photographed with a stereomicroscope (Nikon, SMZ800N with DS-Fi2 camera) and ImageJ was used to scale images and measure the diameter of each spat at the widest point crossing the primary polyp (SS, 8 days n spat = 34, 60 days n = 44; SD, 8 days n = 47, 60 days n = 49). For settlement, mortality, photochemistry, and spat size analyses the chamber was recorded as a random factor within each depth.

For physiological parameters assessment, between four and eight

spats were combined into one replicate sample depending upon live coral availability. Results were normalised to the number of spat used where appropriate. Triplicate samples per treatment at each time point were collected and frozen at -80°C until processing. Samples were processed as described previously (Scucchia et al., 2021b). Briefly, an electrical homogenizer (HOG-160-1/2, MRC-labs, Israel) was used to homogenise the spat samples, after adding 500 μ L of filtered seawater. The homogenate was centrifuged for 5 min at 4°C (5000 $\times g$) to separate the symbiont cells and the debris from the coral host tissue. Aliquots of the supernatant (100 μ L) were used to determine the concentration of soluble protein of the coral host using the QPRO-BCA kit standard (Cyanagen), following the manufacturer's protocol. After resuspending the pellet, aliquots (50 μ L) of the homogenate were used to determine the density of Symbiodiniaceae algae using a Neubauer-Improved hemocytometer (BOECO) by means of microscopic fluorescence counts (Nikon Eclipse Ti). Chlorophyll-*a* concentration was measured in 1 mL of the homogenate, after an overnight incubation with 90 % cold acetone at 4°C . After incubation, samples were centrifuged for 5 min at 4°C (5000 $\times g$), and the optical density was determined in a polypropylene 96-well plate (200 μ L subsample in triplicate) at wavelengths of 630, 647, 664 and 691 nm, based on a previously described method (Ritchie, 2008), using a spectrophotometer (PerkinElmer, 2300 EnSpire R, United States). Pathlength was adjusted accordingly.

Remaining spats (12–14 per treatment, SS and SD) were gently removed from the chambers into 100 % ethanol for scanning electron microscopy as in Martinez et al. (2021). Images taken using scanning electron microscopy were used to measure septa width (the diameter across the middle of the septa), diameter of the primary polyp calyx, and the ratio of the area of rapid accretion deposits to spine base area (RAD: Base) (Scucchia et al., 2021b).

2.3. Nucleic acid extraction, processing, and sequencing

Samples from deep and shallow adult colonies, deep and shallow origin planulae, 8 day old spat from the SS and SD treatments, and 60 day old spat from the SS and SD treatments were collected as described above. On day 8, 6–8 spats were combined for each replicate and on day 60, due to a lower number of survivors, 2–3 spats were combined to each replicate. Samples with RNA/DNA shield were left at room temperature for 30 min before storage at -20°C until processing. Not enough DD and DS spat survived *in situ* to complete any RNA or DNA extraction for these treatments. Samples were processed at random to remove the possibility of batch effects influencing trends with developmental stages through the experiment. Total DNA and RNA were extracted from thawed coral tissue samples in tandem (Quick-DNA/RNA Miniprep Plus Kit, Zymo Research) according to the manufacturer's protocol with the following details. Before nucleic acid extraction, 5–10 2 mm beads were added to each sample and placed into a bead beater for 60-s on maximum speed. DNase treatment was performed in the tube as per kit instructions. DNA was eluted in warmed (60°C) 10 mM Tris-HCl and RNA eluted in DNase/RNase free water. Each eluted sample was divided to three aliquots before storage at -80°C . Nucleic acid concentration and purity was tested using a NanoDrop 2000 (Thermo Fisher Scientific, USA) and RNA quality was tested using a TapeStation (Agilent Technologies, USA). Samples for RNAseq all had an RNA integrity number (RIN) >7.7 (average 8.76). Sequencing libraries were prepared using a standard in house mRNA-Seq protocol at The Crown Genomics Institute of the Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Israel. The polyA fraction (mRNA) was purified from 500 ng of total RNA per sample, followed by fragmentation and the generation of double-stranded cDNA. Unique molecular identifiers were added prior to PCR amplification steps to reduce errors and quantitative bias introduced by amplification. 150 bp paired-end reads were sequenced on an Illumina NovaSeq SP with 100 cycles.

2.4. Differential gene expression analysis

All read processing was conducted using the HIVE computer cluster at the University of Haifa, Israel, using publicly available scripts (Scucchia et al., 2021a, 2023). Read quality was assessed with FastQC and compiled with MultiQC. Adapters were removed from the reads using Cutadapt (Martin, 2011) and reads with a quality score of <25 were discarded using Trimmomatic (Bolger et al., 2014). Prior to alignment to the reference genome, we identified the dominant algal symbiont types by performing a BLASTx search using Diamond (Buchfink et al., 2015) against open-access proteome databases for Symbiodiniaceae species (as described in Scucchia et al., 2021a, 2023) and reported in Supplementary Table 2. Reads were then aligned to the *S. pistillata* host genome assembly (NCBI GCA_002571385.1) using Star (Dobin et al., 2013). MultiQC was then repeated. A total of 823.5 million raw reads were generated ranging from 22.3 to 49.3 million reads per sample. Mean raw gene count was 34.3 million with 81.55 ± 6.98 % (mean $\pm$ standard deviation) successful alignment to the host genome. Average sequence length was 113 bases, with a mean PHRED score over 35 along the entire sequence length. No samples were removed from any downstream analyses. Alignment to the two most dominant Symbiodiniaceae taxa was also conducted, however successful alignment was low and prohibited any further analyses (*Cladocopium goreaui*: 3.33 ± 2.59 %, *Symbiodinium microadriaticum*: 3.32 ± 1.33 %). Raw gene counts and mapping efficiencies are listed for each sample in Supplementary Table 3.

