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Identifying the optimum temperature for the most efficient photosynthetic rate in the scleractinian coral *Stylophora pistillata* in the Gulf of Eilat, Red Sea

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Abstract

The Gulf of Eilat (GoE), Red Sea, is a unique region that harbours one of the northernmost coral reef ecosystems in the world, and possesses high biodiversity and endemism. Global climate change is associated with an increase in sea surface temperatures that can lead to bleaching and mortality in scleractinian corals. However the corals in the GoE differ to other reefs at the same high latitude because of their unique resilience to thermal stress, which suggests that this region may be a refuge for reefs under a period of environmental change. This study aimed to determine the temperature at which the photosynthetic rate of the branching coral *Stylophora pistillata* is at its optimum capacity within this refuge. The effects of gradually increasing temperatures on the photochemical capability and oxygen evolution in the endosymbiotic zooxanthellae were measured, as well as the effect of the current ambient (26°C) and the known bleaching threshold (32°C) temperatures on the zooxanthellae density and chlorophyll content. As photosynthetic rate is widely considered to be a good indicator of coral health, these results suggest that photosynthesis in *S. pistillata* functions most efficiently between 27-29°C, and that there is a significant effect on this rate at the known bleaching threshold at 32°C. There were also higher values of both zooxanthellae density and chlorophyll content at 26°C compared to 32°C. Comparing these results to predictions of future SSTs in the GoE suggests that the local average summer maxima will reach ~28°C by 2030, and ~29°C by 2050. This provides guidelines for scientists and conservation managers as to when corals will reach their optimum temperature and enabling improved ability to predict and mitigate the effects of other anthropogenic stressors, so that coral reefs can thrive in their optimal environment.

Keywords: climate change, coral reefs, coral health, photosynthesis, optimum temperature, Gulf of Eilat, Red Sea

Introduction

Coral reefs are one of the most diverse ecosystems in the marine environment, but have been dramatically declining in recent years due to a rise in sea surface temperature (SST), which has been linked to global warming, and the subsequent increase in intensity and frequency of mass coral bleaching (Veron et al., 2009). Coral bleaching typically occurs when thermal stress causes the coral host to expel its endosymbiotic algae (zooxanthellae) from its tissues, making it transparent, and hence exposes the white carbonate skeleton (Gates et al., 1992; Brown, 1997). Bleaching has led to mass loss in coral cover, change in communities, decline in coral health, as well as loss of critical habitat for associated species, such as reef fish (Baker et al., 2008).

Sea surface temperatures (SSTs) have increased by 0.4-1°C across most of the tropical regions since the 1970s (Cantin et al., 2010), which is unprecedented to historical environmental changes (Veron et al., 2009). If SSTs rise by 2°C as predicted by the middle of this century, it could exceed the upper thermal tolerances of many tropical reef-building scleractinian corals, and the majority would be unable to adapt physiologically or genetically to this rapid change (Glynn, 1993). Other stressors can also impact coral reefs individually or synergistically, such as ocean acidification (De'ath et al., 2009; Veron et al., 2009), increases in ultraviolet (UV) radiation (Ferrier-Pagès et al., 2007), diseases (Brown, 1997), tourism and SCUBA diving (Gladstone et al., 2013), and nutrient pollution from sewage and fish farm waste (Kotb et al., 2004).

Chronic temperature anomalies over the next few decades will lead to reefs only being composed of the hardiest species that are more resilient and can adapt to higher temperatures, which can because of a number of factors (Fitt et al., 2001; Baker et al., 2008). It may depend on the thermal acclimatisation history of corals within a region because of genotypic adaptation to the environment over time (Faxneld et al., 2011; Carilli et al., 2012; Howells et al., 2013), or a specific thermotolerant type of zooxanthellae that the corals harbour (Buddemeier & Fautin, 1993). In high latitude regions, some predictions show that corals in cooler areas have lower upper-thermal-limits than those in tropical and equatorial reefs, and so will reach marginal conditions which are close to their upper thermal limits (Dalton & Carroll, 2011).

