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Sexual reproduction and physiology of the soft coral *Sclerophytum eilatenis* along depth

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Abstract

The ability of species to thrive across environmental gradients relies on their ability for sexual reproduction and physiological adaptations to varying conditions. Advances in diving technologies have enabled in-depth research into corals ecology and biology along depth gradients, from shallow-water habitats to mesophotic coral ecosystems (MCEs; 30-150 m). Findings from such studies suggest that MCEs may act as refugia for some species, providing shelter from threats such as global climate change and local stressors. However, effective refugia necessitate that individuals within a population can both reproduce sexually and adapt physiologically along their depth range. This study investigates the reproductive biology and physiological traits of the zooxanthellate octocoral *Sclerophytum eilatenis* along a depth gradient in the Gulf of Aqaba/Eilat. By examining sexual reproduction characteristics and the physiological parameters of both host and symbiont, this study provides a comprehensive view

into this octocoral's adaptive nature. The current study's findings indicate that MCEs may serve as reproductive refugia for *S. eilatenis*, offering favorable conditions for sexual reproduction. However, significant variations in reproductive and physiological characteristics along depth underscore the necessity for careful evaluation of the potential for MCEs to assist in the recovery of shallow reef populations. This study highlights the importance of integrating reproduction and physiological data in order to advance our understanding of coral adaptation and resilience in dynamic marine environments.

Introduction

Studies of mesophotic coral ecosystems (MCEs; Hinderstein et al., 2010), particularly of their light-dependent communities, located at depths of 30 - 150 m, have received relatively little attention compared to the shallower reefs (0-30 m), that are traditionally explored by SCUBA. Accordingly, our knowledge of the ecology of benthic organisms, such as corals, is primarily based on their relatively shallow assemblages (Menza et al., 2008; Kahng et al., 2010). Enabled by major advancements in diving technologies and remotely operated vehicles (ROVs), the research of MCEs has revealed extensive, diverse, and rich coral communities, which may differ from their shallow-water counterparts (e.g., Pyle, 1996; Lesser et al., 2009; Benayahu et al., 2019; Shlesinger & Loya, 2019; Muir & Pichon, 2019). These findings have promoted hypotheses suggesting that MCEs may play a critical role in the resilience of coral populations during episodes of acute stress; and, in the aftermath, support the replenishment of shallow coral communities (Bongaerts et al., 2010; Hinderstein et al., 2010). However, such theories have also been criticised for being mostly based on the findings of high coral cover and abundances in the deep reef, which do not necessarily indicate their reproduction ability or the connectivity between the shallow and the MCE populations (Bongaerts et al., 2017). Such knowledge gaps

have highlighted the need to explicitly examine the potential adaptability of corals to the prevailing conditions along their entire depth of occurrence. It is considered that this approach will provide a better understanding of species' life-history traits and the role that MCEs play in maintaining the resilience of marine benthic ecosystems (Bongaerts & Smith, 2019; Shlesinger & Loya, 2019).

Coral-reef inhabiting octocorals encompass zooxanthellate and azooxanthellate taxa, both of which contribute to ecosystem diversity and function (Fabricius & Alderslade, 2001). The zooxanthellate taxa are quite common on the hard-bottom substrates of the tropical upper-MCEs (30-70 m: Benayahu et al., 2019). This depth zone includes some shallow-water species that feature an extended depth of distribution (i.e., depth generalists; Kahng et al., 2014), as well as species restricted to the deeper environments alone (i.e., depth specialists; Kahng et al., 2014). Notably, zooxanthellate octocorals have rarely been found in the lower-MCEs (>70 m; but see Slattery & Lesser, 2021). Despite the recent increase in the research on tropical and temperate MCEs, knowledge on their octocorals remains limited, with only a few studies having examined their reproductive biology and physiology along depth (Liberman et al., 2018; Benayahu et al., 2019; Ferrier-Pagès et al., 2022; Liberman, et al., 2022).

The life-history traits of coral communities include aspects of sexual reproduction, which are essential to their maintenance and evolution (Harrison, 2011; Randall et al., 2020; Roff, 2021). Octocorals have adapted and evolved in response to abiotic (e.g., temperature, light intensity, sedimentation, etc.) and biotic (e.g. competition, predation, diseases, etc.) gradients and their life-history strategies likely correspond to the combination of conditions in their habitat (Sammarco & Coll, 1992; Coffroth et al., 2023). Octocorals feature the following three modes of sexual reproduction: broadcasting of eggs and sperm, internal brooding of planula-larvae, and

external surface brooding (Kahng et al., 2011). Broadcasters release large numbers of male and female gametes into the water column, where fertilization occurs. Brooding is associated with internal fertilization and the subsequent release of planulae-larvae. External surface brooding is characterized by internal egg-fertilization and their release to the colony surface, where they undergo development to a planula-stage. A potentially unique type of putative brooding strategy was recently described for soft corals, wherein zygotes are released into the water (Jamodiong et al., 2024). Previous studies have indicated that the timing of octocoral reproduction is often associated with and synchronized by environmental conditions such as lunar phase, water temperature, and depth (Benayahu et al., 1990; Hellström et al., 2010; Baran & Baria-Rodriguez, 2021; Liberman, et al., 2022; Jamodiong & Reimer, 2023). Octocoral life-history traits such as fecundity and gonad diameter have been shown to decrease from the shallow waters to the MCEs, and population sex ratio may also change (Grinyó et al., 2018; Liberman et al., 2018). For light-dependent taxa such as octocorals associated with photosynthetic algal symbionts, the exponential decrease in light intensity with depth (Kahng et al., 2019), may limit their available energetic reserves which may, in turn, influence processes such as colony growth and reproduction ability (Benayahu et al., 2019). Examination of colony physiology along with their reproduction features is therefore likely to highlight adaptations to the environmental conditions along the species' depth of distribution.

The upper-MCE octocoral communities of the Gulf of Aqaba/ Eilat (northern Red Sea) are well developed, and have been shown to exhibit a substantial species diversity and abundance (Shoham & Benayahu, 2017; Benayahu et al., 2019; Liberman et al., 2022). Intrigued by these findings, the present study engages with the ocotocoral *Sclerophytum eilatenis* (Malacalcyonacea, Sarcophytidae), which was originally described from the Gulf of Aqaba/ Eilat

(van Ofwegen et al., 2013) and is currently considered as endemic to the Red Sea. Aspects of *S. eilatenis* trophic ecology and host-symbionts association have been previously reported (Ferrier-Pagès et al., 2022; Liberman et al., 2022). The present study is the first to examine this coral's sexual reproduction, addressing the hypothesis that the upper-MCE population of *S. eilatenis* will exhibit a viable sexual reproduction alongside physiological modifications along depth, from the shallow reef to the upper-MCE. Accordingly, the aims of this study were: (1) to determine the duration of the annual gonad development cycle and the reproduction season from shallow waters to the upper-MCEs; and (2) to examine the physiological traits along depth. The findings from this study enable us to confront the intriguing question of whether the MCE populations may serve as refugium for their shallow-water counterparts.