To visualise experiment-wide patterns in gene expression, an NMDS plot was produced based on sample-to-sample global gene expression with Bray Curtis distances using the R package ‘vegan’ (stress ≤ 0.041). Differences in overall expression (life stage * origin depth * transplant depth) were tested for using a permutational analysis of variance (PERMANOVA) via the *adonis2* function in ‘vegan’ (permutations = 999, method = “euclidean”). Differential gene expression analysis was conducted on the genome-mapped reads using DESeq2 (Love et al., 2014). The model for DESeq2 included all experimental factors and their interactions (life stage * origin depth * transplant depth). Pairwise differences in gene expression were estimated for each life stage separately, i.e., between shallow versus mesophotic adults, shallow versus mesophotic planulae, SS versus SD at 8 days, and SS versus SD at 60 days using the Wald model in DESeq2 (Love et al., 2014), based on host genes with ≥ 5 reads in ≥ 2 samples. The resulting number of expressed genes with an adjusted $p < 0.05$ were identified as differentially expressed and overlap in these genes between life stages was visualised through a Venn diagram (Heberle et al., 2015). The R package ‘goseq’ (Young et al., 2010) was used to perform Gene Ontology (GO) term enrichment analysis based on the DESeq2 output, as described previously (Malik et al., 2020; Scucchia et al., 2021a). Gene ontology terms were assigned based on Uniprot *S. pistillata* (<https://www.uniprot.org/>), KEGG *S. pistillata* (<https://www.kegg.jp/>) and *S. pistillata* Trinotate annotations (Bryant et al., 2017). Resulting enriched terms were visualised with a word cloud (‘ggwordcloud’ {ggplot2} (Wickham, 2020)), after merging redundant terms.

2.5. Statistical analyses

All data exploration, graphics, and statistical analyses were performed using RStudio v4.0.3 (R Core Team, 2020). Functions and packages used are denoted as (‘function’ {‘package’}) in text. Parametric assumptions of homogenous variance between groups and normally distributed data within groups were tested using Levene’s Test (‘leveneTest’ {car} (Fox and Weisberg, 2019)) and a Shapiro-Wilk Normality Test (‘shapiro.test’ {stats} (R Core Team, 2020)), respectively.

Light and temperature data are reported separately for each year of experiments (2021 and 2022). Daily mean light and temperature as well as diurnal range in these parameters (maximum - minimum) were tested for significant differences between shallow and mesophotic reefs using a

two-sided *t*-test (‘t.test’ {stats} (R Core Team, 2020)), with a hypothesised mean difference of zero.

Settlement and mortality data were first visualised graphically to check for prominent differences between 2021 and 2022 experiments. Due to breaches in the parametric assumption of normally distributed data, statistical differences between years were assessed with Wilcoxon tests (‘wilcox.test’ {rstatix} (Kassambara, 2021)). Testing revealed there are no significant differences between years, therefore both year’s data were combined for further analysis and are presented together hereafter. Kaplan-Meier survival curves were created (‘survfit’ {survminer} (Kassambara et al., 2021)) and visualised (‘ggsurvplot’ {survminer}) to assess differences in time to settlement and mortality rates between the four treatments (Surv ~ treatment, log rank test) over the entire experimental period. Pairwise treatment differences were tested for using ‘pairwise_survdif’ {survival} (Therneau, 2020). Thereafter, pairwise Wilcoxon tests were used to detect settlement and mortality differences between treatments at each sampling time point (day 8 and day 60 separately).

Due to limited survival in DD and DS treatments (see results), all other analyses were conducted on the two remaining treatments, SS and SD. Juvenile physiology data (8 day and 60 day) met parametric assumptions; *t*-tests were used to compare the two treatments within each sampling point. Where parametric assumptions were not met (e.g., spat diameter), Wilcoxon tests were used. Algal photochemistry data had multiple non-parametric data groups which were not significantly improved after transformation; Wilcoxon tests were used on all photochemical data. Algal photochemistry and coral physiology data were also explored using Principal Component Analysis (‘prcomp’ {factoextra}; Kassambara and Mundt, 2020).

All graphics were produced {ggplot2} (Wickham, 2020) and arranged {cowplot} (Wilke, 2020) with R Studio. Final aesthetic modifications on graphics were made with the freeware GIMP. Data are mean $\pm$ standard error unless otherwise written. Differences are described as significant when $p \leq 0.05$.

3. Results

3.1. Water temperature and light intensity

Mean daily temperature and light intensity on the shallow reef consistently tracked above the deep reef and had greater diurnal variability (max - min) throughout the experimental period (Supplementary Fig. S1, Supplementary Table 4). In 2021, mean water temperature was 24.44 ± 0.50 °C on the shallow reef (mean $\pm$ variance) and 23.64 ± 0.46 °C on the deep reef. In the same year, mean irradiance was over ten times greater on the shallow reef with >12 times greater diurnal variation compared to the deep reef. In 2022, mean water temperature was 23.69 ± 2.65 °C on the shallow reef (mean $\pm$ variance) and 22.83 ± 1.35 °C on the deep reef. The greater thermal variation in 2022 is due to the extended experimental period relative to 2021. In 2022, mean irradiance was >27 times greater on the shallow reef with >35 times greater diurnal variation compared to the deep reef. In both years, mean daily temperature and diurnal variability were significantly greater on the shallow compared to the deep reef (*t*-test, $p < 0.000001$, Supplementary Table 4).

3.2. Survival, physiology, and morphology

Following Kaplan-Meier plots, log-rank testing indicated significant treatment differences in both time to settlement and survival probability (Fig. 1A, C; $p < 0.0001$). Kaplan-Meier plots suggested that shallow origin larvae were more likely than deep origin larvae to settle within 8 days and overall had attained higher settlement success at the end of the experiment (SS: 45.8 ± 14.1 %, SD: 61.3 ± 21.8 % versus DS: 5.0 ± 5.8 %, DD: 7.5 ± 15.0 % mean settlement success after 60 days) (Fig. 1C). Similarly, average mortality was higher in deep origin larvae (shallow

