

1 **RUNNING HEAD: More than a depth gradient**

2 **Depth alone is an inappropriate proxy for physiological**
3 **change in the mesophotic coral *Agaricia lamarcki*.**
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16 *ABSTRACT:*

17 *The physiology of mesophotic Scleractinia varies with depth in response to environmental*
18 *change. Previous research has documented trends in heterotrophy and photosynthesis with*
19 *depth, but has not addressed between-site variation for a single species. Environmental*
20 *differences between sites at a local scale and heterogeneous microhabitats, because of*
21 *irradiance and food availability, are likely important factors when explaining the occurrence*
22 *and physiology of Scleractinia. Here, 108 colonies of *Agaricia lamarcki* were sampled from*
23 *two locations off the coast of Utila, Honduras, distributed evenly down the observed 50 m*
24 *depth range of the species. We found that depth alone was not sufficient to fully explain*
25 *physiological variation. Pulse Amplitude -Modulation fluorometry and stable isotope*
26 *analyses revealed that trends in photochemical and heterotrophic activity with depth varied*
27 *markedly between sites. Our isotope analyses do not support an obligate link between*
28 *photosynthetic activity and heterotrophic subsidy with increasing depth. We found that *A.**
29 *lamarcki colonies at the bottom of the species depth range can be physiologically similar to*
30 *those nearer the surface. As a potential explanation, we hypothesise sites with high*
31 *topographical complexity, and therefore varied microhabitats, may provide more*
32 *physiological niches distributed across a larger depth range. Varied microhabitats with*
33 *depth may reduce the dominance of depth as a physiological determinant. Thus, *A. lamarcki**
34 *may 'avoid' changes in environment with depth, by instead existing in a subset of favourable*
35 *niches. Our observations correlate with site-specific depth ranges, advocating for linking*
36 *physiology and abiotic profiles when defining the distribution of mesophotic taxa.*

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38 **KEYWORDS:** Mesophotic, Physiology, Depth, Isotopes, Photosynthesis, Heterotrophy

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1 INTRODUCTION:

Mesophotic coral ecosystems (MCEs) are zooxanthellate coral reefs widely considered to occur from between 30 - 40 m to at least 150m depth (Puglise *et al.*, 2009; Kahng *et al.*, 2014; Baker *et al.*, 2016). Deeper reefs are typically darker, colder and further offshore (Lesser *et al.*, 2009). Recently, MCEs in the Caribbean have been recognised as their own distinct biological assemblage, characterised by the absence of shallow-specialist taxa and the presence of depth-generalists (Semmler *et al.*, 2016; Laverick *et al.*, 2017). The upper and lower boundaries of MCEs may therefore be considered variable, with distributions likely underpinned by physiological responses to the environment.

Photosynthetic scleractinian corals derive a significant portion of their energy from sunlight (Hatcher, 1988). However, as depth increases, photosynthetically active radiation (PAR) declines (Sathyendranath & Platt, 1988). The depth-generalist profile typical of mesophotic Scleractinia in the Caribbean (Semmler *et al.*, 2016; Laverick *et al.*, 2017) therefore poses a significant physiological challenge. Photosynthetic corals may increase their photosynthetic efficiency to accommodate changing light profiles (Anthony & Hoegh-Guldberg, 2003; Hennige *et al.*, 2008). This can be achieved through: symbiont switching (Bongaerts *et al.*, 2015), increased symbiont densities (Pim Bongaerts *et al.*, 2011) or pigment concentrations (Cohen & Dubinsky, 2015; Borell *et al.*, 2016), changing growth form (Graus & MacIntyre, 1982), or even by modifying the reflective properties of the coral skeleton (Enríquez *et al.*, 2017) with differences noted between shallow and mesophotic taxa (Kahng *et al.*, 2012). Additionally, scleractinian corals sit on a spectrum of mixotrophy, with variable rates of heterotrophic feeding (Palardy *et al.*, 2005). Heterotrophic subsidy may be used as a strategy to survive coral bleaching events, when the energy contribution from photosynthesis declines (Grottoli *et al.*, 2014). Heterotrophic subsidy, therefore, has also been recognised as a possible mechanism permitting the depth-generalist distribution of mesophotic hard corals (Alamaru *et al.*, 2009; Lesser *et al.*, 2010; Crandall *et al.*, 2016). A third conceivable physiological adaptation to low light levels is a reduced metabolic rate, and so energy requirement (Davies, 1980). Though mass specific respiration rates at rest (basal metabolic rates) appear remarkably consistent across biology (Suarez *et al.*, 2004; Makarieva *et al.*, 2008), energy could be saved by reduced investment in reproduction (Feldman *et al.*, 2017; Shlesinger *et al.*, 2018) or growth.

Pulse Amplitude Modulated (PAM) fluorometry is an established method for studying photochemistry (Schreiber, 2004) and can be used to calculate a variety of metrics, such as photosynthetic efficiency and capacity, light-related stress, and other features (Jassby & Platt, 1976; Juneau *et al.*, 2005). Further, the ratios of heavy to light nitrogen isotopes in coral tissue can provide a measure of heterotrophic feeding (Peterson & Fry, 1987), providing environmental differences are accounted for (Heikoop *et al.*, 2000; Baker *et al.*, 2010). Discrimination between carbon isotopes is partly dependent on photosynthetic activity in the absence of feeding (Alamaru *et al.*, 2009); the translocation of carbon from the zooxanthellae symbionts to the coral host may be affected by depth and produce an isotopic signature

1 (Muscatine *et al.*, 1989). However, lipid content may also affect bulk $\delta^{13}\text{C}$ measurements
2 (Alamaru *et al.*, 2009).

3

4 Stable isotope analyses and PAM fluorometry of a number of mesophotic Scleractinia,
5 including *Agaricia lamarcki* (Crandall *et al.*, 2016) and *Montastraea cavernosa* (Lesser *et al.*,
6 2010, 2014; Crandall *et al.*, 2016) in the Caribbean, and *Favia fava* (Alamaru *et al.*, 2009)
7 and *Stylophora pistillata* (Alamaru *et al.*, 2009; Einbinder *et al.*, 2009; Brokovich *et al.*,
8 2010; O. Nir *et al.*, 2011; Cohen & Dubinsky, 2015; Einbinder *et al.*, 2016) in the Red Sea,
9 have revealed between-species variation in the changing rate of heterotrophy and
10 photosynthetic efficiency with depth. However, there has been little effort to investigate intra-
11 species variation between sites with different abiotic conditions, such as light levels and
12 slope.