Methods

Study site and sampling procedure

Samples of *S. eilatensis* were obtained during 2018-2019 from the reef across from the Interuniversity Institute for Marine Sciences in Eilat (IUI), northern Gulf of Aqaba, Red Sea (29°30′N, 34°55′E), along the species' depth distribution of 5-65 m. This reef features a continuous hard substrate down to 10 m depth, followed by a rather a moderate slope to 30 m comprising patches of calcareous substrate over a sandy bottom. In the upper MCEs (30-70 m), the seabed is fairly flat, featuring a similar mixture of hard substrate and sandy patches occasionally covered with gravel. At this site, colonies of *S. eilatensis* may reach 10-20 cm in width and usually appear as random solitary colonies (Fig. 1). Sampling of *S. eilatensis* was carried out under a permit from the Israel Nature and Parks Authority (permit numbers: 2018-41893 and 2019-42159), limited to five fragments (~5 x 5 cm each) per month during May, June, July, and November of 2018 and 2019. The fragments were collected using SCUBA from the

shallow reef (5-15 m) and the upper MCE (40-45 m). Additional samples were collected in October 2019 from 55-65 m by a remotely operated vehicle (ROV, ECA H800) controlled from the IUI R/V Sam Rothberg using the ROV manipulator arm. All samples were individually placed in labeled Ziploc bags and transported to the IUI laboratory for further examination.

Reproductive studies

To determine the sex and reproduction status of the samples, they were fixed in 4% formaldehyde in seawater overnight, then rinsed three times using DDW, and finally preserved in 70% EtOH for further examination at Tel Aviv University. To dissolve their sclerites, the samples were decalcified for 24 hours, using a mixture composed of a 1:1 ratio of 50% formic acid in distilled water and 20% trisodium citrate in DW, washed three times using DDW, and placed back in 70% EtOH. The polyp cavities of the decalcified samples were examined under a dissecting microscope (Nikon SMZ800) and mesenteries with gonads were removed using fine scissors and forceps for wet-mount preparation. The sex of each sample was determined under a light microscope (Nikon ECLIPSE 80i) based on the observed texture of the gonads (males: granular, females: opaque, see also Mandelberg & Benayahu, 2015). When no gonads were found, the samples were classified as an indeterminate group (e.g., Liberman et al., 2018). Measurements of the diameter of oocytes and spermaries followed the sex determination and were also conducted on the wet mounts under a light microscope to a precision of 10 μm. For histological examination, decalcified samples were embedded in paraffin blocks (56° C). Serial cross-sections 6-7 μm thick, were prepared from the blocks using a microtome (MIR, SHANDON) from each block and mounted on microscope slides. The prepared slides were then stained with hematoxylin and eosin (Carson, 1997), and the gonads were examined to verify of sex determination, and their maximal diameter was taken.

Physiological studies

To examine the physiological features of *S. eilatensis,* five additional fragments were collected from both the shallow reef and the upper-MCE in early March (winter) and June (summer) 2019. In addition, in October 2019 samples were collected at 55-65 m and treated as noted above.

For the physiological studies, subsamples, 1x1 cm each, were removed from the polypary of each sample, rinsed with DDW to reduce salt concentration on the fragment surface, placed in 2 ml Eppendorf vials, then flash-frozen using liquid nitrogen, and stored at -80° for further analysis (see below). Determination of algal symbiont density, chlorophyll a concentration, protein content, and ash-free dry weight (AFDW) was performed following Pupier et al. (2018). Briefly, frozen samples were lyophilized, crushed into powder using mortar and pestle, and weighed for determination of total dry weight (DW). Powdered samples were homogenized with 1 ml of DDW and a subsample of 100 µl was extracted for the analysis of total protein. The algal cells were then separated from the host-tissue using centrifugation (700 G, 5 cycles). The chlorophyll content was extracted from the algal cells using 100% acetone, followed by 17 hours dark-incubation at 4°C, and its concentration was measured in a spectrometer at 630, 664, and 750 nm and normalized per sample DW (Jeffrey & Humphrey, 1975). The density of symbiodiniacean cells was measured using a hemocytometer under a light microscope and normalized per sample DW.

The amount of inorganic and organic matter (biomass) was determined in additional samples of *S. eilatensis* randomly collected during June-October 2019 (summer season) from three depth zones: 5-15, 40–45, and 50-65 m (12 colonies from each zone, 2-3 two cm long lobes from each colony). Following collection, the samples were stored at -80°C. The samples were Then lyophilized as described above and individually placed on aluminum foil and dried at 60°C

for two hours to determine their DW. Next, the samples were combusted at 450°C (Carbolite Gero Oven) for six hours and the remaining ash weight (AW) was determined. The samples' ash-free dry weight (AFDW) was calculated by subtracting the AW from the DW.

TEM Studies

To study the microstructural features of the symbiodiniacean cells, in particular their chloroplasts along depth gradient, several lobes were removed from the polypary of freshly collected samples of *S. eilatensis* (5-15 and 40-45 m; May 2019). Following collection, the samples were placed on ice and immediately fixed in 4% glutaraldehyde in 0.45 filtered seawater (FSW) for an overnight period (4ºC). The samples were then washed three times in DDW and transferred to 70% EtOH. They were decalcified as described above, washed three times in DDW, and placed back in 70% EtOH. The samples were later postfixed in 1% OsO4 for 2 h at 4ºC, washed in PBS, dehydrated in graded series of EtOH, incubated in propylene oxide, and then embedded in glycid ether. Thin sections were mounted on carbon-coated grids, stained with uranyl acetate and lead citrate, and observed on Jem 1200ex TEM (JEOL, Japan). Chloroplast diameter belonging to symbiodiniacean cells from *S. eilatensis* along the depth gradient was then measured (n=10 cells in each depth, 4 measurements per cell).

Statistical analysis

All statistical analyses were performed using the software R and R studio (R Core Team, 2013). Parametric tests were performed on all datasets that had satisfied the assumptions of normality of residuals and equal variance. The nonparametric Kruskal-Wallis test was used on datasets that failed to satisfy the above-noted assumptions (R package 'rstatix'; Kassambara, 2021). *S. eilatensis* oocyte diameters of 2018 and 2019 peak reproduction seasons (May-June) were

compared using a nonparametric Kruskal-Wallis test. Data on the physiological parameters of *S. eilatensis* were first log-transformed and then examined using two fixed factors: water depth and seasonality, and the interaction between them. Kruskal-Wallis test was used to examine the amount of inorganic content present in colonies collected from three depths: shallow (5-15 m), upper-MCE (40-45 m), and MCE (50-65 m). Post-hoc Dunn's test was used to assess differences among the depth groups. A linear mixed-effects model was done using the nlme package (v3.1-164; Pinheiro et al., 2021), to determine differences in the algal symbionts chloroplast diameters across depth, using depth as a fixed factor and replicates nested within colony as a random factor.