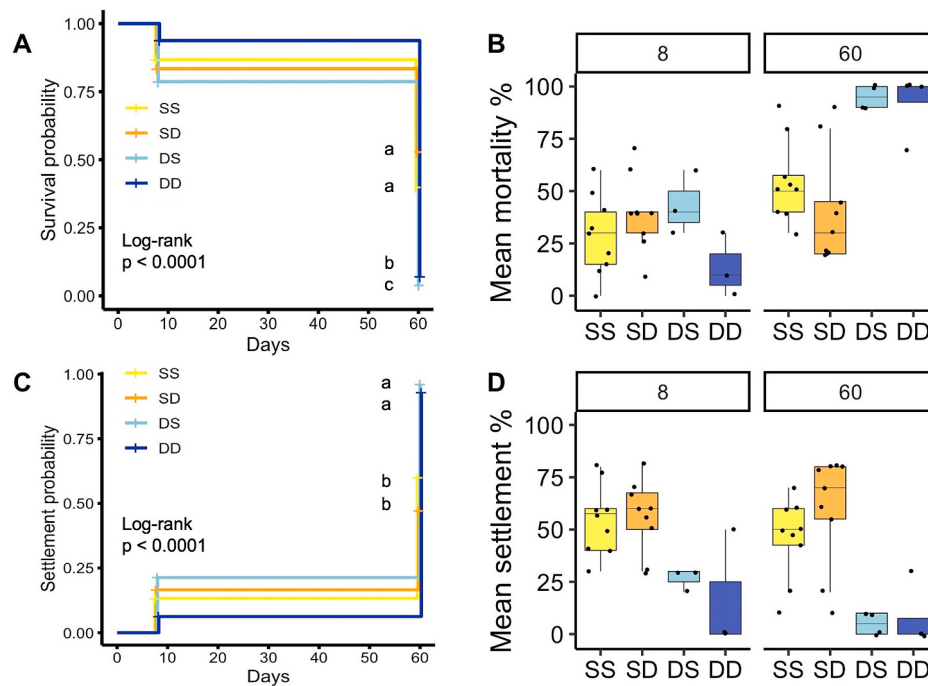


Fig. 1. Settlement success and mortality of shallow to shallow (SS) and shallow to deep (SD) planulae after 8 and 60 days of *in situ* reciprocal transplantation. (A, C) Kaplan-Meier plots; log-rank testing shows significant treatment differences in both time to settlement and survival probability ($p < 0.0001$). Different lower case letters at the 60 day endpoint indicate significant pairwise differences between treatments for the entire experimental period (two sampling points combined). Note: sampling only occurred on day 8 and day 60 but data have been jittered on the x-axis for clarity. (B, D) Mean mortality and mean settlement on the 8 day and 60 day timepoints. There were no significant pairwise differences between treatments within each experimental day (Wilcoxon Tests, adjusted $p > 0.05$). Horizontal black lines within boxes are median values and box limits represent first and third quartiles. Whiskers represent 1.5 times the interquartile range. Round black points are individual sample data.

origin: $46.5 \pm 17.9\%$ versus deep origin: $93.8 \pm 10.4\%$ mean mortality rate after 60 days (Fig. 1B). No planulae remained swimming at the 60 days' time point. However, pairwise tests between treatments within each day, showed that there were no significant differences in settlement or mortality rates between treatments on day 8 (Fig. 1B, D; Wilcoxon tests, adjusted $p > 0.1$) and statistical differences in settlement and mortality rates between shallow origin larvae (SS and SD) and deep origin larvae (DD and DS) on day 60 were borderline (Wilcoxon tests, adjusted $p < 0.06$). High mortality rates of deep origin larvae ($> 90\%$) meant there were not enough survivors to complete any further analyses for the DD and DS treatments.

After 8 days *in situ*, compared to SD spat, SS settled spat had significantly lower chlorophyll concentration ($T = -5.5$, $df = 3.2$, $p = 0.01$) (Fig. 2A), higher density of algal symbionts ($T = -6.9$, $df = 3.9$, $p = 0.003$) (Fig. 2B), but not significantly different protein concentration ($T = -2.5$, $df = 2.32$, $p > 0.05$) (Fig. 2C). At 60 days, SS spat retained a similar chlorophyll concentration, but SD spat increased concentration > 5 times relative to the 8 days' time point ($T = -7.4$, $df = 2.3$, $p = 0.012$) (Fig. 2A). At 60 days, algal symbiont density ($T = -6.2$, $df = 2.2$, $p = 0.019$) (Fig. 2B) and protein concentration ($T = -10.2$, $df = 3.2$, $p = 0.002$) (Fig. 2C) were 1.6 and 4.8 times higher in SD spat, relative to SS. Chlorophyll concentration separated 60 day old SD spat from other treatments on principal component 1 (PC1) which accounted for 81.4 % of the sample variation (Supplementary Fig. 2a). Other treatments showed separation on PC2 (17.2 % of variation) driven by protein concentration and algal cell count.

At 8 days old, SS spat had significantly larger diameter than SD (Wilcoxon test statistic = 1124, $p = 0.002$; SS 2.41 ± 0.35 mm; SD 2.02 ± 0.52 mm) but SD spat grew significantly between the two time points (Wilcoxon test statistic = 678, $p < 0.001$) and matched those of the SS treatment at 60 days (Fig. 3A). Septa width was greater in SD spat compared to SS at 8 days (Wilcoxon test statistic = 31, $p = 0.003$) but not at 60 days (Fig. 3B). There were no treatment differences in corallite

diameter or RAD:base at either time point (Fig. 3C, D).

3.3. Algal photochemistry

At the first time point, day 8, there were significant treatment differences with alpha and F_v/F_m being higher in SD spat (Fig. 4B, D). This difference, as well as its direction was maintained at the 60-day time point. Mean F_v/F_m in SS spat was 0.47 ± 0.05 compared to 0.54 ± 0.05 in SD spat during the whole experiment. Initially, on day 8, there was no significant difference in ETRmax between treatments but on day 60, SD spat had significantly higher ETRmax (Fig. 4A). There were no treatment differences in E_k at either time point (Fig. 4C). However, E_k was a strong driver of PC2 (41.8 % of variation) within the photochemistry data (75 % contribution). PC1 (47.9 % variation) separated SS and SD samples predominantly driven by F_v/F_m and alpha (Supplementary Fig. 2b).

3.4. Algal symbiont genotype by life stage

Symbiont species identification analysis, based on all reads BLASTed against Symbiodiniaceae species, reveals that shallow planulae and adults, as well as SS and SD spats predominantly host *S. microadriaticum* and *S. linucheae*, whereas mesophotic planulae and adults mainly host *Cladocopium* sp., formerly referred to as "clade C" (*Cladocopium goreau* and *Symbiodinium* clade C) (Fig. 5). Mesophotic corals also host *Durudinium* sp. (approximately 15 % of BLASTed reads). *Durudinium* sp. was also present in a similar percentage in the SD corals at 60 days.

3.5. Differential gene expression

A total of 27,575 genes were identified. Differential gene expression analysis was conducted on 25,795 genes which had ≥ 5 reads in ≥ 2 samples. Adult corals from deep versus shallow reefs had 3568 differentially expressed genes (DEG's, adjusted $p < 0.05$), 13.8 % of the