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14 The relationship between these factors and the cellular physiology of corals across depth
15 gradients has also yet to be examined in detail. The intracellular ratio between the secondary
16 metabolites dimethylsulphoxide and dimethylsulphoniopropionate (DMSO:DMSP) has been
17 previously used as an early indicator for cellular oxidative ‘stress’ in the cordgrass *Spartina*
18 *alterniflora* (Husband & Kiene, 2007; McFarlin & Alber, 2013) because of their role in
19 cellular antioxidant cascades (Sunda *et al.*, 2002). Since corals (and associated symbionts)
20 harbour significant quantities of DMSP (Raina *et al.*, 2013; Burdett *et al.*, 2014), it may be
21 hypothesised that the DMSO:DMSP ratio is also a useful oxidative stress indicator for these
22 organisms. Elevated DMSP concentrations have been observed coinciding with a stressful
23 light environment for *S. pistillata* in the Red Sea (Borell *et al.*, 2016).

24

25 Here, we consider the physiology of the mesophotic depth-generalist scleractinian *A.*
26 *lamarcki*, one of the dominant mesophotic taxa at our Caribbean study sites. *A. lamarcki* is a
27 brooding species, which has shown limited symbiont switching around the mesophotic-
28 shallow reef boundary (Bongaerts *et al.*, 2015). We sampled two sites down a continuous
29 depth gradient to assess the consistency of physiological patterns with depth. We use
30 techniques which have already been used to assess physiological change across the shallow-
31 mesophotic depth gradient (Alamaru *et al.*, 2009; Einbinder *et al.*, 2009; Lesser *et al.*, 2010;
32 Crandall *et al.*, 2016); PAM fluorometry, oxygen flux, stable isotope analyses, and also
33 investigate trends in intracellular DMSP:DMSO ratios.

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36 2 MATERIALS AND METHODS:

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39 2.1 Research Site-

40 Utila is one of the Honduran Bay Islands on the southern end of the meso-American barrier
41 reef. A quantitative benthic description exists to a maximum depth of 85 m (Laverick *et al.*,
42 2017). Of the five sites described, two are considered here: ‘The Maze’ on the north shore
43 (TMA, N 16.112, W-86.949, WGS84 format) and ‘Little Bight’ on the south shore (LB,

1 16.079, W-86.929). Generally, south shore reefs are slopes ending in sand at ~45 m depth. In
2 contrast, north shore reefs are typically walls extending deeper than 100 m. Though we do
3 not have light data, the sites are known to have differing maximum depth ranges for *A.*
4 *lamarcki* and the transition depths from shallow to mesophotic communities are known to be
5 deeper at TMA than LB (Laverick *et al.*, 2017).

6 7 **2.2 Collection-**

8 Coral fragments were collected (Permit number: ICF-261-16) by SCUBA divers using mixed
9 gas closed circuit rebreathers during July 2015. During collection dives, *A. lamarcki* colonies
10 were identified as plating and encrusting agariciid colonies with white, star-shaped polyps
11 (Humann & Deloach, 2013). Species identity was verified by the alternation of long and short
12 septo-costae following examination under a microscope in the field (Veron *et al.*, 2016).

13
14 Sampled depths were from 10 m to 45 m at the site LB and 16 m to 60 m at TMA. These
15 depths reflect the shallowest and deepest observed colonies of *A. lamarcki* at each site. We
16 are confident the whole depth range of *A. lamarcki* was sampled for the following reasons.
17 The lower limit of LB coincides with a sandy plain, and concurrent ecological studies at
18 TMA, which reported no Scleractinia deeper than 85m with maximum dive depths of 100m
19 (Laverick *et al.*, 2017), did not report deeper incidences of *A. lamarcki* than sampled here.
20 Additionally, roaming divers reported no *A. lamarcki* deeper than 60 m. Sampled coral
21 colonies were >40 cm in diameter, to minimise damage to newly-recruited colonies, and 5+
22 m from their nearest sampled neighbour to minimise the sampling of clones. All colonies
23 were sampled as they were found, so long as they satisfied these selection criteria, with up to
24 12 colonies per 10 m vertical depth band. Half the samples were collected by swimming with
25 the reef on the divers' left side from the dive site mooring buoy, half with the reef on the right
26 side.

27
28 To sample a suitable colony, a thumb sized fragment was excised using a chisel from the
29 plate margin. This was placed in a labelled zip-lock bag and stowed in a PVC tube that was
30 opaque to light. Fragments were kept in the dark prior to analysis to mitigate light associated
31 stress during the divers' ascent. Once stowed, the fractured margin of the colony was lined
32 with pre-mixed Milliput modelling putty to prevent infection or fouling (Downs, 2011). The
33 samples were returned to a temperature-controlled field lab and placed within an opaque
34 plastic aquarium filled with water from the fore-reef. The aquaria were heated to 28°C (=
35 ambient in situ temperature), aerated, and covered in four layers of plastic tarp to allow dark
36 acclimation of fragments. Samples were acclimated in the dark for 12 hours prior to analysis
37 for photosynthetic characteristics and dissolved oxygen consumption.

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39 Water (LB n= 4, TMA n = 18) and sediment samples (LB n=14, TMA n = 24) were collected
40 from both sites at 5, 15, 25, 40, and also at 55 and 70 m at TMA. These samples provide an
41 environmental isotope signature for context when interpreting the trends in coral values
42 (Heikoop *et al.*, 2000; Baker *et al.*, 2010). Sediment was collected in sediment traps deployed
43 on the reef for 4 weeks before the contents were drained and desiccated. Water was collected
44 by SCUBA divers and poisoned in the lab with 10 µl of mercuric chloride solution per 12 ml
45 of water, and stored without headspace in exetainer vials (Labco Ltd).

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2 **2.3 Laboratory Methods-**

3 2.3.1 OXYGEN INCUBATIONS

4 Coral fragments were removed from their aquarium, in the dark, and isolated in 450 ml
5 plastic chambers. Chambers were filled with fresh, unfiltered, sea water from the fore-reef in
6 the same container, at the same time of day. The chambers were left for an hour, deemed a
7 suitable time for generating a detectable signal after pilot tests. The lab was kept in darkness
8 during the incubations. Water samples were taken at the beginning and end of the incubation.
9 The change in dissolved oxygen (DO₂) content was quantified using the same Fibox oxygen
10 optode sensor spot system calibrated with a two-point calibration according to the
11 manufacturer's instructions (PreSens Precision Sensing GmbH). The probe was held steady
12 until the reading plateaued before recording. The change in dissolved oxygen during the
13 incubation (ΔDO_2) was standardised to 10g of coral tissue, measured when later removed
14 from the fragment (details below), and to the hour.

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16 2.3.2 PAM FLUOROMETRY

17 Immediately after the oxygen incubation, Rapid Light Curves (RLCs) were conducted on the
18 submerged, polyp-bearing side of the coral fragment using PAR levels of 2, 3, 5, 8, 12, 19,
19 37, 64, 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ set on a Diving-PAM (Heinz Walz GmbH). The fibre optic was
20 positioned 5 mm away from the coral surface using the Surface Holder attachment during all
21 RLCs. PAR levels for the RLCs were chosen based on a balance between avoiding rapid light
22 saturation of mesophotic fragments, whilst still achieving a detectable response from shallow
23 fragments.