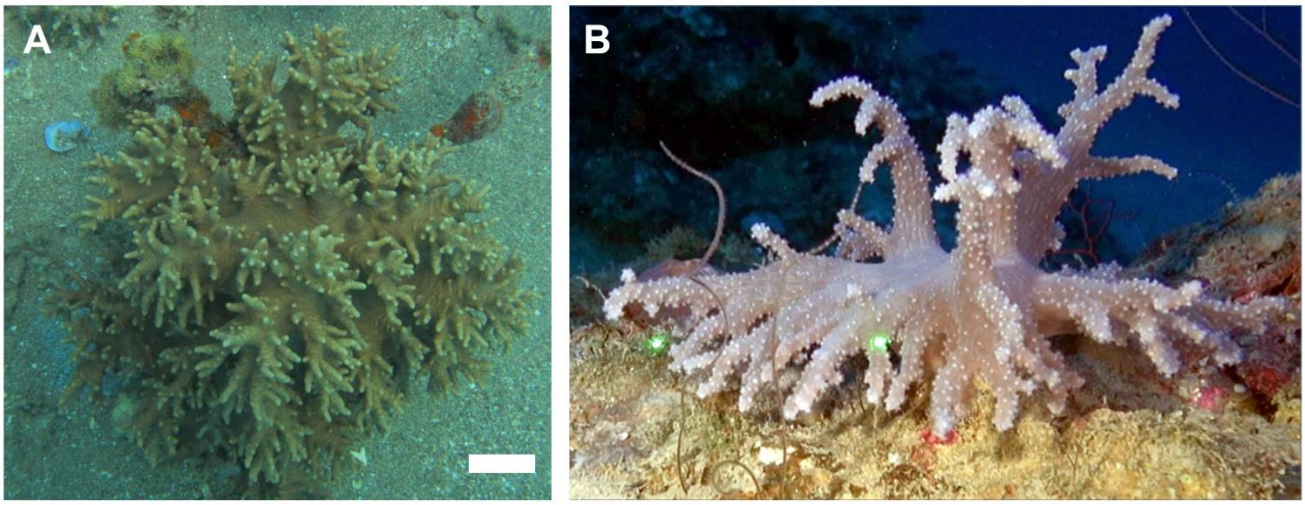


Figure 1. Sclerophytum eilatensis. Images of live colonies from (A) shallow (6 m) and (B) MCE (50 m). Scale bar in A = 5 cm. Distance between the two green points in B = 7 cm.

Results

Reproductive studies

196 The sampling of *S. eilatensis* colonies revealed their annual gonadal development (Fig. 2, Table
197 1). Oocytes were found in the samples during October 2018-July 2019 and again in November
198 2019 (Fig. 2). Concomitantly, spermaries were observed during October 2018-June 2019 (Fig.
199 2). The highest monthly mean diameter of the oocytes among the shallow colonies was recorded
200 in May 2018 (530±211 µm, n = 26 oocytes), while among the upper MCE colonies only two
201 oocytes were found in June 2018 (750±50 µm, n = 2). The highest monthly mean diameter of the
202 spermaries among the shallow colonies was recorded in May 2018 (459±66 µm, n = 11), while
203 among the upper MCE ones it was recorded in May 2019 (378±32 µm, n = 14). Maximal oocyte
204 diameter of the shallow *S. eilatensis* colonies was significantly higher than that of the upper-
205 MCE ones during both 2018 and 2019 (Fig.2, Kruskal-Wallis test, p= 0.006 and p= 0.001,
206 respectively).

207

208 Table 1. *Sclerophytum eilatensis*. Comparison of the reproductive features of the shallow-water
209 colonies (5-15 m) with the upper MCE (40-45 m) colonies.

Reproductive feature	Shallow colonies	Upper MCE colonies
Duration of spermatogenesis	November-June	October-May
Duration of oogenesis	October[†]-July	October-June
Maximal average spermary diameter (µm)	459±66, n=11*	378±32, n=14*
Maximal average oocyte diameter (µm)	530±211 (n=26)	750±50 (n=2)
Sex ratio (males: females)	1:1.36	1:3.75

Estimated spawning time	May-June	May-June

[†]Estimated month, * significantly different ($p < 0.05$, for details see Materials and Methods).