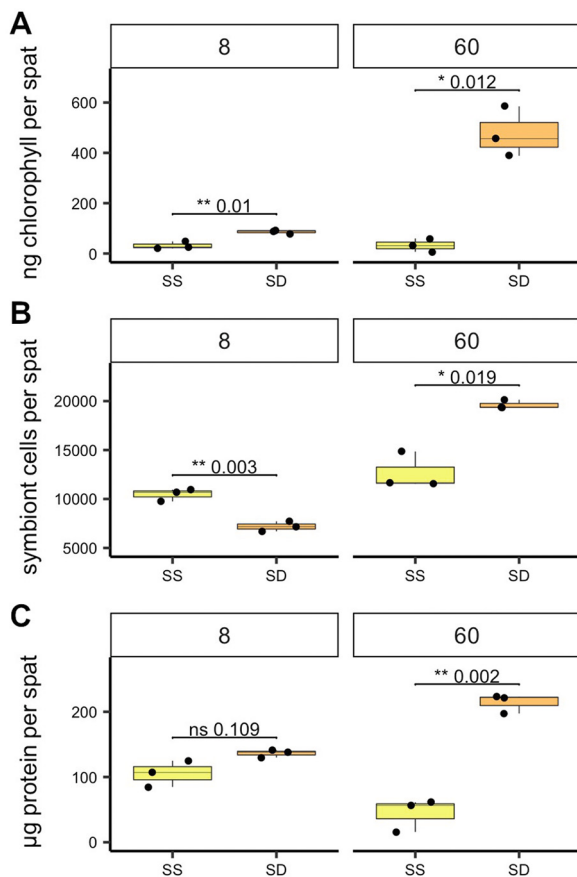


Fig. 2. Physiological parameters of SS and SD spat after 8 and 60 days of *in situ* reciprocal transplantation *in situ* translocation. Horizontal black lines within boxes are median values and box limits represent first and third quartiles. Whiskers represent 1.5 times the interquartile range. Round black points are individual sample data. Statistical tests (Wilcoxon test) compare data for SS versus SD juveniles; ns = not significant, * = $p < 0.05$, ** = $p < 0.01$.

transcriptome (Fig. 6A and B). Twenty-two of these genes were also differentially expressed between SS and SD spat 8 days after *in situ* depth separation with a total of 95 DEG's between the SS and SD treatments at 8 days (0.37 % of transcriptome). By 60 days, there were 1670 DEG's between SS and SD spat (6.5 % of transcriptome). A total of 366 of these genes were also differentially expressed between deep and shallow adult colonies; this number of shared genes is statistically significant (Fisher's exact test, two-sided, $p < 0.0001$). There were 7284 DEG's between deep and shallow planulae (28.2 % of transcriptome), the greatest contrast found in all the life stages tested. 1080 of these genes were also differentially expressed in deep versus shallow adults. Few planulae DEG's were shared with juveniles at 8 days (19 genes) but this number increased to 441 shared DEG's at 60 days. An NMDS plot indicated a clustering of gene expression patterns primarily based upon life stage rather than a separation of the samples based upon reef depth, i.e., all adult, planulae, and juvenile samples formed separate clusters (Fig. 6C). Adult and juvenile samples were closer on MDS1 but separated by MDS2, whereas adult versus planulae samples were closer on MDS2 but separated by MDS1. A permutations test indicated that 53 % of the sample variation was due to the life stage ($R^2 = 0.53$, $F = 17.51$, $p < 0.001$) with a further 24 % accounted for by the interaction of the life stage with origin depth ($R^2 = 0.24$, $F = 23.34$, $p < 0.001$).

Functional annotation revealed 181 significantly enriched gene ontology terms (GO terms, $p < 0.01$) between deep and shallow adults (Fig. 7). Categories such as gene silencing, sorocarp development, oxidoreductase activity, and ubiquitination were significantly down regulated in deep colonies compared to shallow colonies whereas ion

channel and transmembrane processes were typically upregulated relative to shallow colonies. In planulae, 3250 significantly enriched GO terms were identified. Developmental processes including cell differentiation, cell development, and Wnt signaling were strongly upregulated in deep planulae and metabolic processes were downregulated relative to shallow planulae. At 8 days, only 6 significantly enriched GO terms were identified, all of which indicated downregulation of metabolic processes in SD spat versus SS. At 60 days, 705 GO terms were significantly different between SS and SD spat. A downregulation of metabolic processes including heme proteins, mitochondrion, respiration, and amino acid and fatty acid metabolism remained in SD spat. GO terms upregulated in SD relative to the shallow control spat (SS) included signaling, membrane processes, and immune response.

4. Discussion

4.1. Mesophotic planulae have high mortality rates

Planulae from the reef building coral *S. pistillata* were collected from 5 m (shallow) and 40 m (deep/ mesophotic) reef depth in Eilat, Israel, reciprocally transplanted *in situ* between depths in settlement chambers, and monitored for 60 days. Deep planulae had significant upregulation of 'signaling' and 'binding' GO terms relative to shallow planulae (Fig. 7). These terms were correlated with the onset of settlement behaviour and metamorphosis in *Acropora millepora* (Strader et al., 2018) suggesting that such processes have a role in successful recruitment. Yet, over the experimental period, significant mortality (>90 %) was observed for deep origin individuals irrespective of whether they were placed at 5 m or 40 m reef depth (Fig. 1B). It is possible that deep planulae from this study settled, but subsequently died before the 8-day monitoring point. However, these GO terms were also significantly upregulated in deep adults relative to shallow adults and therefore, the expression of these genes in deep planulae may be remnants of the maternal transcriptome.

Deep origin planulae also displayed an upregulation of 'development' GO terms relative to shallow planulae which, in contrast to the above, may indicate that they are not yet fully competent when released (Fig. 7). Concomitantly, metabolic processes (GO terms 'metabolism', 'amino acid metabolism', 'mitochondrion development') required for metamorphosis and settlement were downregulated in deep planulae suggesting that shallow planulae had greater potential to settle immediately upon release. Finally, previous studies of *S. pistillata* in Eilat report significantly smaller planulae released by mesophotic colonies compared to shallow colonies throughout the reproductive season (Bellworthy et al., 2023) and reduced size is often linked with higher mortality in nature. Taken together, these data suggest reduced survival, fitness, and recruitment for deep planulae relative to shallow conspecifics. Though this is the first study attempting to settle deep origin *S. pistillata* planulae *in situ*, deep origin *S. pistillata* planulae have been successfully settled *ex situ* (Bellworthy et al., 2023). Likewise, following *ex situ* settlement, deep origin *Stylophora kuehlmanni* juveniles survived when transferred back to the reef at both shallow and deep sites, though survival was significantly lower when not at parental depths (Shlesinger and Loya, 2021). Thus, further studies are required to determine whether the lack of settlement and high mortality rates of deep origin planulae found in this study are reflective of natural rates or represent an experimental artefact.