The Gulf of Eilat (GoE) in the northernmost part of the Red Sea is one of these particular regions, as they remained relatively healthy even when SSTs exceeded the local average summer maximum temperature by 2.0°C in 2010 and 2012 (Fine et al., 2013). Although other studies imply that reefs at the same high latitude of 28-29.5°N (Dalton & Carroll, 2011; Debose et al., 2013) should reach their bleaching threshold at 27°C, the actual threshold of Eilat corals was found to be 5°C higher, at 32°C. This is not due to the high-latitude location, *Symbiodinium* diversity, or previous repetitive exposures to varying temperatures but because of their unique thermal acclimatisation history (Howells et al., 2013). During the dispersal of coral larvae over a geological period of time, the ones which were able to survive passing through the extreme environmental conditions of the Red Sea before they settled in the cooler waters of the GoE were selected for and so are naturally resilient (Fine et al., 2013).

There are a number of ways that reef health under a changing climate can be studied. These include coral growth rates (Edinger et al. 2000), or calcification rates (De'ath et al., 2009; Cantin et al., 2010), and energy reserves (Schoepf et al., 2013). However, the effect of temperature stress on photosynthetic rate in the zooxanthellae is also considered to be a good indicator, as the thermal sensitivity of some types is thought to be one of the primary causes of bleaching (Brown, 1997; Warner et al., 1999), and they also provide a critical source of nutrients and energy for metabolism, which essentially drives the major biochemical processes in corals (Grottoli et al., 2006).

Stress induced by temperature has been shown in many studies to decrease the photosynthetic performance of the zooxanthellae (Jones et al., 1999; Maxwell & Johnson, 2000; Ralph et al., 2005). During photosynthesis, the by-products of fluorescence, heat and photochemistry occur after the absorption of light photons by chloroplasts in the zooxanthellae, and these are pathways that "compete" for the de-excitation of chlorophyll (Zartler, 2012). The same excitation states that result in fluorescence emission also contribute to photochemical energy conversion (Schreiber, 2004).

Fluorescent quenching is the increase in the rate at which electrons are transferred away from photosystem II during the light-dependent stage of photosynthesis, when the light photons activate the enzymes involved in carbon metabolism, and results in photochemical quenching. However, non-photochemical quenching (qN) occurs when there is a rise in the efficiency of the conversion of light to heat energy, or if it is re-emitted in the form of fluorescence (F). The degree to which the amount of energy converts into each of the states depends on the environmental conditions, so if there is an increase in the efficiency of one of them, the other two will decrease. Therefore, it is critical when studying photosynthetic performance to determine the extent of both photochemical and non-photochemical processes (Maxwell & Johnson, 2000). The effective quantum yield (Y(II)) is a good indicator of thermal stress in corals as they represent the effective amount of light acquisition that occurs in PSII at any given time. A decline in Y(II), caused by thermal stress therefore reflects a decrease in the photochemical efficiency of zooxanthellae (Zartler, 2012)

Maxwell & Johnson (2000) review the many benefits of using a "modulated" measuring system to determine photosynthetic efficiency. One system is the Imaging-PAM chlorophyll fluorometer which can determine the effective quantum yields from quenching analysis and rapid light curves, but it also allows multiple samples to be tested simultaneously, as multiple Areas of Interest (AoI) can be selected (Ralph et al., 2005). Another similar fluorometer includes the Dual-PAM-100, which enables simultaneous measurements of Chlorophyll fluorescence and P700 absorbance changes (Klughammer & Schreiber, 2008).

The Gulf of Eilat may be a suitable refuge for corals, as it is a site of extreme high-frequency thermal variability that has selected corals which are physiologically pre-adapted to cope with regional temperature increases (Pineda et al., 2013). Another example of a high-latitude reef refuge is the Gulf of California which has stable and thermotolerant symbiotic combinations (LaJeunesse et al., 2008). Identifying high-latitude marginal reefs regions such as this in a period of global climate change is

critical for gaining a better global perspective of predicting responses to environmental change (Kleypas et al., 1999), as these regions harbour thermally-resistant corals that are able to delay the onset of bleaching and mortality. However, many of the potential sites are either under-studied or under threat from other natural or anthropogenic stresses (Beger et al., 2013).

Although the general consensus is that action is needed now to prevent significant loss of coral reefs (Veron et al., 2009; Maynard et al., 2013), it may be better to plan the timing of conservation schemes more effectively with these refugia. In addition, one study suggested that the general optimum temperature range for coral reef formation is 25-29°C (Vaughan & Wells, 1943), so some reefs may already be in this range or have gone beyond it in more tropical regions (Hume et al., 2013). In the GoE, it is not expected that the thermal threshold (31°C) of some coral species will not be reached until at least 2100 as the onset of bleaching and mortality is delayed in this region. Therefore it may be that the ideal conditions for this region have not been reached yet as current predictions suggest they will have higher growth rates in warmer waters (Fine et al., 2013).