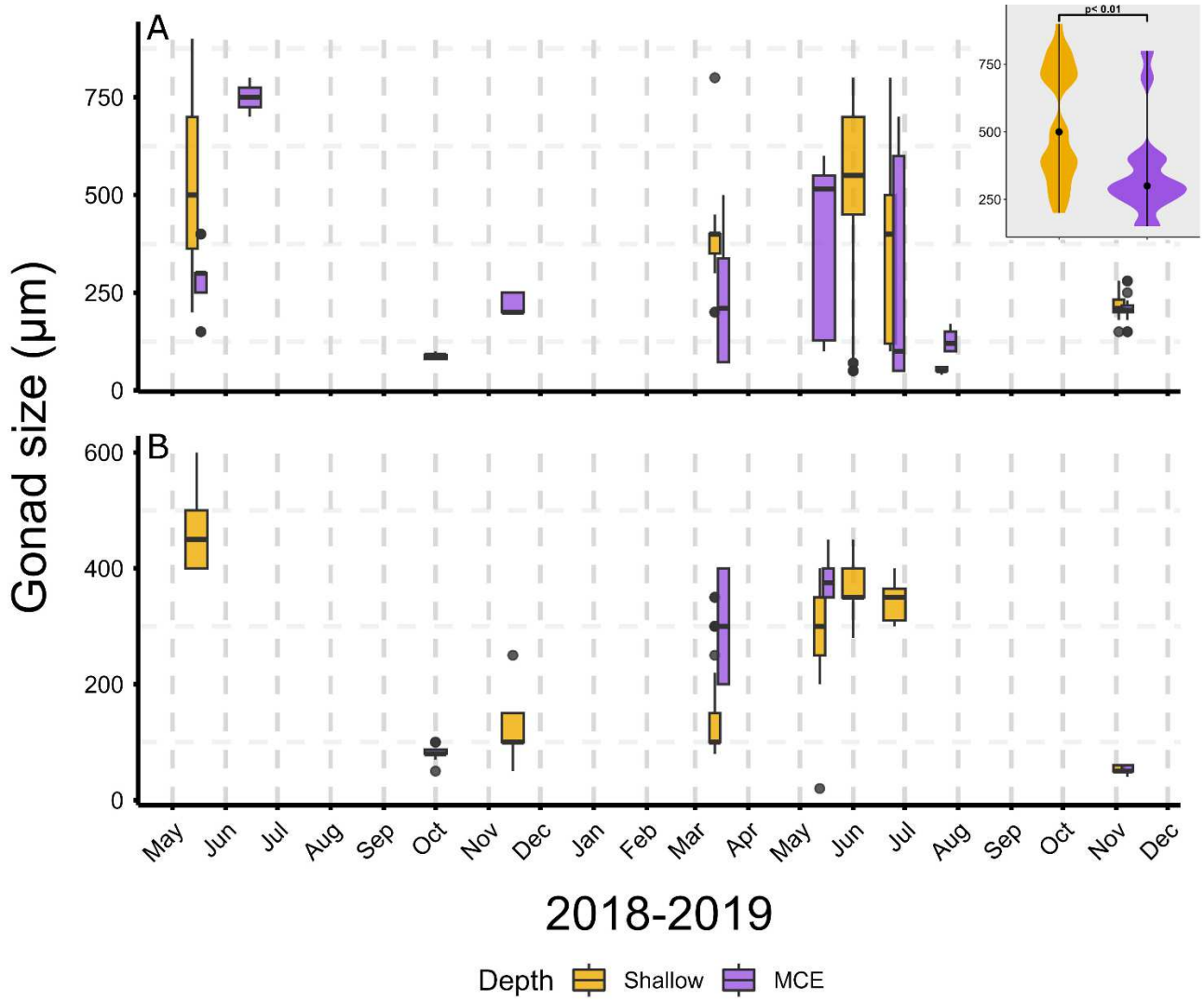


Figure 2. *Sclerophytum eilatensis*. Monthly average gonad diameter of shallow-water (in orange) and upper-MCE (in purple) colonies during May 2018-December 2019. (A) Oocytes and (B) Spermaries. Box limits represent 25th and 75th percentiles, whiskers span upper and lower limits of data, horizontal lines within boxplots represent medians, and above x-axis number of gonads measured each month. The upper-right density plot represents all the data for oocytes recorded in 2019 peak reproduction season (May-June); points represent average value.

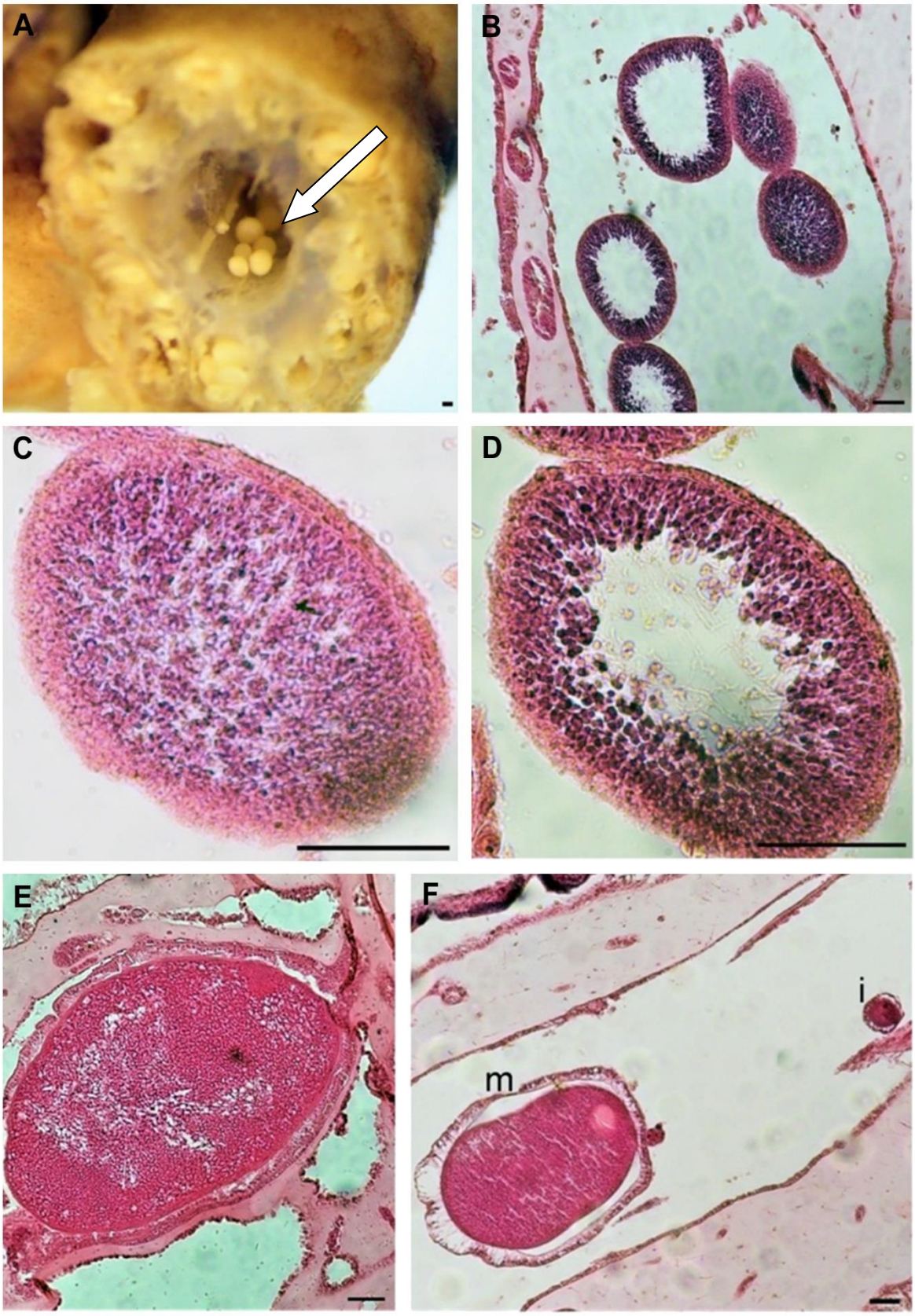

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Figure 3. Gonads of shallow-reef and upper-MCE *Sclerophytum eilatensis*. (A) Colony containing spermaries in gastrovascular cavity (March 2018). (B–D) Histological section of gastrovascular cavity with spermaries. C. Pre-mature spermary. D. Mature spermary with spermatids located at its periphery (E) Cross-section of oocyte (May 2019). (F) Mature oocyte and its nucleus. All scale bars are 50 μm.

Physiology of *S. eilatensis*

Data normalized to DW revealed that Symbiodiniaceae cell density in *S. eilatensis* tissues was significantly higher during the summer season for both study depths (Fig. 4A, Two-way ANOVA test, $p= 0.03$), while protein, chlorophyll a content, and chlorophyll a per algal cell remained similar across depth and sampling dates (Fig. 4B-D, Two-way ANOVA test). In contrast, the findings revealed a constant increase in the average inorganic matter of the tissues along the depth gradient, and, consequently, a decrease in the organic content (Fig. 4E, Kruskal-Wallis test, $df = 36$, chi square = 25.3, $p< 0.001$). A pairwise comparison of the inorganic content in *S. eilatensis* for the three depth zones revealed that colonies at 5-15 m and 40-45 m had significantly less inorganic matter compared to those at 50-65 m (Fig. 4E, Dunn's test, $n= 12$, $p< 0.001$ for both test groups).

TEM examination of chloroplast diameter within *S. eilatensis* symbiotic algal cells revealed significant differences between shallow waters and upper-MCE colonies (Fig. 4F, Linear mixed-effects model, $F = 55.25$, $p< 0.001$). The mean chloroplast diameter of the algal cells in the upper-MCE was significantly higher than that found in the shallow reef ones (Fig. 4F, 0.91±0.34, 1.49±0.5 μm, respectively).