The planulae output of deep colonies in this study was notably reduced compared to shallow colonies. Per unit sampling effort (planulae trap), shallow colonies released on average 30.3 planulae net⁻¹ (N planulae = 1515, N nets = 50), whilst deep colonies averaged 2.7 planulae net⁻¹ (N planulae = 250, N nets = 91), 11.2 times lower (Supplementary Table 1). It is worthwhile to note that this shallow output is approximately 30 % lower than the average 44.3 planulae net⁻¹ reported from collections in 2015 and 2016 which spanned the entire reproductive period for this species, including less fertile periods (Shefy

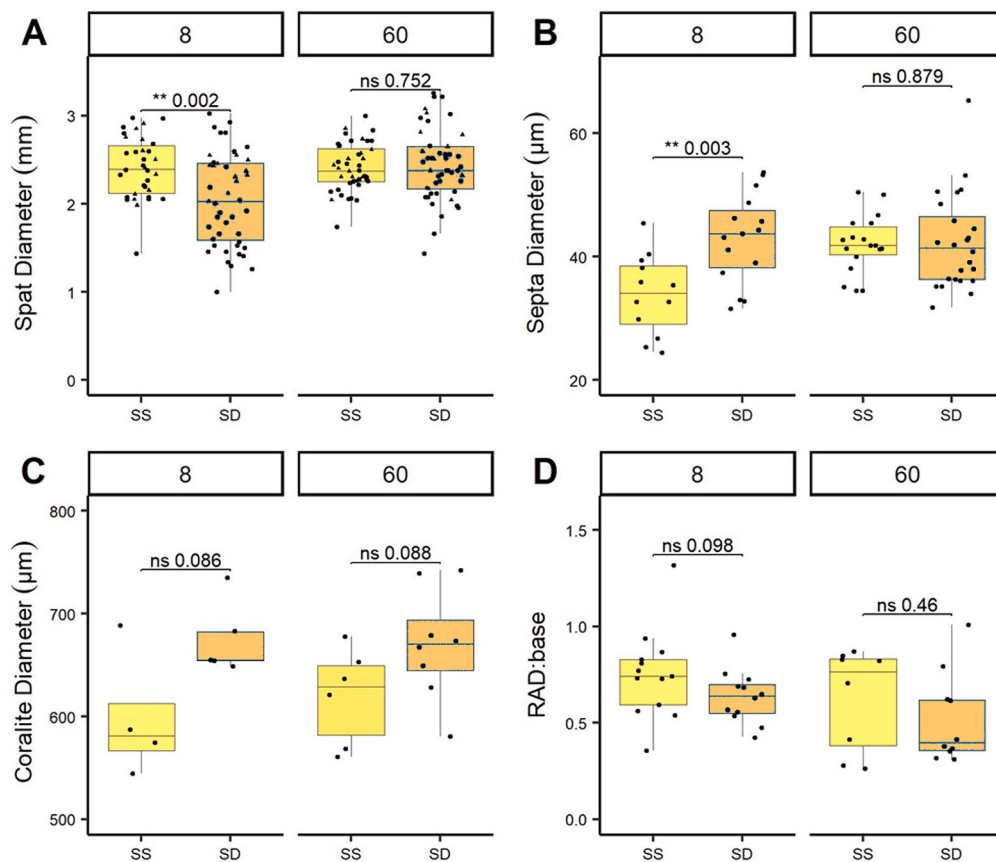


Fig. 3. Skeletal growth parameters of SS and SD spats after 8 and 60 days of *in situ* reciprocal transplantation. Horizontal black lines within boxes are median values and box limits represent first and third quartiles. Whiskers represent 1.5 times the interquartile range. Round black points are individual sample data. Statistical tests (Wilcoxon test) compare data for SS versus SD juveniles; ns = not significant, ** = $p < 0.01$.

et al., 2018). Significantly decreased planulae output has been noted from shallow colonies of *S. pistillata* that survived a local reef devastating storm in March 2020 (*personal observation*). A temporal delay in peak reproductive fitness for mesophotic colonies relative to shallow colonies, both in terms of colony fecundity and offspring survival rates, has been reported for multiple coral species in Eilat (Feldman et al., 2018; Liberman et al., 2018, 2022; Shlesinger et al., 2018). Differences in seasonal planulation patterns across the reef scape were also found for the brooding coral *Porites astreoides* in Bermuda, where peak planulation for shallow inshore colonies occurred one month earlier than deeper offshore colonies (de Putron and Smith, 2011). Whilst planulae release of *S. pistillata* peaks in March and April on the shallow reef (Shefy et al., 2018), Shlesinger and Loya (2021) found mesophotic colonies released little to no planulae in February and March. Here, we found higher numbers of planulae released by mesophotic colonies during the later sampling dates (e.g., May versus March, Supplementary Table 1), suggesting that the timing of planulae collections for this study may have pre-empted peak fecundity for mesophotic colonies and that collections later in the season might have resulted in greater planulae output from mesophotic colonies. Larval competency and physiology can be affected by release date, where release at suboptimal times results in lower survival (Cumbo et al., 2013). Physical aspects of the settlement environment also impact settlement success and long-term survival, such as an appropriate chemical cue, without which metamorphosis will not occur (Pechenik, 1990). This is unlikely to have impacted settlement of the mesophotic larvae in this study as the settlement plugs had acquired a bacterial film and crustose coralline algae, documented to induce settlement of coral larvae, from water from an intermediate depth (Morse et al., 1994; Quinlan et al., 2023). Rather, the reduced fitness of mesophotic larvae found here is putatively due to limited light

availability for mesophotic adult colonies, reducing the energy allocated to reproduction, particularly early in the season, and resulting in smaller larvae with lower rates of settlement and survival (Baird, 2001; Gleason and Hofmann, 2011).

4.2. Symbiont photochemical acclimation to depth is rapid; coral host acclimation needs more time

Low settlement success and high mortality rates in DD and DS chambers meant that not enough corals remained to assess juvenile acclimation in these treatments. Photochemical acclimation of Symbiodiniaceae in transplanted shallow origin planulae (SD) to the lower light intensity and narrow light spectrum at 40 m (Supplementary Fig. 1, Supplementary Table 4) appeared to be underway within 8 days. This is evidenced with a significantly higher F_v/F_m and photosynthetic alpha in SD compared to SS spat at 8 days, though saturation irradiance (E_K) was not different between treatments even after 60 days (Fig. 4C, D). This acclimation is faster than that observed in an *ex situ* study with this species and with similar experimental design that reported photochemical acclimation within 35 days (Bellworthy et al., 2023). It is likely that differences in the light intensity between SS and SD treatments were greater in the current *in situ* experiment compared to the former aquaria experiment (Bellworthy et al., 2023), resulting in a more rapid acclimation. Individual SD spat in this study had significantly increased chlorophyll concentration at day 8 despite being smaller in diameter and having significantly lower algal symbiont density (Fig. 2A, B). By day 60, where spat diameter is no longer significantly different between treatments, SD spat had higher chlorophyll concentration and higher symbiont density than SS spat. Both changes are further indications of the symbionts' ability to acclimate to depth.