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25 For RLCs a steady state is not reached during each light step (Ralph & Gademann, 2005),
26 unlike traditional light curves. Therefore, results from RLCs yield information on the actual,
27 rather than optimal, photosynthetic state as suggested by traditional light curves (Ralph &
28 Gademann, 2005). Additionally, comparing RLCs from different species or under different
29 environmental conditions should be conducted with care, as the irradiance absorption of a
30 photosynthetic organism may change, affecting electron transport rates (Saroussi & Beer,
31 2007; Einbinder *et al.*, 2016). Comparisons between sites and/or depths may therefore be
32 affected by changes in the coral's irradiance absorption, such as changes in coral optics
33 (Chalker *et al.*, 1983; Anthony & Hoegh-Guldberg, 2003; Wangpraseurt *et al.*, 2019), and
34 have been taken into account when interpreting the PAM fluorometry results. Our results
35 represent an integrated photosynthetic and bio-optical response, providing relative
36 comparisons of the same species between sites. Variations are likely to have arisen in
37 response to a varied environmental regime, thereby enabling comparison between sites, albeit
38 without the capacity to identify if any observed changes are as a result of photosynthetic or
39 bio-optical characteristics.

40

41 2.3.3 STABLE ISOTOPE ANALYSES

42 All stable isotope samples were prepared as described below before shipping to the UK for
43 analysis at the NERC Life Sciences Mass Spectrometry Facility in East Kilbride. In the field,
44 following RLCs, coral fragments were patted dry and their mass recorded. Surface coral
45 tissue was removed using a Waterpik filled with sea water (Johannes & Wiebe, 1970). The

1 mass of the air-dried skeleton was later recorded to allow the mass of coral tissue to be
2 determined (= original mass – mass of bare skeleton). Air-dried skeleton was ground into a
3 powder using a pestle and mortar and sealed in micro-centrifuge tubes. Coral slurry was left
4 to settle and then pipetted into micro-centrifuge tubes. The tubes were centrifuged at
5 14,000rpm for 60 seconds and the supernatant removed. This was repeated three times,
6 topping with more slurry between spins to maximise material recovery. The resulting
7 material was left standing for 10 hours, in an aluminium tray under a sheet of glass in direct
8 sunlight, to allow desiccation before storing at -20°C. Upon return to the UK these samples,
9 and sediments, were further dried at 50°C overnight. We were unable to separate symbiont
10 and host tissue in the field, we therefore interpret our results at the level of the holobiont, as
11 has been done in similar studies (Crandall *et al.*, 2016). When host and symbiont have been
12 analysed independently, the results tend to show a shift in mean values between the two
13 fractions, but similar relationships with increasing depth (Alamaru *et al.*, 2009; Einbinder *et*
14 *al.*, 2009; Lesser *et al.*, 2010).

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16 2.3.4 SEDIMENTS AND TISSUE SAMPLES ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$)

17 Samples were weighed (0.7 mg for organic tissues, 5 mg for sediment) into tin capsules and
18 loaded into an Elementar (Hanau, Germany) Pyrocube elemental analyser (EA) run in NC
19 mode. Samples were combusted and gases purified such that N_2 (for $\delta^{15}\text{N}$) and CO_2 (for
20 $\delta^{13}\text{C}$) were admitted consecutively into a Thermo (Bremen, Germany) Delta XP isotope ratio
21 mass spectrometer (IRMS). The protocol loosely follows simultaneous nitrogen, carbon, and
22 sulphur analysis (Fourel *et al.*, 2014) with the following deviations: we did not run for
23 sulphur and so did not use a SO_2 trap; oxidation and reduction reactors were cooler at 950°C
24 and 600°C respectively; the oxidation reactor was centrally filled with CuO as a catalyst,
25 succeeded by a plug of silver wool filtering Cl species. Three standards were used to correct
26 for linearity and drift of a range of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Werner & Brand, 2001; Newton, 2010): a
27 gelatine solution (GEL), a ^{13}C -enriched alanine/gelatine solution (ALAGEL), and a ^{15}N -
28 enriched glycine/gelatine solution (GLYGEL). All standard solutions were dispensed into tin
29 capsules and oven dried at 70°C prior to analysis. C and N abundance, and an independent
30 evaluation of isotope ratio, was provided by four USGS40 standards (Qi *et al.*, 2003).
31 Measurement error of all four reference materials can be found in the supplementary
32 information (Supplementary 1).

33

34 2.3.5 DISSOLVED INORGANIC CARBON (DIC, $\delta^{13}\text{C}$)

35 Two drops of 103% Phosphoric acid were added to exetainers (Labco Ltd), which were then
36 flushed with helium. 1 ml of each water sample was added to the exetainer via a syringe
37 through the septum. The phosphoric acid liberated gaseous CO_2 from the sample into the
38 headspace of the exetainer. Standards were treated differently as these were solid sodium
39 bicarbonate and calcium carbonate powders (Waldron *et al.*, 2014). Standards were loaded
40 into dry exetainers with 1 ml of dilute phosphoric acid, ensuring the H_3PO_4 concentration was
41 the same as for the samples. For both standards and samples, the headspace CO_2 was dried in
42 a Gas Bench (Thermo, Bremen, Germany) and the $\delta^{13}\text{C}$ measured on a Thermo (Bremen,
43 Germany) Delta V IRMS (Torres *et al.*, 2005; Yang & Jiang, 2012).

44

1 2.3.6 SKELETAL CARBONATES ($\delta^{13}\text{C}$)

2 $\delta^{13}\text{C}$ of skeletal carbonates were analysed on an 'Analytical Precision' sampler/mass
3 spectrometer (de Groot, 2008). 1 mg samples of each powdered coral skeleton were sealed in
4 vavutainers and flushed with helium. Phosphoric acid was injected through the septum in
5 excess by the autosampler. The reaction was left at 70°C for 20 minutes to liberate CO_2 into
6 the headspace. The resulting gas was analysed by the instrument's IRMS.

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8 2.3.7 DIMETHYLATED SULPHUR ANALYSES

9 Approximately 1 mg of centrifuged tissue was diluted to 5 ml volume with MilliQ 18 Ω water
10 with 1 ml 10M NaOH and stored in 20ml chromatography vials (Fisher Scientific) sealed
11 with Pharma-Fix septa (Fisher Scientific), to hydrolyse DMSP into DMS. Samples were
12 stored in the dark and transported back to the University of St Andrews for analysis. The
13 sample headspace was analysed by direct injection using an SRI-8610C gas chromatograph
14 (GC) (SRI Instruments UK) fitted with a 15 m 5.0U MXT-1 capillary column (N_2 carrier gas
15 @ 8 psi, 45°C), and a sulphur-specific flame photometric detector (air pressure: 2 psi, H_2
16 pressure: 27 psi, 150°C).