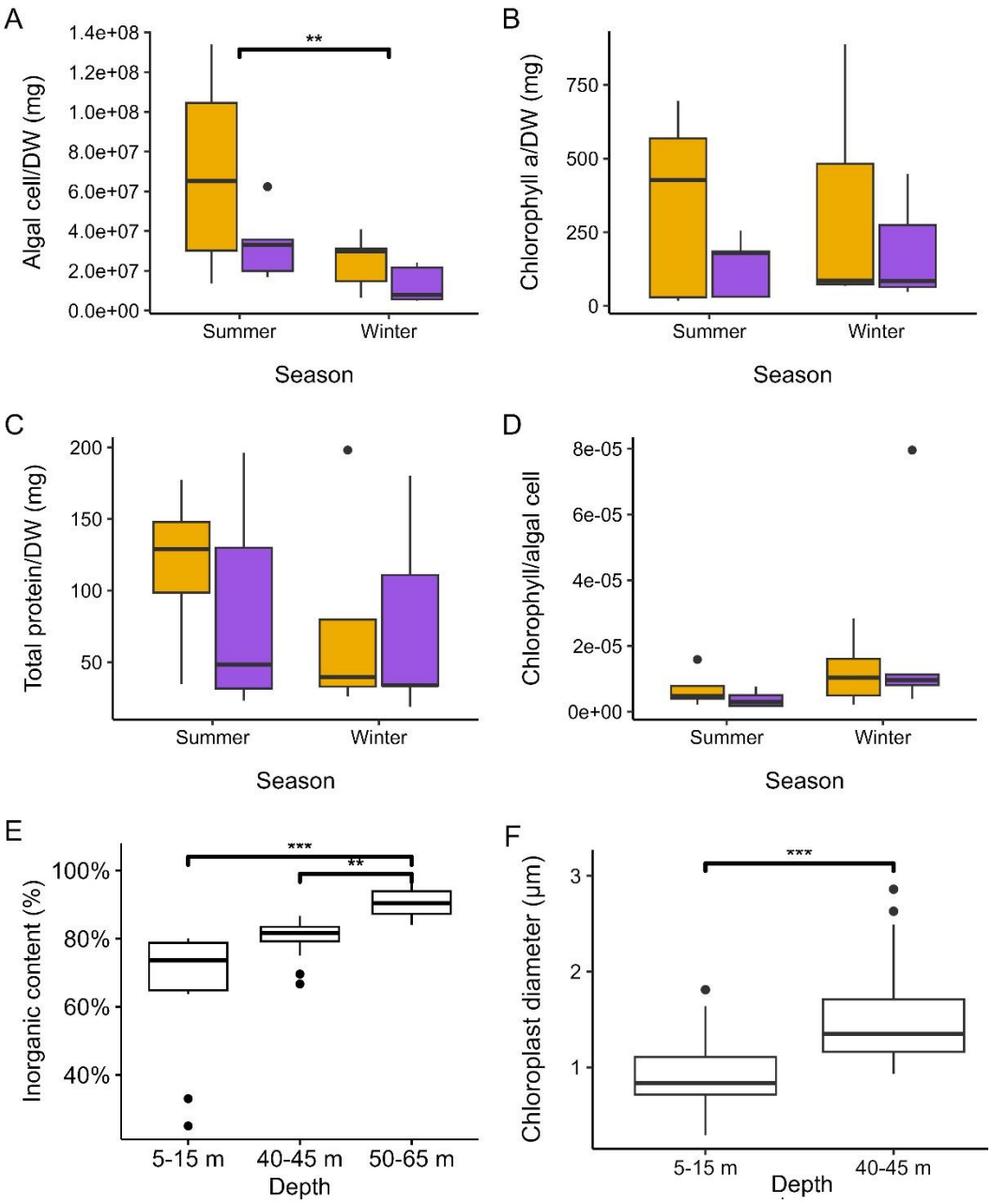


Figure 4. *Sclerophytum eilatensis*. Physiological parameters of shallow-water (in orange) and upper-MCE (in purple) (A) Symbiodiniaceae cell density per dry weight, (B) Chlorophyll a per dry weight; (C) Total protein content per dry weight (D) Chlorophyll a per Symbiodiniaceae cell normalized to dry weight (E) Percentage of inorganic matter per sample. (F) Chloroplast diameter. Specific sampling months: March (Winter) and June (Summer). **p< 0.05; ** p< 0.001, Kruskal-Wallis test.

Discussion

This study provides for the first time comparative data on the reproduction and physiological features of the soft coral *S. eilatensis* for the shallow and upper MCE, down to 65 m, in the Gulf of Aqaba/Eilat. Although traits are often studied separately, an integrated analysis of sexual reproduction traits alongside parameters pertinent to the host and symbiont physiology, as carried out here, offers a comprehensive understanding of species' adaptations along environmental gradients (Gori et al., 2012; Grinyó et al., 2018). Using this approach, the findings from this study provide evidence supporting the assumption that MCEs could indeed serve as reproductive refugium for the soft coral *S. eilatensis*. The discernible differences in several reproductive and physiological characteristics, however, call for careful consideration when evaluating the potential of such refugia in maintaining the resilience of populations in shallow-reef environments.

Reproduction studies

S. eilatensis is a gonochoric species featuring an annual gonad development and their inferred maturation in May-June. The annual male and female gonad development of the shallow and upper-MCE populations (5-15 and 40-45 m, respectively) were found to be closely synchronized, with initiation of annual oocyte development in October, achieving maturity during May-June (Fig. 2, Table 1). A similar synchronicity of gamete development along the depth gradient was recorded for the internal brooder *Ovabunda* sp. (Xeniidae) in the Gulf of Aqaba/Eilat (=*Xenia macrospiculata*: Benayahu & Loya 1984, Liberman et al. 2018). Synchronous timing of gonad development was also found in the northwestern Mediterranean population of the gorgonian *Eunicella singularis* (Gori et al., 2012). In contrast, gonad development of the surface-brooder

Rhytisma fulvum exhibits a delay in maturation from shallow to mesophotic depths (Liberman et al., 2018, 2022). A time difference in gonad maturation across depth was also reported for the soft coral *Sarcophyton glaucum* colonies in the northern Red Sea (Benayahu & Loya, 1986). The results of the current study suggest that annual gonad development of *S. eilatensis* populations exhibit a synchronicity along depth that is comparable to certain other octocoral species, and to most scleractinian ones whose sexual reproduction has been studied from shallow to mesophotic depths (Shlesinger & Loya, 2019). The findings indicate that the shallow female colonies of *S. eilatensis* produce significantly larger oocytes than the upper-MCE ones (Fig. 2, Table 1). A decrease in the maximal oocyte diameter from shallow to mesophotic depth is common among both octocorals and scleractinians (Benayahu et al., 2019; Shlesinger & Loya, 2019). For example, the Caribbean *Briareum asbestinum* at 35 m features smaller oocytes compared to those at 5 m (West et al., 1993). A significant decrease in oocyte size was also recorded in four species of scleractinian corals from the Gulf of Aqaba/Eilat (Shlesinger et al., 2018). As light is the primary energy source for zooxanthellate corals, its logarithmic decrease with depth has been suggested to affect several coral reproduction parameters, including oocyte size (Lesser et al., 2009; Shlesinger & Loya, 2019). Furthermore, whilst some corals may compensate through increased heterotrophy in lower light (Slattery et al., 2011), this does not occur in *Sclerophytum* sp. from the Gulf of Aqaba/ Eilat (Ferrier-Pagès et al., 2022). Therefore, it is suggested here that the reduced oocyte size found with depth clearly indicates a reduced reproductive performance for the MCE population of *S. eilatensis* due to energetic constraints.