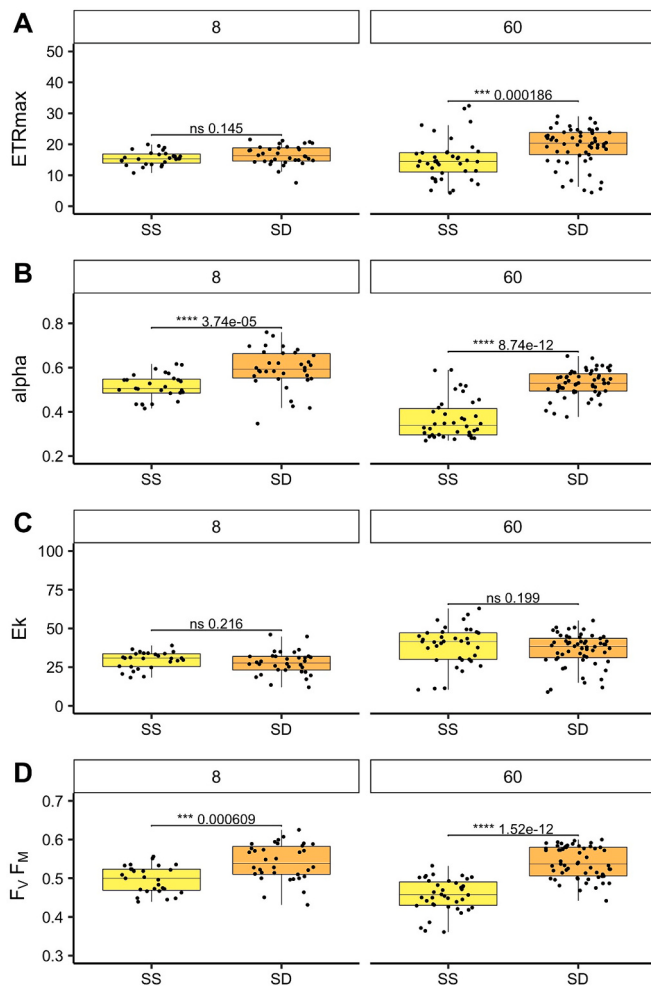


Fig. 4. Photosynthetic parameters of SS and SD spat after 8 and 60 days of *in situ* reciprocal transplantation. Horizontal black lines within boxes are median values and box limits represent first and third quartiles. Whiskers represent 1.5 times the interquartile range. Round black points are individual sample data. Statistical tests (Wilcoxon test) compare data for SS versus SD juveniles; ns = not significant, *** = $p < 0.001$, **** = $p < 0.0001$.

These physiological and photochemical changes at day 8 were not a result of symbiont shuffling - a change in the relative abundance of different Symbiodiniaceae genera or species *in hospite*. Both adults and planulae of *S. pistillata* from this site host predominantly *S. microadriaticum* on the shallow reef and *C. goreaui* on mesophotic reefs (Fig. 5 (Byler et al., 2013; Scudchia et al., 2020)). In the current study, acclimation towards deep photochemical traits at day 8 did not occur alongside symbiont genera shuffling, i.e., from typical shallow *S. microadriaticum* to deep typical *C. goreaui*. This result raises the question as to when and how the depth specific symbiont types develop in this population. By day 60, though the dominant symbiont species did not change from the origin (*S. microadriaticum*), SD spat acquired *Durussinus* sp. in an approximately similar proportion to mesophotic adult corals, a shift that was not present in SS spat. This is the first indication of initial symbiont shuffling following depth translocation and occurred before a shift in the dominant algal symbiont species. A previous study of symbiont shuffling during juvenile development for *P. astreoides* in Bermuda found that shallow planulae translocated to a deep reef adjusted their symbiont consortia to be more similar to adults from the deep reef than the shallow reef after four weeks (Reich et al. 2017). This demonstrates that different species have different levels of fidelity to their maternal symbionts and therefore shuffling time to match the settlement environment is variable. *S. pistillata* takes at least longer than

60 days to shuffle algal symbionts to match the settlement environment. Although vertical genetic connectivity of symbiont associations appears to be limited for this species in Eilat (Scudchia et al., 2021b), it is present in other locations for other coral species (Bongaerts et al., 2017; Prada and Hellberg, 2013; Scudchia et al., 2023; Serrano et al., 2014; Studivan and Voss, 2018), warranting further investigation.

Coral host physiological and morphological acclimation was less evident than symbiont photochemical acclimation. The only statistically significant depth dependent difference detected was greater protein concentration in SD spat after 60 days (Fig. 2C). This occurred despite similar spat diameter (Fig. 3A), thus suggesting a greater tissue thickness on SD relative to SS spat. The skeletal morphological differences previously reported between deep and shallow adult colonies of this population (Einbinder et al., 2009; Kramer et al., 2022; Malik et al., 2020) were not observed in transplanted spat within the timeframe of this experiment. Such changes would have included greater corallite diameter in SS corals and likely differences in spat diameter reflecting differences in growth rate/linear extension. In a previous study, it was suggested that a lack of morphological acclimation after 45 days *ex situ* may in part be due to an absence of certain abiotic parameters such as greater wave action and flow on shallow reefs (Bellworthy et al., 2023). However, these parameters were present in this *in situ* study so instead the conclusion that 60 days is not yet sufficient time to observe skeletal morphological acclimation in early ontogeny is more likely. Alternatively, again, the shallow and deep populations of this species at this site may be so isolated that their skeletons are morphologically distinct, and acclimation does not occur even with a much longer time frame, suggesting limited morphological plasticity and a separation into two populations.

Finally, whilst acclimation to the deep phenotype is not yet complete in SD spat at 60 days old, transplanted juveniles had survival rates matching or exceeding those of SS spat (Fig. 1B), indicating that individuals could survive if shallow propagules settle on the mesophotic reef. Whether these juveniles survive to adulthood and can reproduce remains to be seen.