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18 Samples were then analysed for DMSO concentration, using the reductase enzyme method
19 (Hatton *et al.*, 1994). Samples were purged of DMS with N_2 following addition of Tris-buffer
20 and neutralisation to pH 7.0. Where samples could not be analysed within 24hrs of
21 preparation, they were frozen (-20°C) until analysis. 2 ml of flavin mononucleotide solution
22 was added to each vial and irradiated with 3 x 60 W bulbs for one hour to catalyse the
23 reaction of DMSO to DMS, following Hatton et al (1994). Samples were left for 12 hours to
24 allow DMS equilibration in the vial headspace, before direct-injection GC analysis, as
25 described above. All sample concentrations were quantified from DMSP standard calibration
26 curves (DMSP standard from Research Plus Inc.). The limit of detection for both DMSP and
27 DMSO samples was 1 μg S per 100 μl headspace injection; standard and sample precision
28 was within 1%.

29

30 2.4 Statistical Analyses-

31 All statistical analyses and data manipulation were conducted in the programming language R
32 (R-Core-Team, 2013). The minimum saturating irradiance (RLC[Ek]) and initial
33 photosynthetic rate (RLC[alpha]) were calculated for each fragment by fitting rapid light
34 curve (RLC) data to the equations of Jassby & Platt (Jassby & Platt, 1976) in the package
35 Phytotools (Silsbe & Malkin, 2015). Maximum Relative Electron Transport Rate
36 (RLC[rETRmax]) was calculated as $\text{RLC}[\text{Ek}] * \text{RLC}[\text{alpha}]$. As the $\delta^{13}\text{C}$ skeletal value attains
37 an equilibrium with the environment (McConnaughey *et al.*, 1997), and the difference to
38 tissue $\delta^{13}\text{C}$ represents a metabolic effect, we calculate a $\delta^{13}\text{C}$ differential as $\delta^{13}\text{C}$ tissue - $\delta^{13}\text{C}$
39 skeleton. We rely on sea water $\delta^{13}\text{C}$ DIC as an additional control for potential between site
40 variation in $\delta^{13}\text{C}$ sources, as it provides the basis for coral carbonate production (Allison *et*
41 *al.*, 2014).

42

43 Linearity, normality, heteroskedasticity and influential outliers were assessed using residual
44 plots. Statistical tests were not used to assess these as the large number of data points caused
45 spurious rejection of assumptions with high *P* values associated with only small deviations.
46 Tests are robust to the assumption of homogeneity of variance across groups because of

1 balanced sample sizes between sites. Data remained untransformed to ensure fair
2 comparisons between tests.

3

4 In turn, RLC[Ek], RLC[alpha], RLC[rETRmax], change in dissolved oxygen during
5 incubation (ΔDO_2), tissue molar C:N, DMSO:DMSP, $\delta^{13}\text{C}$ tissue differential, tissue $\delta^{15}\text{N}$,
6 sediment $\delta^{15}\text{N}$, and sea water $\delta^{13}\text{C}$ DIC were all fitted as the dependent variable of a linear
7 model with depth as the independent variable, site as a grouping factor, and an interaction
8 term. RLC[rETRmax], ΔDO_2 , tissue molar C:N, $\delta^{13}\text{C}$ tissue differential, tissue $\delta^{15}\text{N}$ were
9 also fitted in the same way against DMSO:DMSP. For the $\delta^{13}\text{C}$ tissue differential, tissue
10 molar C:N was included as a control variable to account for possible fluctuations in lipid
11 content. These models show the physiological profile of *A. lamarcki* with depth at Utila, in
12 terms of photosynthesis, heterotrophy, respiration, and oxidative stress:

13
$$Y \sim (\text{Depth or DMSO:DMSP}) + \text{Site} + \text{interaction}$$

14 Additional models included:

15
$$\text{Tissue } \delta^{15}\text{N} \sim r\text{ETRmax} + \text{Site} + r\text{ETRmax:Site}$$

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$$\delta^{13}\text{C differential} \sim \text{Tissue } \delta^{15}\text{N} + \text{Tissue molar C:N} + \text{Site} + \text{Tissue } \delta^{15}\text{N:Site}$$

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18 Statistically significant model elements were detected with heteroskedastically constant
19 variance using ‘Anova(white.adjust = HC3)’ (Long & Ervin, 2000). The final data file can be
20 found in supplementary material (Supplementary 2).

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23 3 RESULTS:

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26 The changes in physiology recorded for *A. lamarcki* with increasing depth differ markedly
27 between TMA and LB. A summary of ANCOVA results and model parameters are presented
28 in Tables 1 and 2 respectively. Plots of environmental controls can be found in the
29 supplementary material (Supplementary 3, Supplementary 4), as well as Residual plots
30 (Supplementary 5). Residual plots revealed no systematic deviations from model
31 assumptions. For analyses considering the $\delta^{13}\text{C}$ tissue differential, molar C:N was included as
32 an additional parameter to control for lipid content. Molar C:N did not vary with depth ($F =$
33 $2.40 P = 0.12$) or tissue $\delta^{15}\text{N}$ ($F = 8.94 P = 0.54$).

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35 During our sampling we did not encounter any intermediate, general colony-level,
36 morphologies. Though morphological variation and change in growth form down depth
37 gradients has been documented in some species of Scleractinia (Dustan, 1975; Amaral, 1994;
38 O. Nir *et al.*, 2011; Goodbody-Gringley & Waletich, 2018), we observed only modest
39 plasticity in growth form. At the extremes of *A. lamarcki*'s depth range, within a given site,
40 there was a tendency toward smaller encrusting colonies. Plating forms were most common
41 between ~20-55m depth.

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3.1 Physiological Variation with Depth-

Three coral measures significantly varied with depth: $\delta^{15}\text{N}$ as a signal of heterotrophy ($F = 19.38 P < 0.001$), $\delta^{13}\text{C}$ differential as a signal of long term photosynthetic activity ($F = 5.53 P = 0.02$), and RLC[rETRmax] as an instantaneous measure of potential photosynthetic capacity ($F = 8.46 P = 0.004$). ΔDO_2 , molar tissue C:N, and DMSO:DMSP did not significantly vary with depth (Table 1). Mean values of tissue $\delta^{15}\text{N}$ differed between sites for a given depth ($F = 25.59 P < 0.001$) – the mean at LB was 0.53‰ higher. Mean DMSO:DMSP values were 0.08 higher at TMA than LB ($F = 4.23 P = 0.04$). Differing slopes with depth were detected for RLC[rETRmax] and tissue $\delta^{15}\text{N}$ (Figure 1) between sites ($F = 8.87 P = 0.004$, $F = 14.13 P < 0.001$, Table 1). We found potential photosynthetic capacity (RLC[rETRmax]) significantly declined with depth (Table 1), however, this appears to only be true at LB (Table 2, Figure 1). To further understand how photosynthetic profiles vary with depth, RLC[Ek] and RLC[alpha] were tested independently against depth, as RLC[rETRmax] is a composite of these two quantities. While RLC[alpha] significantly increased with depth, RLC[Ek] significantly decreased. Only RLC[Ek] returned a significant interaction term (Table 1), suggesting the observed differences in photosynthetic capacity with depth between the two sites were caused by differing RLC[Ek] values, i.e. the minimum saturating irradiance.