The finding of differing sex ratios in *S. eilatensis* populations along a depth gradient, with balanced ratios (i.e., not different than 1:1) in the shallow-reef population, and a female-skewed one in the upper MCE population, is indeed intriguing (1:1.6 and 1:3.75, respectively. Table 1).

297 Such differences are suggested to result from differences in the energetic requirements needed
298 for both reproduction and growth for each sex along the depth gradient, a factor that was
299 reported to drive skewed sex ratios among octocorals (Santangelo et al., 2003; Porcu et al., 2017;
300 Liberman et al., 2018). In general, octocoral population sex ratios have been shown to vary
301 among taxa, although a balanced or female-skewed ratio is also commonly observed (Kahng et
302 al., 2011). It should be noted that only a limited number of octocoral species have been studied
303 along depth, and a common pattern has not yet been defined. For example, in contrast to the
304 current results, *Rhytisma fulvum* was reported to shift from a balanced sex ratio in Eilat's shallow
305 reefs to a male-skewed one in the upper MCE there (Liberman et al. 2018). In contrast,
306 *Corallium rubrum* in the Mediterranean Sea exhibits a female-skewed sex ratio in the upper-
307 MCE, becoming balanced at the lower-MCE depths (Porcu et al., 2017). In addition to energetic
308 constraints, it should be noted that asexual propagation resulting in clone colonies which may
309 also result in a skewed sex ratio (Brazeau & Lasker, 1989; Kahng et al., 2011). Further research
310 quantifying reproduction and growth costs for each sex along depth gradients, together with
311 sampling over longer time periods, is undoubtedly needed in order to elucidate the mechanisms
312 generating the overall reproductive features of *S. eilatensis*.

313 **Physiological studies**

314 The current findings have revealed only slight differences in several key physiological
315 parameters of *S. eilatensis* between the shallow and the mesophotic depths, during the different
316 sampling months. Algal symbiont density, chlorophyll a content, protein content, and chlorophyll
317 a per symbiont cell showed no significant differences between the shallow and MCE populations
318 (Fig. 4A-D). This lack of major differences is suggested to be of adaptive value to *S. eilatensis,*
319 enabling its adjustment to lower light without altering its fundamental physiological

characteristics. Notably, these characteristics of *S. eilatensis* in the upper-MCE closely resemble those of the depth-specialist *Sclerophytum mesophoticum*, described from Eilat at 62-65 m (Benayahu et al., 2017; Tamir, 2023), thus indicating that S. *mesophoticum* is adapted to the MCE environment. Such findings stand in contrast to previous studies that found depth-related differences in algal density and chlorophyll content for the octocorals *R. fulvum* and *Litophyton* sp. in the Gulf of Aqaba/Eilat (Pupier et al., 2019). However, the latter species has a narrower depth distribution compared to *S. eilatensis* (pers. obs.). Thus, whereas the prevailing light conditions at 40-45 m may still be sufficient for *S. eilatensis,* they are limiting for other zooxanthellate octocorals. Despite similar chlorophyll content, evidence of increased chloroplast diameter with depth was found for *S. eilatenis* (Fig. 4E), corresponding to the finding of expanded thylakoid membranes under low light conditions in the MCE population of the scleractinian *Stylophora pistillata* (Martinez et al., 2020), as well as in cyanobacteria under experimental conditions (Kolodny, 2020). Such finding reflects a phenotypic plasticity that may enable the maintenance of photosynthesis under attenuated light conditions through photophysiological adaptation (Kahng et al., 2019; Lesser et al., 2009). Furthermore, a recent study revealed that *S. eilatensis* colonies host different Symbiodiniaceae populations across depth, with ITS2 profile C65/C65a dominating shallow colonies and C1-C3dx dominating the upper-MCE ones (Liberman et al., 2022). Further examination is still clearly required in order to determine whether the depth adaptations are driven by the differences in the algal symbiont taxa, or by environmental conditions. Taken together, S. *eilatensis* exhibits an adaptation ability capacity across its broad depth range, which likely facilitates its niche expansion into the mesophotic zone and thus makes this species a depth-generalist.

342 The current study has revealed a significant decrease in organic content in *S. eilatensis*
343 with depth (Fig. 4F), reflecting morphological differences along depth. While depth-related
344 adaptations have been studied in scleractinians, there is only limited information pertaining to
345 octocorals, leaving the adaptive nature of their morphology along depths unclear. A study by
346 Ferrier-Pages et al. (2022) revealed high C:N ratios in deeper (60-65 m) *Sclerophytum* sp.
347 colonies compared to the known ratios in shallower scleractinians and those at similar depths,
348 indicating nitrogen limitation. Hence, our findings suggest that the upper MCE colonies of *S.*
349 *eilatensis* may experience energy limitation, thus leading to decreased organic content in the
350 tissue with increasing depth. Reciprocal transplant experiments between shallow and deep
351 habitats are needed in order to confirm this hypothesis. Overall, our findings reveal depth-related
352 physiological changes in *S. eilatensis*, aligning with preliminary evidence of resource constraints
353 in MCE octocorals that may shape their morphological adaptation along the depth gradient.

354 The present work emphasizes the need for further comprehensive studies on the reproduction and
355 physiology of octocorals across their entire range of distribution, in order to validate the
356 implications of the respective traits. While the present findings offer a promising avenue for
357 future investigations, they also highlight the need for continuing research in this field in order to
358 more fully unravel the complexities of octocoral adaptations across different reef environments.

359 **Author contributions**

360 RL assisted in the field work, analyzed the data, and wrote the manuscript, with contributions
361 from all authors. YB led the research topic, provided financial support, contributed to data
362 interpretation and to the writing of the manuscript. ST designed and conducted the field and
363 laboratory work and contributed to data analysis.

References

Baran, C. C., & Baria-Rodriguez, M. V. (2021). Sexual reproduction in the soft coral *Lobophytum schoedei* in Bolinao-Anda reef complex, Pangasinan, northwestern Philippines. *Invertebrate Biology*, *140*(2), e12316. https://doi.org/10.1111/ivb.12316

Benayahu, Y., & Loya, Y. (1984). Life history studies on the Red Sea soft coral *Xenia macrospiculata* Gohar, 1940. I. Annual dynamics of gonadal development. The Biological Bulletin, 166(1), 32-43.