4.3. Gene expression patterns are life stage and depth dependent

Gene expression analysis indicated minimal overlap in genes that are differentially expressed between shallow and deep corals between each life stage (Fig. 6A and C). In other words, whilst there are significant gene expression differences between depths within each life stage, the specific genes that are differentially expressed are typically not the same, e.g., between planulae and adults. This indicates that gene expression patterns significantly depend upon life stage and that ontology likely has a stronger influence than depth upon gene expression. This finding, which is consistent with the pattern observed between planulae and adults of the coral *P. astreoides* across depths in Bermuda (Scudchia et al., 2023), concurs with previous studies that report differential gene expression with an organisms' developmental age (Arenas-Mena, 2010; Strader et al., 2018) and is reflective of the contrasting physiological processes that are most crucial at different life stages. For example, adult corals sampled in this study, were likely highly invested in reproduction and homeostasis in contrast to the planulae that would be more invested in sensing environmental cues, metamorphosis, and development, as observed in *P. astreoides* planulae compared to parental colonies (Scudchia et al., 2023). The low number of DEGs at day 8 likely indicates the presence of basic developmental processes occurring at this stage which were not influenced by transplantation (Fig. 6). However, the 53 DEGs that appear uniquely between SS and SD spat at 8 days may putatively be genes involved in the initial 'stress' response following translocation away from the parental environment (Fig. 6). The 20 DEGs shared between 8-day and 60-day spat are likely indicators of longer-term processes that are involved in depth acclimation or a lingering stress response following translocation (Fig. 6). These genes were all related to a downregulation of metabolism

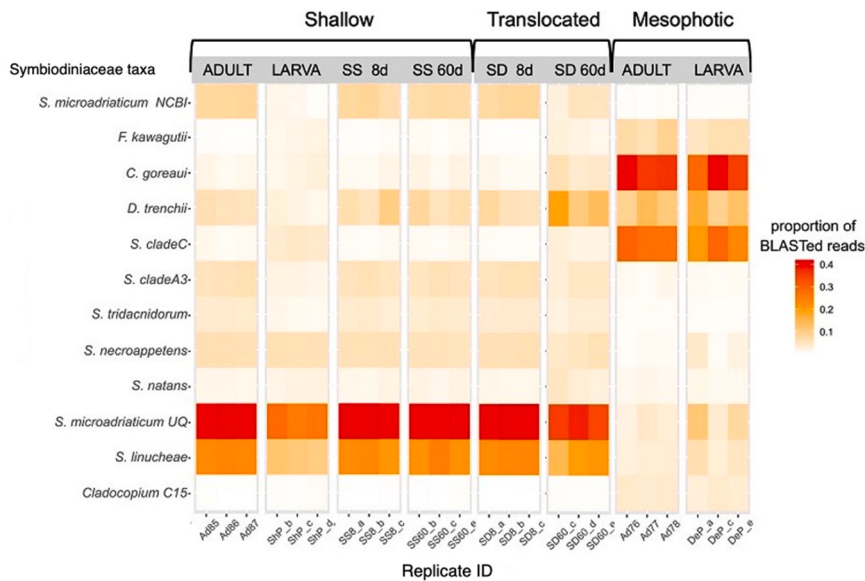


Fig. 5. Algal symbionts species identification. Origin of symbiont species sequences were detected by performing a BLASTx search using high-quality reads against open-access proteome databases of Symbiodiniaceae species. The names of the symbionts on the left correspond to the names of the species/group of each proteome source employed for the analysis (see Supplementary Table 3). According to LaJeunesse et al., 2018 these would correspond to *Symbiodinium microadriaticum* (*S. microadriaticum* NCBI, *S. clade A3*, *S. microadriaticum* UQ), *Fugacium kawagutii* (*F. kawagutii*), *Cladocopium goreau* (*C. goreau*, *S. clade C*), *Durudinium trenchii* (*D. trenchii*), *Symbiodinium tridacnidorum* (*S. tridacnidorum*), *Symbiodinium necroappetens* (*S. necroappetens*), *Symbiodinium natans* (*S. natans*), *Symbiodinium linucheae* (not present in LaJeunesse et al., 2018), *Cladocopium* sp. (*Cladocopium* C15).

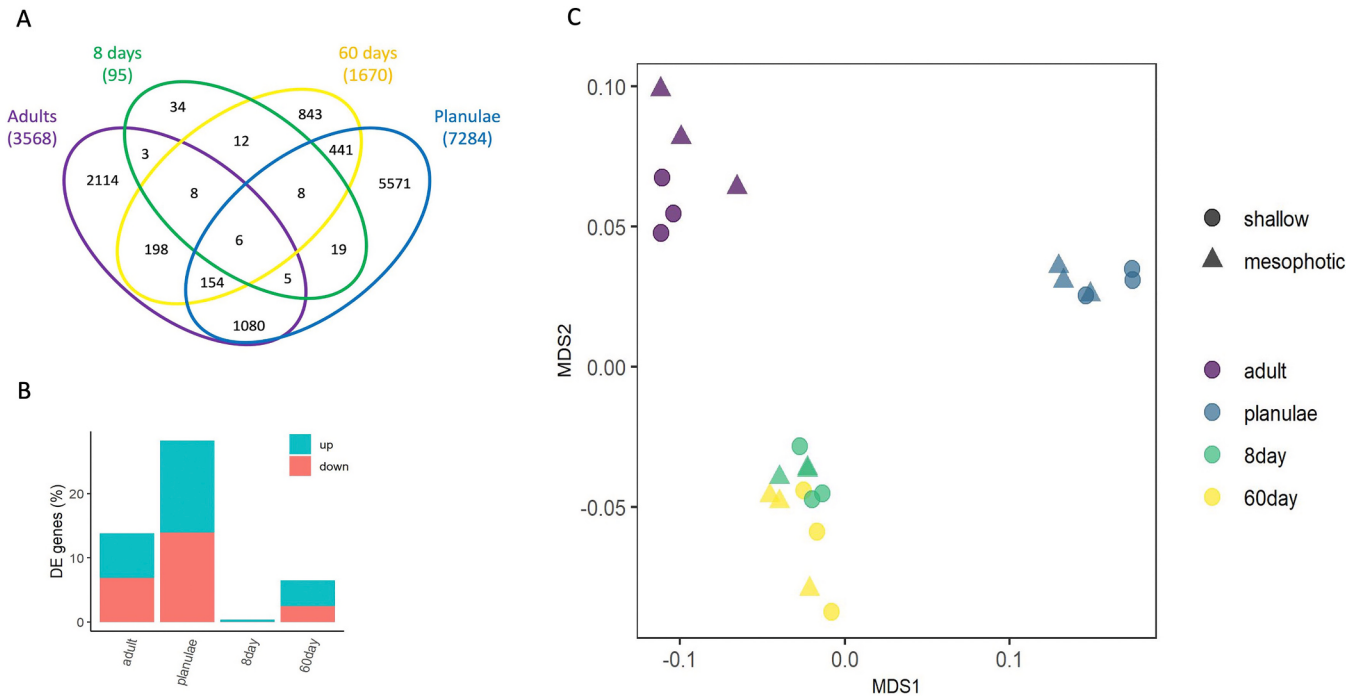
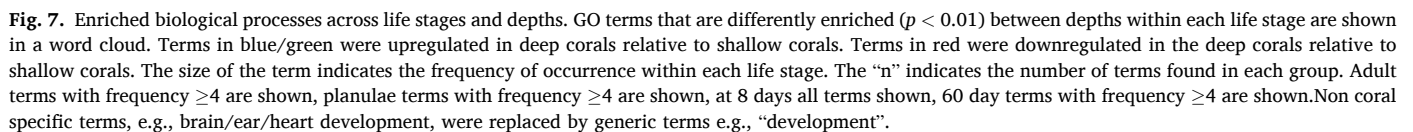