3.2 Environmental Controls-

No differences in environmental baselines were observed between the two sites. $\delta^{15}\text{N}$ of sediment samples at LB and TMA (Supplementary 3) were collected as environmental baselines for comparison to changes in tissue values which may be affected by local enrichment (Heikoop *et al.*, 2000; Baker *et al.*, 2010). Both the environmental signal and tissue showed a significant relationship with depth (Table 1). No significant difference was detected in mean $\delta^{15}\text{N}$ of sediments between sites, though parameter estimates indicated mean $\delta^{15}\text{N}$ was slightly enriched at LB compared to TMA (+0.36‰).. Though this was not statistically significant (Table 1), this value is close enough to the difference in mean levels between sites for tissue $\delta^{15}\text{N}$ that we conclude there is little biological meaning to the result. It is not believed that environmental patterns drive the relationship in the tissue since the gradients in tissue $\delta^{15}\text{N}$ with depth are in opposing directions, whereas the environmental signal is consistently positive (Table 2). $\delta^{13}\text{C}$ DIC of seawater (Supplementary 4) was collected and analysed as an environmental comparison to coral $\delta^{13}\text{C}$. Though DIC exhibits a statistically significant relationship with depth, this was in the opposite direction to the coral holobiont $\delta^{13}\text{C}$ measurement, and the effect size was at least an order of magnitude lower at each site (Table 2). Similarly to the sediment data, no statistically significant site differences were detected in $\delta^{13}\text{C}$ (Table 1); though LB had a mean $\delta^{13}\text{C}$ 0.05‰ higher than TMA. These results suggest the trends we see between sites result from physiological variation, and not differing environmental baselines between sites

3.3 Relationships Between Photosynthesis (RLC[rETRmax] and $\delta^{13}\text{C}$), Inferred Heterotrophy ($\delta^{15}\text{N}$), and Stress (DMSO:DMSP) -

We detect two statistically supported relationships between DMSO:DMSP and other physiological variables. Firstly a reduction in tissue molar C:N with increasing DMSO:DMSP ($F = 4.16 P = 0.04$). Secondly an effect of site on mean levels of tissue bulk $\delta^{15}\text{N}$ ($F = 18.25 P < 0.001$), with higher $\delta^{15}\text{N}$ values for a given DMSO:DMSP at LB than

1 TMA (Table 1). With $P = 0.051$, a notable effect of DMSO:DMSP on mean levels of the $\delta^{13}\text{C}$
2 tissue differential is identified, with higher $\delta^{13}\text{C}$ values at TMA than LB (Table 1).

3
4 A significant site and depth interaction for RLC[rETRmax] and for tissue $\delta^{15}\text{N}$ (Table 1,
5 Figure 1) could be driven by two factors. Either, certain physiological relationships within the
6 coral holobiont were not constant, or unmeasured sources of variation were confounded
7 differently with depth at the two sites. To aid interpretation, tissue $\delta^{15}\text{N}$ was plotted against
8 RLC[rETRmax] (Figure 2) and statistically assessed, determining whether the physiological
9 relationships remained constant between sites. Site affected mean values at the two sites, but
10 only to the degree expected by the sediment control (Supplementary 3). There is no
11 statistically supported relationship between RLC[rETRmax] and tissue $\delta^{15}\text{N}$ ($F = 1.79$ $P =$
12 0.18 , Table 1), nor a significant interaction.

13
14 As there was, unexpectedly, no relationship between RLC[rETRmax] and tissue $\delta^{15}\text{N}$, we
15 further explored the $\delta^{13}\text{C}$ differential result. When controlling for variability in molar C:N,
16 the difference in slope between LB and TMA $\delta^{13}\text{C}$ with depth (Figure 1) was no longer
17 statistically significant, $P = 0.08$ (Table 1). A relationship might be expected between
18 photosynthetic parameters and the degree of heterotrophy, based on previous work (Alamaru
19 *et al.*, 2009; Lesser *et al.*, 2010; Crandall *et al.*, 2016). We therefore plot the $\delta^{13}\text{C}$ differential
20 against tissue $\delta^{15}\text{N}$ (Figure 2) and statistically assessed the relationships. Whilst there was no
21 overall relationship between the two variables, a significant interaction term (Table 1)
22 revealed opposing gradients at the two sites and differing group means (Table 2).

23 24 **3.4 Variability of Physiological Measures Between Sites-**

25 Despite differences in physiology with depth between LB and TMA, the probability
26 distributions of parameter values are broadly comparable (Figure 3). If the physiological
27 parameters in Figure 3 were linearly correlated with depth, we would expect the probability
28 distributions to reflect the sampling effort with depth. The distributions at both sites return a
29 modal value in close agreement and are more tightly grouped around this value than expected
30 with sampling effort, despite sampling different depth ranges at the two sites. The exception
31 is a shift in DMSO:DMSP between the two sites, consistent with the differences in mean
32 levels detected by linear models (Table 1). The probability distribution for TMA is
33 consistently narrower than for LB, despite TMA being sampled over a larger depth range
34 which we would expect to necessitate greater physiological variation.

35 36 37 **4 DISCUSSION:**

38 39 40 **4.1 *Agaricia lamarcki* Expresses Site-Specific Physiological Profiles With Depth-**

41 This study aimed to assess the constancy of physiological patterns with depth between sites in
42 the depth-generalist mesophotic coral, *Agaricia lamarcki*. We found clear site-specific trends,
43 both in terms of PAM fluorometry and stable isotope analyses. At LB, *A. lamarcki* exhibited

1 a reduction in potential photosynthetic capacity and an increase in heterotrophic feeding with
2 depth (Figure 1). These patterns were absent at TMA, despite a wider vertical depth range.
3 These observations highlight the variability of scleractinian physiology, and the importance
4 of taking into consideration local/regional scale variation when attempting to generalise
5 biological response. We have shown that the same species of coral will not necessarily
6 behave in the same way down a depth gradient in different locations. Depth alone may
7 therefore be an inappropriate proxy for physiological change through the mesophotic zone.
8 We should instead consider more explicitly the role of the underwater light field when
9 explaining mesophotic coral physiology (Lesser *et al.*, 2018), and how this can interact with
10 reef topography (Muir *et al.*, 2018; Kramer *et al.*, 2019).