Benayahu, Y., & Loya, Y. (1986). Sexual reproduction of a soft coral: Synchronous and brief annual spawning of *Sarcophyton glaucum* (quoy & gaimard, 1833). *The Biological Bulletin*, *170*(1), 32–42. https://doi.org/10.2307/1541378

Benayahu, Y., Weil, D., & Kleinman, M. (1990). Radiation of broadcasting and brooding patterns in coral reef alcyonaceans. *Advances in Invertebrate Reproduction*, *5*, 323–328.

Bongaerts, P., Ridgway, T., Sampayo, E. M., & Hoegh-Guldberg, O. (2010). Assessing the 'deep reef refugia' hypothesis: Focus on Caribbean reefs. *Coral Reefs*, *29*(2), 309–327. https://doi.org/10.1007/s00338-009-0581-x

Benayahu, Y., Bridge, T. C. L., Colin, P. L., Liberman, R., McFadden, C. S., Pizarro, O., Schleyer, M. H., Shoham, E., Reijnen, B. T., Weis, M., & Tanaka, J. (2019). Octocorals of the Indo-Pacific. In Y. Loya, K. A. Puglise, & T. C. L. Bridge (Eds.), *Mesophotic Coral Ecosystems* (pp. 709–728). Coral Reefs of the World, vol 12. Springer, Cham. https://doi.org/10.1007/978-3-319-92735-0_38

Bongaerts, P., Riginos, C., Brunner, R., Englebert, N., Smith, S. R., & Hoegh-Guldberg, O. (2017). Deep reefs are not universal refuges: Reseeding potential varies among coral species. *Science Advances*, *3*(2), e1602373. https://doi.org/10.1126/sciadv.1602373

Bongaerts, P., & Smith, T. B. (2019). Beyond the “Deep Reef Refuge” Hypothesis: A Conceptual Framework to Characterize Persistence at Depth. In Y. Loya, K. A. Puglise, & T. C. L. Bridge (Eds.), *Mesophotic Coral Ecosystems* (pp. 881–895). Coral Reefs of the World, vol 12. Springer, Cham. https://doi.org/10.1007/978-3-319-92735-0_45

Brazeau, D. A., & Lasker, H. R. (1989). The Reproductive Cycle and Spawning in a Caribbean Gorgonian. *The Biological Bulletin*, *176*(1), 1–7. https://doi.org/10.2307/1541882

Carson, F. L. (1997). Histotechnology: A Self-Instructional Text. Chicago, IL: ASCP Press.

Coffroth, M. A., Buccella, L. A., Eaton, K. M., Lasker, H. R., Gooding, A. T., & Franklin, H. (2023). What makes a winner? Symbiont and host dynamics determine Caribbean octocoral resilience to bleaching. *Science Advances*, *9*(47), eadj6788. https://doi.org/10.1126/sciadv.adj6788

Ferrier-Pagès, C., Bednarz, V., Grover, R., Benayahu, Y., Maguer, J.-F., Rottier, C., Wiedenmann, J., & Fine, M. (2022). Symbiotic stony and soft corals: Is their host-algae relationship really mutualistic at lower mesophotic reefs? *Limnology and Oceanography*, *67*(1), 261–271. https://doi.org/10.1002/lno.11990

Gori, A., Viladrich, N., Gili, J.-M., Kotta, M., Cucio, C., Magni, L., Bramanti, L., & Rossi, S. (2012). Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea). *Coral Reefs*, *31*(3), 823–837. https://doi.org/10.1007/s00338-012-0904-1

Grinyó, J., Viladrich, N., Díaz, D., Muñoz, A., Mallol, S., Salazar, J., Castillo, R., Gili, J.-M., & Gori, A. (2018). Reproduction, energy storage and metabolic requirements in a

mesophotic population of the gorgonian *Paramuricea macrospina*. *PLOS ONE*, *13*(9), e0203308. https://doi.org/10.1371/journal.pone.0203308

Harrison, P. L. (2011). Sexual Reproduction of Scleractinian Corals. In Z. Dubinsky & N. Stambler (Eds.), *Coral Reefs: An Ecosystem in Transition* (pp. 59–85). Springer Netherlands. https://doi.org/10.1007/978-94-007-0114-4_6

Hellström, M., Kavanagh, K. D., & Benzie, J. A. H. (2010). Multiple spawning events and sexual reproduction in the octocoral *Sarcophyton elegans* (Cnidaria: Alcyonacea) on Lizard Island, Great Barrier Reef. *Marine Biology*, *157*(2), 383–392. https://doi.org/10.1007/s00227-009-1325-8

Hinderstein, L. M., Marr, J. C. A., Martinez, F. A., Dowgiallo, M. J., Puglise, K. A., Pyle, R. L., Zawada, D. G., & Appeldoorn, R. (2010). Theme section on "Mesophotic Coral Ecosystems: Characterization, Ecology, and Management." Coral Reefs, 29(2), 247–251. https://doi.org/10.1007/s00338-010-0614-5

Jamodiong, E.A., Tabalanza, T.D., Azuma-Malsol, M.A. et al. (2024). Spawn release of a *Sclerophytum* soft coral species in Okinawa Island, Japan. Mar. Biodivers. 54, 24. https://doi.org/10.1007/s12526-024-01417-9

Jamodiong, Emmeline A., and James Davis Reimer. (2023). Reproductive characteristics and gamete development of the soft coral *Sclerophytum cf. heterospiculatum* in Okinawa Island, Japan. Invertebrate Biology, 142.2: e12404.

Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemie Und Physiologie Der Pflanzen*, *167*(2), 191–194. https://doi.org/10.1016/S0015-3796(17)30778-3

Kahng, S., Benayahu, Y., & Lasker, H. (2011). Sexual reproduction in octocorals. *Marine Ecology Progress Series*, *443*, 265–283. https://doi.org/10.3354/meps09414

Kahng, S., Copus, J., & Wagner, D. (2014). Recent advances in the ecology of mesophotic coral ecosystems (MCEs). *Current Opinion in Environmental Sustainability*, *7*, 72–81. https://doi.org/10.1016/j.cosust.2013.11.019

Kahng, S., Akkaynak, D., Shlesinger, T., Hochberg, E. J., Wiedenmann, J., Tamir, R., & Tchernov, D. (2019). Light, Temperature, Photosynthesis, Heterotrophy, and the Lower Depth Limits of Mesophotic Coral Ecosystems. In Y. Loya, K. A. Puglise, & T. C. L. Bridge (Eds.), *Mesophotic Coral Ecosystems* (pp. 801–828). Coral Reefs of the World, vol 12. Springer, Cham. https://doi.org/10.1007/978-3-319-92735-0_42

Kahng, S., Garcia-Sais, J. R., Spalding, H. L., Brokovich, E., Wagner, D., Weil, E., Hinderstein, L., & Toonen, R. J. (2010). Community ecology of mesophotic coral reef ecosystems. *Coral Reefs*, *29*, 255–275.

Kassambara, A. (2021). rstatix: Pipe-friendly framework for basic statistical tests. R package version 0.7. 0.