Fig. 6. Differentially expressed genes (DEGs) between corals growing at 40 m (mesophotic) and 5 m (shallow) depths at different life stages. Corals from 8 and 60 days old all originated from planulae produced at 5 m depth, with half transplanted to 40 m before settlement. (A) Venn diagram representing the total number of DEGs (up and down regulated combined) shared between life stages and treatments (number brackets = n DEG per life stage). (B) DEGs as percentage of the total transcriptome of 25,795 genes (adults 13.8 %, 8 days 0.37 %, 60 days 6.5 %, planulae 28.2 %). For each life stage ‘up’ means number of genes upregulated in deep corals relative to the shallow control and ‘down’ means downregulated in deep corals relative to the shallow control. (C) NMDS plot based on Bray Curtis dissimilarity of gene expression for all samples run on a single DEseq model (log10 FPM values, stress = 0.04081). Points are individual samples (triplicate/life stage/depth).

in deep juveniles relative to shallow juveniles (Fig. 6). Finally, 352 DEGs shared between 60-day spat and adult colonies indicate processes involved in living at mesophotic versus shallow depth. Functional

annotation indicates that this environmental effect upon the transcriptome includes upregulation of membrane processes and a down-regulation of metabolic processes in deep corals relative to shallow



Despite the strong influence of ontogeny on gene expression, there were also indications of the corals' acclimation to depth and how this progressed with age. Firstly, adults and planulae (which did not experience transplantation) had the greatest number of DEGs between depths. The overexpression of gene groups such as 'development', 'cell differentiation', and 'cell fate' in deep planulae relative to shallow planulae may be reflective of reduced settlement competency for deep origin planulae (Fig. 7); deep planulae may have still been undergoing significant developmental processes after release. Concomitantly, 'immune response' was upregulated in deep planulae relative to shallow, a group of genes we putatively link to environmental stress response. In Bermudan corals, immune response genes are upregulated in shallow versus deep *Porites asteroides* planulae (Scucchia et al., 2023) - the opposite direction to *S. pistillata* in this study. However, in Bermuda, it is shallow larvae which appear less fit and have increased mortality (Goodbody-Gringley et al., 2021; Scucchia et al., 2023) thereby linking 'immune response' to reduced survival in both studies.

particularly crucial since it has been previously established that deep planulae are smaller than shallow in this population (Bellworthy et al., 2023; Scucchia et al., 2020). In contrast, shallow planulae have an upregulated metabolism, which primes them for the metabolically demanding processes of settlement and metamorphosis. Metabolism and growth are differentially regulated in coral planulae across the depth gradient, even in the case of coral populations that are genetically connected (Scucchia et al., 2023). In the Eilat population of *S. pistillata*, metabolic differences may also arise due to the different Symbiodiniaceae populations that deep and shallow planulae host (Fig. 5). Further evidence of the influence of depth upon gene expression was the increase in the number of DEGs between 8- and 60-day old juveniles (day 8, n DEG = 6; day 60, n DEG = 705) (Fig. 6). This was witnessed despite minimal physiological and morphological acclimation even at 60 days. Juveniles at 8 days old that all originated from shallow parents had the fewest DEGs between depths but by 60 days old the number of DEGs increased, indicating the start of acclimation to distinct environments. Gene regulation plays a considerable role in phenotypic plasticity and acclimation (Rivera et al., 2021). Future studies could assess gene expression plasticity in juvenile corals as they acclimate to a settlement environment distinct from the parental environment (Kenkel and Matz,

2017).

The downregulation of metabolic functions in SD relative to SS spat present both at 8 and 60 days old may signify a reduced rate of development for SD juveniles. Though not observed within the timeframe of this experiment, this reduced metabolism may accrue to reduced growth rate, reduced size, and delayed onset of sexual maturity in deep corals. Signs of environmental stress e.g., upregulation of 'immune response', remained present at 60 days and yet were not overexpressed in deep adult colonies (Fig. 7).

5. Conclusions

Using a cross-depth settlement approach, it was found that planulae produced by mesophotic *S. pistillata* colonies had significantly lower settlement and survival compared to planulae released by shallow colonies, suggesting reduced overall fitness for mesophotic planulae. Shallow origin juveniles growing for 60 days at mesophotic depth (SD), though surviving, did not show complete physiological or morphological acclimation to reflect that of mesophotic adults. Although photochemical acclimation appears to be occurring via documented changes in photophysiology, associated shuffling of symbiont species towards the dominant symbionts hosted by mesophotic adults was incomplete within the course of this study. In addition, relative to the shallow control, shallow origin juveniles growing at mesophotic depth (SD) showed a reduced expression of metabolic genes, which likely hindered their development. Furthermore, the expression of genes such as 'immune response' and 'response to stimulus' may indicate residual environmental stress absent from native deep adult colonies. Future studies should aim to include mid-depth settlement points since, in nature, migration and acclimation to depth gradients potentially occur in stepping stones, and thus may be more successful. Such further examinations may explore coral acclimation to depth by, for example, looking at overall gene expression and phenotypic plasticity among corals and their symbionts (Kenkel and Matz, 2017; Rivera et al., 2021). Taken together, these results suggest that the mesophotic reef of Eilat is likely serving as a sink for shallow planulae rather than a source of propagules up the slope, but should recruitment down the slope occur, it may result in reduced overall fitness and resilience. Overall, this study refutes the Deep Reef Refugia Hypothesis (Bongaerts et al., 2010) for this species and location since deep origin larvae of *S. pistillata* did not survive on the shallow reef of Eilat. Indeed, deep origin larvae had high mortality rates regardless of their settlement depth. Our study further highlights the importance of mesophotic reefs as vulnerable habitats that are a critical source of genetic and phenotypic diversity for coral populations.

CRediT authorship contribution statement

Jessica Bellworthy: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Federica Scucchia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Gretchen Goodbody-Gringley:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Tali Mass:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

RNA-seq data are available on NCBI, accession number GSE250215. All other raw data and code are available on Github: https://github.com/JessicaBellworthy/Bellworthy_planulae_insitu_translocation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.172090>.

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