12 **4.2 The Deepest Colonies of *A. lamarcki* can be Comparable to Those More Shallow**

13 In addition to the trends in tissue $\delta^{15}\text{N}$ and RLC[rETRmax], there were no detected trends in
14 respiration (as inferred from ΔDO_2), and no trend in the molar C:N ratio (Table 1,
15 Supplementary 5). The lack of trend in respiration and molar C:N is of interest when taking
16 into account the significant reduction in RLC[Ek] with depth (Table 2), which underpins a
17 reduction in potential photosynthetic capacity (Figure 1). The change in RLC[Ek] clearly
18 indicates that deeper *A. lamarcki* colonies are acclimated to lower light levels; they do not
19 exhibit lower fat stores or rates of energy consumption at the light levels used in this study.
20 This leads us to believe that the potential adaptation of deeper colonies may not prevent
21 connectivity between shallow and mesophotic reefs and may permit a deep-water refuge for
22 *A. lamarcki* at this location. This is supported by the lack of an observed effect of depth on
23 the cellular oxidative stress indicator DMSO:DMSP, and by no impact of collection site on
24 the survival of the same colonies sampled here during a transplant experiment (Laverick &
25 Rogers, 2018). A similar situation has been noted for *E. paradivisa* in the Red Sea (Eyal *et al.*,
26 2015). ΔDO_2 in the dark, however, approximates basal metabolic rate which is expected
27 to be largely constant (Suarez *et al.*, 2004; Makarieva *et al.*, 2008). In situ measurements of
28 net-photosynthesis year round are necessary to better understand the importance of
29 respiration in balancing energy budgets with increasing depth.

31 Interpreting our stable isotope data, with respect to photosynthetic activity, comes with
32 caveats. Coral growth rates can vary with increasing depth (Baker & Weber, 1975), and this
33 in turn can leave isotopic signals (Patzold, 1984). Further, coral growth rates can vary with
34 light exposure, independent of changes in photosynthesis (Eyal *et al.*, 2019). Growth signals
35 can also correlate with light exposure in skeletal carbon fractionation (Shimamura *et al.*,
36 2008). Further studies which could quantify the variation in *A. lamarcki* growth rates with
37 depth would be valuable. This would allow the isotopic signatures of growth and
38 photosynthesis to be disentangled but would also be helpful for demographic studies. For
39 $\delta^{15}\text{N}$, symbiodinium growth rate does not affect nitrogen isotope fractionation (Muscatine &
40 Kaplan, 1994).

42 The observed negative relationship between C:N and DMSO:DMSP supports the role of
43 tissue C:N as an indicator of holobiont health (Szmant & Gassman, 1990) and further
44 supports the use of DMSO:DMSP as an indicator of cellular stress (Husband & Kiene, 2007;
45 McFarlin & Alber, 2013). Between-site differences in mean DMSO:DMSP supports the
46 hypothesis that spatial variation in environmental conditions is impacting the local-scale
47 physiology of *A. lamarcki*.

1

2 **4.3 Physiological Profiles are Coincident With Differences in Ecological Patterns-**

3 Our findings also reveal a connection between physiological parameters and ecological
4 patterns. If we interpret the difference between mean tissue $\delta^{15}\text{N}$ at the two sites (Figure 1,
5 Table 2) as resulting from differing environmental baselines (Supplementary 3, Table 2), then
6 there was no difference in the mean value of any physiological parameter between the two
7 sites, except DMSO:DMSP. This observation is despite the larger depth range at TMA.
8 Significant interaction terms for tissue $\delta^{15}\text{N}$ and RLC[rETRmax] show that it is the rates of
9 change with depth which vary, such that the same physiological limits are reached for these
10 parameters. In fact, the minimum saturating irradiance (RLC[Ek]) reduces at a rate three
11 times faster at LB than TMA with depth, while RLC[alpha] increases at more than twice the
12 rate with depth at TMA ($0.28\% \text{ m}^{-1}$) than LB ($0.13\% \text{ m}^{-1}$), although this relationship was too
13 noisy to return a statistically significant interaction term. Both quantities are considered key
14 photoadaptations with increasing depth in coral (Chalker *et al.*, 1983). We expect corals from
15 low light environments to have higher alpha values, and lower Ek values, as they optimise
16 themselves to quickly capture the small amount of light available. Although we are able to
17 detect variation in the rates of photoadaptation with depth, the use of RLCs prevents us from
18 distinguishing between photosynthetic characteristics and bio-optical properties as the
19 causative factor in these observations.

20

21 These findings do suggest that depth ranges and physiological change are related. If site-
22 specific environmental conditions are the root cause of differing distributions for coral taxa
23 between sites (Anthony & Hoegh-Guldberg, 2003), then we may have an explanation for
24 observations of mesophotic taxa at 'unusual' depths (Muir & Wallace, 2015; Laverick *et al.*,
25 2017). Increasingly in mesophotic ecology there are calls for a biologically-informed, rather
26 than depth-lineated, definition of mesophotic reefs (Laverick *et al.*, 2016; Loya *et al.*, 2016;
27 Semmler *et al.*, 2016; Lesser *et al.*, 2018), as intended (E. Baker *et al.*, 2016). Coral species
28 typical of the mesophotic zone are known to prefer shaded microhabitats at shallower depths
29 (Muir *et al.*, 2018; Kramer *et al.*, 2019). Meanwhile, the potential role the underwater light
30 field could play in controlling the distribution of mesophotic reefs has been highlighted
31 through simulations of varying reef structure (Lesser *et al.*, 2018). Lesser *et al.* suggested that
32 a more nuanced definition of MCEs may be in reach if the light-field can be connected to the
33 intrinsic properties of coral communities. We have shown how physiological patterns
34 between sites could translate into differing depth distributions for *A. lamarcki* on Utila. We
35 now suggest that considering the interaction between physiology and the light field, at a
36 community level, could allow us to expand the concept of mesophotic habitats.

37

38 **4.4 *Agaricia lamarcki* Appears to be a Mixotroph-**

39 Previous studies have attempted to interpret physiological data and claim particular species
40 are primarily heterotrophic or photoautotrophic (Lesser *et al.*, 2010; Crandall *et al.*, 2016).
41 Specifically *A. lamarcki* has been previously identified as a heterotrophic coral (Crandall *et al.*,
42 2016). Our high degree of replication within sites, in conjunction with a cross-site
43 comparison, provides robust evidence for notable mixotrophy in *A. lamarcki*. Our trends in
44 bulk tissue $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ at LB indicate heterotrophy (Figure 1), in agreement with
45 previously published research (Crandall *et al.*, 2016). The trends detected at TMA, however,
46 are more similar to those reported by others for *Montastraea cavernosa* (Lesser *et al.*, 2010;
47 Crandall *et al.*, 2016). Though we do not have the compound-specific stable isotope analysis

1 of sterols used by Crandall et al. (2016), we do detect a decrease in $\delta^{13}\text{C}$ without a
2 commensurate increase in $\delta^{15}\text{N}$. This suggests a primarily photosynthetic strategy at TMA.
3 Further, the statistically significant trends in RLC[Ek] and RLC[alpha] with depth indicate
4 photoadaptation (driven by changes in photosynthetic characteristics and / or bio-optical
5 properties) is occurring. As RLC[alpha] increases at twice the rate with depth at TMA, and
6 over a larger depth gradient, it may be that photoadaptation is sufficient to maintain an
7 autotrophic strategy at this location, but not at LB. This difference in strategy between the
8 two sites is interesting, as we observed no differences by site in the relationship between
9 photosynthetic capacity and tissue $\delta^{15}\text{N}$ (Figure 2). Site differences in the change in
10 environmental conditions by depth may therefore have been responsible for the apparent
11 switch in hetero/autotrophic strategy with depth between the two sites.