Kolodny, Y. (2020). *Marine cyanobacteria tune energy transfer efficiency in their light-harvesting antennae by modifying pigment coupling—Kolodny—2021—The FEBS Journal—Wiley Online Library*. https://febs.onlinelibrary.wiley.com/doi/10.1111/febs.15371

Lesser, M. P., Slattery, M., & Leichter, J. J. (2009). Ecology of mesophotic coral reefs. *Journal of Experimental Marine Biology and Ecology*, *375*(1–2), 1–8. https://doi.org/10.1016/j.jembe.2009.05.009

Liberman, R., Benayahu, Y., & Huchon, D. (2022). Octocorals in the Gulf of Aqaba exhibit high photosymbiont fidelity. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.1005471

Liberman, R., Shlesinger, T., Loya, Y., & Benayahu, Y. (2018). Octocoral Sexual Reproduction: Temporal Disparity Between Mesophotic and Shallow-Reef Populations. *Frontiers in Marine Science*, *5*, 445. https://doi.org/10.3389/fmars.2018.00445

Liberman, R., Shlesinger, T., Loya, Y., & Benayahu, Y. (2022). Soft coral reproductive phenology along a depth gradient: Can "going deeper" provide a viable refuge? *Ecology*, *103*(9). https://doi.org/10.1002/ecy.3760

Martinez, S., Kolodny, Y., Shemesh, E., Scucchia, F., Nevo, R., Levin-Zaidman, S., Paltiel, Y., Keren, N., Tchernov, D., & Mass, T. (2020). Energy Sources of the Depth-Generalist Mixotrophic Coral Stylophora pistillata. *Frontiers in Marine Science*, *7*. https://www.frontiersin.org/articles/10.3389/fmars.2020.566663

Menza, C., Kendall, M., & Hile, S. (2008). The deeper we go the less we know. *Revista de Biología Tropical*, *56*(S1), 11–24.

Muir, P. R., & Pichon, M. (2019). Biodiversity of Reef-Building, Scleractinian Corals. In Y. Loya, K. A. Puglise, & T. C. L. Bridge (Eds.) *Mesophotic Coral Ecosystems*. Coral Reefs of the World, vol 12. Springer, Cham. https://doi.org/10.1007/978-3-319-92735-0_33

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2021). nlme: Linear and Nonlinear Mixed Effects Models. https://CRAN.R-project.org/package=nlme

Porcu, C., Follesa, M. C., Cannas, R., Cau, Al., Soldovilla, G., Pesci, P., & Cau, An. (2017). Reproductive patterns in deep versus shallow populations of the precious Mediterranean

476 gorgonian *Corallium rubrum* (Linnaeus, 1758) (Sardinia, central-western Mediterranean).
477 *Mediterranean Marine Science*, *18*(1), 64. https://doi.org/10.12681/mms.1854
478 Pupier, C. A., Bednarz, V. N., & Ferrier-Pages, C. (2018). Studies with soft corals–
479 recommendations on sample processing and normalization metrics. Frontiers in Marine
480 Science, 5, 348.
481 Pupier, C. A., Fine, M., Bednarz, V. N., Rottier, C., Grover, R., & Ferrier-Pagès, C. (2019).
482 Productivity and carbon fluxes depend on species and symbiont density in soft coral
483 symbioses. *Scientific Reports*, *9*(1), 17819. https://doi.org/10.1038/s41598-019-54209-8
484 Pyle, R. (1996). How much coral reef biodiversity are we missing? *Global Biodiversity*, *6*, 3–7.
485 R Core Team, R. (2013). R: A language and environment for statistical computing.
486 Randall, C. J., Negri, A. P., Quigley, K. M., Foster, T., Ricardo, G. F., Webster, N. S., Bay, L. K.,
487 Harrison, P. L., Babcock, R. C., & Heyward, A. J. (2020). Sexual production of corals for
488 reef restoration in the Anthropocene. *Marine Ecology Progress Series*, *635*, 203–232.
489 Roff, G. (2021). Evolutionary history drives biogeographic patterns of coral reef resilience.
490 *BioScience*, *71*(1), 26–39. https://doi.org/10.1093/biosci/biaa145
491 Sammarco, P. W., & Coll, J. C. (1992). Chemical adaptations in the Octocorallia: Evolutionary
492 considerations. *Marine Ecology-Progress Series*, *88*, 93–93.
493 Santangelo, G., Carletti, E., Maggi, E., & Bramanti, L. (2003). Reproduction and population
494 sexual structure of the overexploited Mediterranean red coral *Corallium rubrum*. *Marine*
495 *Ecology Progress Series*, *248*, 99–108. https://doi.org/10.3354/meps248099
496 Shlesinger, T., Grinblat, M., Rapuano, H., Amit, T., & Loya, Y. (2018). Can mesophotic reefs
497 replenish shallow reefs? Reduced coral reproductive performance casts a doubt. *Ecology*,
498 *99*(2), 421–437. https://doi.org/10.1002/ecy.2098

Shlesinger, T., Loya, Y. (2019). Sexual Reproduction of Scleractinian Corals in Mesophotic Coral Ecosystems vs. Shallow Reefs. In: Loya, Y., Puglise, K., Bridge, T. (eds) *Mesophotic Coral Ecosystems*. Coral Reefs of the World, vol 12. Springer, Cham. https://doi.org/10.1007/978-3-319-92735-0_35

Shoham, E., & Benayahu, Y. (2017). Higher species richness of octocorals in the upper mesophotic zone in Eilat (Gulf of Aqaba) compared to shallower reef zones. *Coral Reefs*, *36*(1), 71–81. https://doi.org/10.1007/s00338-016-1528-7

Slattery, M., & Lesser, M. P. (2021). Gorgonians are foundation species on sponge-dominated mesophotic coral reefs in the Caribbean. *Frontiers in Marine Science*, *8*. https://www.frontiersin.org/articles/10.3389/fmars.2021.654268

Slattery, M., Lesser, M. P., Brazeau, D., Stokes, M. D., & Leichter, J. J. (2011). Connectivity and stability of mesophotic coral reefs. *Journal of Experimental Marine Biology and Ecology*, *408*(1–2), 32–41. https://doi.org/10.1016/j.jembe.2011.07.024

Tamir, S. (2023). A comparison of the reproductive, physiological and morphological traits of mesophotic *Sclerophytum* octocoral species with those of their shallow-water congenerics. Thesis submitted towards the M.Sc. degree in Ecology and Environmental Studies at Tel-Aviv University, 60 pp.

van Ofwegen, L. P., Benayahu, Y., & McFadden, C. S. (2013). *Sinularia leptoclados* (Ehrenberg, 1834) (Cnidaria, Octocorallia) re-examined. *ZooKeys*, *272*, 29–59. https://doi.org/10.3897/zookeys.272.4406

West, J. M., Harvell, C. D., & Walls, A. M. (1993). Morphological plasticity in a gorgonian coral (*Briareum asbestinum*) over a depth cline. Marine Ecology-Progress Series, 94, 61-61.

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