The main aim for this study is to determine the optimum temperature for the most efficient rates of photosynthesis for *Stylophora pistillata*, with a hypothesis that tests the effect of temperature on the rate of photosynthesis. A number of different parameters will be tested in order to do so, such as photochemical efficiency, rate of oxygen production, and the density of zooxanthellae and chlorophyll content. Little is known what the “ideal” conditions for the unique coral reefs found in the Gulf of Eilat should be, so knowing this could enable scientists and environmental managers to make more informed decisions about preserving resilient populations.

Methods

Study Organism and Site

Samples were collected from an underwater coral nursery next to coral reefs in front of the Interuniversity Institute for Marine Sciences (IUI) in Eilat, Israel (Gulf of Eilat, Northern Red Sea, 29°30'N, 34°56'E) at a depth of 6m using free diving techniques. Single-branched fragments, or microcolonies, of 3-5cm were cut from the coral *Stylophora pistillata* (Esper 1797) using cutters. *Stylophora pistillata* was chosen as a model due to its dominance in the Red Sea (Loya, 1972) and because it has been the study species for many other studies on photophysiology and photosynthesis (Falkowski & Dubinsky, 1981; Winters et al., 2003, 2009; Nir et al., 2014).

Sample Collection and Aquarium Maintenance

Four fragments were taken from each of six colonies on the same nursery table, and placed in separate small zip-lock bags, which were removed from the water and placed into the tanks within 20 minutes to minimise the stress of fragmentation and transportation in the bags. The fragments were mixed so there were four from different colonies in each of the six 10-litre aquaria (Aquarium (A) 1-6) before microcolonies were prepared. Microcolony propagation has been described by Al-Moghrabi et al. (1993). The microcolony from each mother colony were labelled with coloured wires, which were tied around the attached fishing wire, so they were easy to identify (i.e. Colony (Co1= Red; Co2=Blue; Co3= Green; Co4= Orange; Co5= Blue & Orange; and Co6= Black).