12
13 An interesting extra area of research for mesophotic coral physiology concerns the seasonal
14 fluctuations in energy availability. In the Red Sea net O_2 production in *S. pistillata* varies
15 through the year (Orit Nir *et al.*, 2014), indicating a shift in the relative contributions of
16 heterotrophy and photosynthesis over time. For *A. lamarcki* in the US Virgin islands different
17 temporal trends in energy content were detected with increasing depth. In contrast to corals at
18 25 m, corals at 63 m were starved in July-September, and compensate through November-
19 April (Brandtneris *et al.*, 2016). It may be possible that the site-specific conditions of TMA
20 on the north, and LB on the south, side of Utila may come from seasonal asynchrony, as
21 opposed to constant differences. Only time series studies at depth, which are highly unusual
22 on MCEs, will be able to help us understand how energy budgets are balanced across the
23 course of a year.

24 25 **4.5 Exploiting Available Microhabitat may Explain Physiological Consistency at The** 26 **Maze, a Hypothesis-**

27 In comparison to LB, very few physiological changes with depth were observed at TMA.
28 This was despite colonies being collected across a 44 m depth range and comparable modal
29 parameter values between both sites (Figure 3). One potential explanation is that the
30 topography of TMA is more complex than the gentle slope of LB, affording more light-
31 equivalent microhabitats for colonies to exploit (Brakel, 1979). Photosynthetic capacity in
32 Scleractinia has been shown to correlate with the light environment of microhabitats
33 (Anthony & Hoegh-Guldberg, 2003; Bessell-Browne *et al.*, 2017). Given the rate of light
34 attenuation with depth, we may expect the relative difference in light intensity between
35 microhabitats (e.g. illuminated vs shaded) to be greater at shallower depths than deeper, and
36 we do not expect mesophotic reefs to be exposed to a higher light intensity than shallower
37 reefs. Given a random distribution of coral colonies across these microhabitats and in situ
38 acclimation, we would expect similar heteroscedasticity in photosynthetic capacity. Our
39 residual plots revealed no notable deviation from the assumption of homoscedasticity in
40 photosynthetic capacity with depth (Supplementary 5A).

41
42 Similarly microhabitats with low flow rates, and therefore food availability, have been shown
43 to impact the growth of *Agaricia tenuifolia* in shallow waters down a depth gradient (Sebens
44 *et al.*, 2003). Low flow rates in the mesophotic favour ciliary mucus feeders such as *A.*
45 *lamarcki* in general (Sebens & Johnson, 1991). Varying flow rates between microhabitats at

1 TMA may permit a more constant heterotrophic contribution to the energy budget with depth
2 (Figure 1).

3
4 Further, Figure 3 show the probability distributions of the parameters with the greatest
5 between site differences, as well as the depth distribution of sampled colonies for a null
6 comparison. In all cases, the colonies at TMA have a tighter distribution around a modal
7 value, despite being sampled over a larger depth range than at LB. Figure 3 shows a tighter
8 distribution at TMA in terms of DMSO:DMSP, suggesting lower inter-colony variability in
9 oxidative stress, and so potentially irradiance. This could also in part be explained by higher
10 variability in irradiance levels at LB as a result of the south-facing nature of the site, in
11 comparison to TMA on the north shore of Utila. Differing site means of DMSO:DMSP with
12 depth may also indicate maintained higher irradiance levels at TMA (Table 1), corroborated
13 by lower rates of change in RLC[Ek] at TMA with depth . This suggests *A. lamarcki* is better
14 able to exist in a sub-set of preferred, stable, microhabitats at TMA.

15
16 Consideration of environmental conditions is already being used to predict the occurrence of
17 mesophotic taxa in the Hawai’ian archipelago (Costa *et al.*, 2015). Environmental data (e.g.
18 temperature, irradiance, sedimentation, turbidity) and holobiont genetic information (e.g.
19 symbiont type, gene regulation) may have provided a mechanism to explain the differences
20 we observed. Because of an absence of environmental measures, we are unable to explain the
21 cause of documented pattern. We do, however, make some suggestions for further research.
22 At TMA, appropriate microhabitats appear to extend deeper than at LB, increasing the
23 vertical range of *A. lamarcki* and mitigating physiological response to depth, at this location.
24 This leads us to hypothesise that sites with greater topographical complexity are more likely
25 to act as depth refuges, though this will require explicit testing across other species. Such
26 sites begin to break the correlation of environmental conditions with depth (Brakel, 1979),
27 allowing suitable microhabitats to exist below surface pressures (Bridge *et al.*, 2013). We
28 have found that site specific conditions may influence physiology to a greater degree than
29 depth for *A. lamarcki*. Future physiology studies should try to record the light environment
30 that colonies are located in, preferably with temporal variation, and relate this to substrate
31 slope and shading.

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14

15 **Fig. 1.** Principle physiological relationships of *Agaricia lamarcki* with depth across two sites:

16 Linear models of bulk tissue $\delta^{15}\text{N}$, RLC[rETRmax], and the $\delta^{13}\text{C}$ differential against depth.
17 Shaded areas are the 0.95 confidence interval. Statistical assessment and model parameters
18 can be found in Tables 1 and 2. LB = Site Little Bight, TMA = Site The Maze.

19

20 **Fig. 2.** Variation in inferred trophic level by photosynthetic capacity and inferred, in-situ,
21 photosynthetic activity of *Agaricia lamarcki* across two sites:

22 Linear model of $\delta^{15}\text{N}$ by RLC[rETRmax]. Linear model of $\delta^{13}\text{C}$ differential by bulk tissue
23 $\delta^{15}\text{N}$. Shaded areas are the 0.95 confidence interval. Statistical assessment and model
24 parameters can be found in Tables 1 and 2. LB = Site Little Bight, TMA = Site The Maze.

25

26 **Fig. 3.** Probability distributions of select parameter values:

27 The height of the curve indicates the relative probability of a particular parameter value. Each
28 curve is scaled so 1 reflects the modal value within a site, the area under each curve sums to a
29 probability of 1. The distributions from left to right show the sampled colonies with depth,
30 values of bulk tissue $\delta^{15}\text{N}$, RLC[rETRmax], $\delta^{13}\text{C}$ differential, and DMSO:DMSP. If
31 physiological parameters were correlated linearly with depth, we would expect distributions
32 to be similar to those shown for sample collections depths. LB = Site Little Bight, TMA =
33 Site The Maze.

34

Table 1. ANCOVA results: Values are reported as they appeared in computer outputs. P values < 0.05 are in bold and followed by an *. LB = Site Little Bight. TMA = Site The Maze

ANCOVA Summaries	Sample size		Effect of X		Effect of Site on Means		Effect of Site on Slope		X variable
	<i>n</i> : LB	<i>n</i> : TMA	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
RLC[Ek]	50	58	17.0856	0.00007251*	0.0135	0.90756	5.7756	0.01802*	Depth
RLC[alpha]	50	58	43.4559	1.835E-09*	0.3176	0.5743	2.1614	0.1445	Depth
RLC[rETRmax]	50	58	8.464	0.00443*	0.0199	0.888178	8.8711	0.003606*	Depth
DMSP:DMSO	44	44	1.7308	0.19158	4.2328	0.04248*	0.0505	0.82277	Depth
Incubation δDO_2	47	41	0.0064	0.9366	0.1543	0.6955	2.7145	0.103	Depth
Tissue molar C:N	48	54	0.0108	0.9176	0.0133	0.9083	2.5248	0.1153	Depth
$\delta^{13}\text{C}$ differential	48	54	5.5309	0.02068*	0.4084	0.52427	3.2029	0.0766	Depth
Tissue $\delta^{15}\text{N}$	48	54	19.378	0.00002711*	25.591	0.000001944*	14.128	0.0002889*	Depth
Sediment $\delta^{15}\text{N}$	14	24	8.1582	0.00726*	2.5446	0.11992	0.2265	0.6372	Depth
DIC sea water	4	18	10.9894	0.003851*	1.5271	0.23243	1.6271	0.218326	Depth
Tissue $\delta^{15}\text{N}$	48	54	1.786	0.1845	17.2591	0.00006928*	0.0972	0.7559	RLC[rETRmax]
$\delta^{13}\text{C}$ differential	48	54	0.3741	0.542172	4.9821	0.027888*	8.5822	0.004223*	Tissue $\delta^{15}\text{N}$
RLC[rETRmax]	44	44	0.0232	0.8794	0.3086	0.5799	1.2962	0.2579	DMSO:DMSP
Incubation δDO_2	43	33	0.0924	0.762	2.2564	0.1371	0.372	0.5437	DMSO:DMSP
Tissue molar C:N	44	44	4.1628	0.04429*	0.1721	0.67921	0.0833	0.77361	DMSO:DMSP
$\delta^{13}\text{C}$ differential	44	44	1.367	0.24545	3.9114	0.05105	1.0363	0.31145	DMSO:DMSP
Tissue $\delta^{15}\text{N}$	44	44	0.7734	0.3815	18.2509	4.839E-05*	0.5592	0.4566	DMSO:DMSP

Model summaries	Little Bight		The Maze		Adjusted R ²	Residuals	X variable	Interpretation
	Intercept	Slope	Intercept	Slope				
RLC[E _k]	206.227352	-2.692050	143.694792	-0.879897	0.1705	N	Depth	Minimum saturating irradiance
RLC[α]	0.492716055	0.001374574	0.449357211	0.002833639	0.228	M	Depth	Initial photosynthetic rate
RLC[rETR _{max}]	100.220204	-1.204346	67.201354	-0.175732	0.1412	A	Depth	Photosynthetic capacity (relative electron transport rate)
DMSP:DMSP	0.75419628	-0.002717019	0.816521507	-0.001934149	0.01403	Q	Depth	Inferred oxidative stress
Incubation δDO ₂	-17.9271223	0.3868667	-64.6352683	-0.7049168	0.0003409	D	Depth	Net photosynthesis during dark trials
Tissue molar C:N	13.6679714	0.0446042	8.9962262	-0.0825296	0.006482	E	Depth	Holobiont condition
δ ¹³ C differential	-15.88822	-0.01171	-13.94745	-0.08405	0.1926	C	Depth	Long term photosynthetic signal
Tissue δ ¹⁵ N	1.63560878	0.03637871	2.29028497	-0.00204339	0.2585	B	Depth	Inferred trophic level
Sediment δ ¹⁵ N	2.86968	0.7132	2.40521	0.69883	0.2104	K	Depth	Environmental control
DIC sea water	0.88	8.29E-19	0.6542	0.005055	0.4287	L	Depth	Environmental control
Tissue δ ¹⁵ N	3.000773	-0.004688	2.9671088	-0.007505	0.1366	O	RLC[rETR _{max}]	Investigating site differences
δ ¹³ C differential	-14.37608	-0.97474	-20.32498	1.10782	0.1584	P	Tissue δ ¹⁵ N	Investigating site differences
RLC[rETR _{max}]	53.35772	16.19293	66.32352	-7.45428	0.01279	F	DMSO:DMSP	Photosynthetic capacity (relative electron transport rate)
Incubation δDO ₂	-6.487366	-3.052744	-13.446896	30.751496	0.007288	I	DMSO:DMSP	Net photosynthesis during dark trials
Tissue molar C:N	17.385736	-3.462559	18.745543	-4.712544	3.32E-05	J	DMSO:DMSP	Holobiont condition
δ ¹³ C differential	-13.212548	-2.262587	-15.850777	-4.685545	0.05017	H	DMSO:DMSP	Long term photosynthetic signal
Tissue δ ¹⁵ N	2.7326381	-0.1110724	2.5391774	-0.5638523	0.0002416	G	DMSO:DMSP	Inferred trophic level

Table 2. Model parameters. (above): Values are reported as they appeared in computer outputs. References to the residual plots in supplementary information for each model are under column 'Residuals'. 'Interpretation' is a brief explanation of what the model represents. LB = Site Little Bight. TMA = Site The Maze

APPENDICES:

Supplementary 1. Measurement error for stable isotope analyses:

The standard deviations of the measurement distribution of four different standards, by isotope, used during stable isotope analyses of coral tissue and sediment.

Supplementary 2. Datafile:

All raw data and calculated metrics used above are contained in the attached CSV file.

Supplementary 3. $\delta^{15}\text{N}$ of Sediment at LB and TMA with depth:

Shaded areas are the 0.95 confidence interval. Statistical assessment and model parameters can be found in Tables 1 and 2.

Supplementary 4. DIC of sea water at LB and TMA with depth:

Shaded areas are the 0.95 confidence interval. Statistical assessment and model parameters can be found in Tables 1 and 2.

Supplementary 5. Residual plots considering the assumptions of linear models:

From top left, clockwise, each plot in a panel reveal deviations from: linearity, normality, outliers, and heteroskedasticity for the relationship mentioned in the attached file.