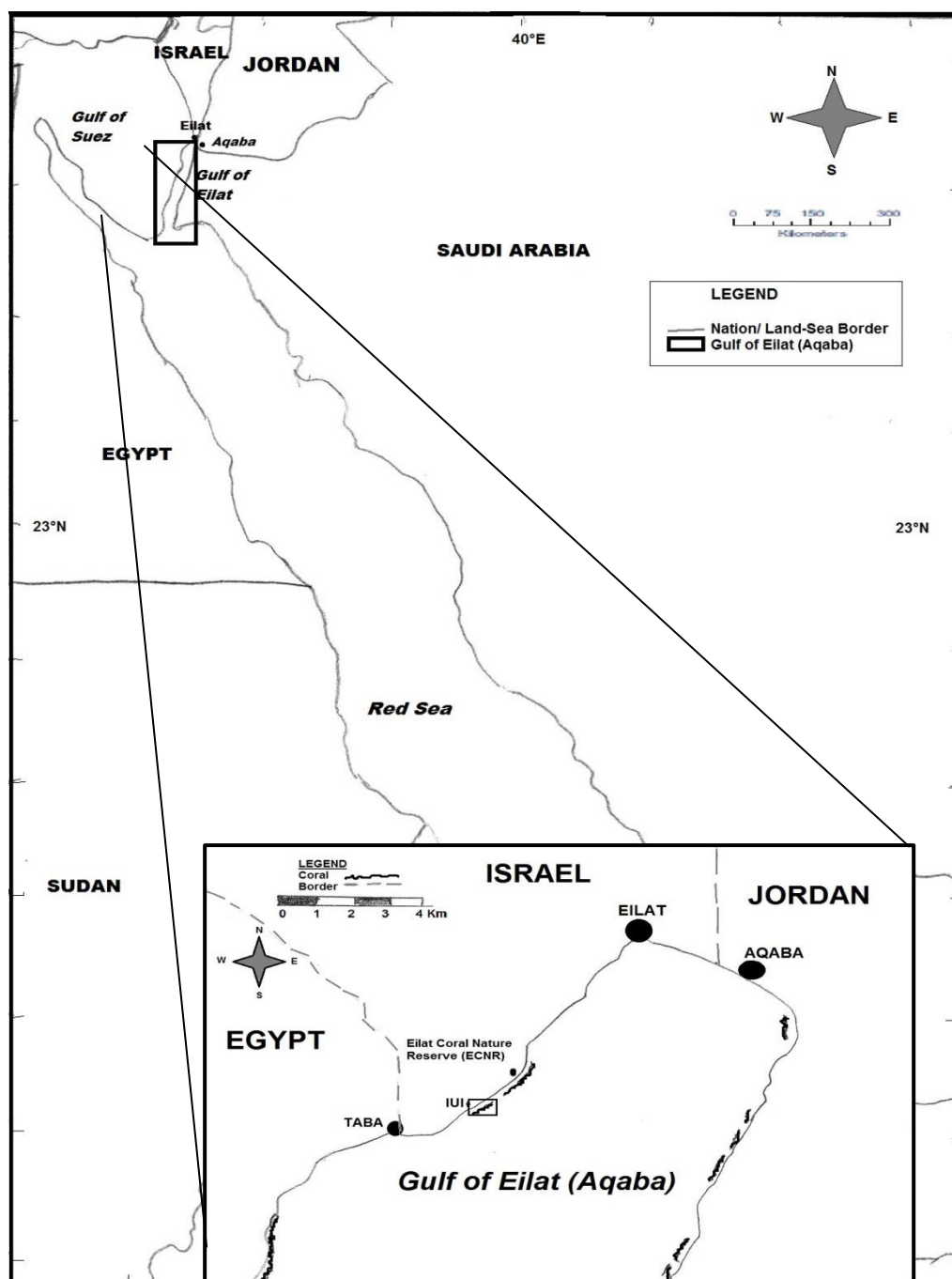


Fig. 1: a) General map of the Red Sea and the surrounding nations, and b) the northern region of the Gulf of Eilat (Aqaba), and locations of coral reefs (IU= Interuniversity Institute for Marine Sciences). a) was modified from Kotb et al. (2004) and b) from Loya (2004).

Any small protrusions or branches from the fragments were removed to keep the sizes (and hence surface areas) as similar as possible. Six plastic cylindrical pipes were cut to fit the length of the tank to hang the fragments from.

The microcolonies acclimated for 12 days before the temperature was increased in A1, A2 and A3 by using thermostat-regulated aquarium heaters (Plenn-Plax Cascade Heart Thermostat, 300W). They were also fed the day after sample collection with *Artemia* spp. nauplii from the Underwater Observatory Marine Park so they could acclimate faster, but because feeding can influence photosynthetic rates under different temperatures, they were not fed for the remainder of the experiment (Hoogenboom et al., 2012). Measurements for pH, temperature, and mean flow rate were recorded daily, and the light intensity was also recorded and adjusted to ensure evenness.

Following the acclimation period, the temperatures in A1-3 were increased by 1°C above the ambient summer SST (26°C) for 1.5 days to allow time to take measurements for photosynthesis after the initial incubation. This process was repeated every 1.5 days as a rolling experiment, progressively increasing the temperature by a degree each time until 32°C was reached, and was maintained at this peak temperature for 14 days. Digital photographs were taken during the acclimation period, 1 day, and 5 days after exposure to 32°C to determine coral health based on colour and length of each fragment, using a Coral WATCH Health Colour Chart.

Photochemical Efficiency

The photochemical efficiency of the symbiotic zooxanthellae living in the tissues of *S. pistillata* was measured at the temperatures 25, 26, 27, 28, 29, and 32°C using an Imaging-PAM M Fluorometer (Heinz Walz GmbH, Effeltrich, Germany) (Ralph et al., 2005). The parameters used were the average effective quantum yield of PS(II) in the zooxanthellae, $Y(II)$, the electron transfer rate (ETR) in PS(II), the amount of energy dissipated as heat during photosynthesis (qN), and fluorescence (F). A Dual-PAM-100 (Heinz Walz GmbH, Effeltrich, Germany) was also used as a comparison of PAM software (Schreiber & Klughammer, 2008) to test for compatibility of the data for $Y(II)$, qN and ETR at 26, 30, 31 and 32°C, due to a temporary malfunction of the Imaging-PAM during the experiment which meant measurements at 30 and 31°C could not be recorded.

The effective quantum yield derives from a ratio of different fluorescent intensities, including the current yield of fluorescence in the light ($F_t=F$), the yield of fluorescence without actinic (photosynthetic) illumination (F_o), the maximum fluorescence (F_m), and the equation for the effective quantum yield could be worked out: ($Y(II) = (F_m' - F)/F_m'$), which were calculated by the Imaging-PAM, and by the Dual-PAM. ETR ($ETR = Y(II) \times PAR \times 0.5 \times 0.84$), and qN ($qN = (F_m - F_m')/(F_m - F_o)$) could also be calculated.

A Rapid Light Curve RLC) was plotted for each parameter over increasing Photosynthetically Active Radiation (PAR) intensities (Mass et al., 2010) (from 0, 21, 111, 281, 396, to 531 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), as the photosystems in the chlorophyll became saturated at this point, and further over-saturation led to photoinhibition. Four fragments were randomly selected from each treatment and were acclimated to

dark conditions for 20 minutes. This was repeated for every experimental temperature that the aquarium seawater was raised to (Maxwell & Johnson, 2000).

Oxygen Evolution

The oxygen levels of three fragments were measured for one hour in sealed flasks (“chambers”) with the Oxy-4 (PreSense, Germany) and four electrodes (with one control heated chamber that contained no coral) after 20-30 minutes under dark conditions to acclimate until the oxygen levels on the graph decreased below the line of the control graph (i.e. when they were no longer photosynthesising and only respiring). Each flask/ chamber was placed on a magnetic heated-stirrer, with a magnet at the bottom of each open flask. Each coral was suspended halfway in the seawater, and the electrode was also positioned halfway into the flask near to the fragment. The opening of the flask was sealed with Parafilm to secure the electrode and to also minimise evaporation.

Coral Tissue Extraction

After the fragments had been exposed to 32°C for 14 days, 4 fragments from both treatment and control aquaria were randomly selected for tissue extraction. The tissue was removed using a handheld compressed airbrush spray filled with Filtered Seawater (FSW) that was attached to a 12L dive cylinder. This was done until 15mL of tissue and FSW were collected in a 50 mL Falcon tube, which was held in a covered ice box. The coral skeleton was dried and used for measuring the surface area of the fragment. The tissue was homogenised with an electronic homogeniser (Heidolph DIAX100 100W, Germany) for 30 seconds and placed in the centrifuge for 5 minutes at 5000rpm at 4°C. The supernatant (coral host tissue) was removed and the pellet was resuspended with 1mL FSW with a vortex (ORNAT BIO Vortex V1-Biosan, DC:12V) and the homogeniser, before centrifuging the samples in 1mL Eppendorf tubes for 5 minutes under the same conditions as before. This process was repeated, but before the samples were centrifuged again, 100µL was removed for a later zooxanthellae count. 1mL of 90% Acetone was used in the fourth and final resuspension of the zooxanthellae pellet, which was then incubated at 4°C in the dark for 15 hours for chlorophyll content (see *below*).

Chlorophyll Content and Zooxanthellae Density

A spectrophotometer (Thermo Scientific-Multiskan Spectrum- Skanit Software 2.4.2) was used to determine the total chlorophyll content of the incubated zooxanthellae samples from the treatment and the control aquaria after 15 hours by checking the Optical Density (OD) at three different wave lengths (630, 664 and 750 nm) for each sample. 1mL of 90% Acetone was added to a glass curvette and used as a blank for the first sample. A spectrophotometric equation was applied to the results to calculate the total chlorophyll content of the zooxanthellae (Jeffrey & Humphrey, 1975):

$$\text{Chlorophyll } (\mu\text{g}) = 11.43 * [\text{OD}(664\text{nm}) - \text{OD}(750)] - 0.64 * [\text{OD}(630) - \text{OD}(750)]$$

After 15 hours of incubation in the dark at 4°C, the 100 µL of zooxanthellae suspension was diluted to 1:3 Filtered Seawater (FSW). 30µL of the well-suspended sample was placed on a haemocytometer and a cover slip was placed over it. The total number of zooxanthellae was counted under a light microscope in each of the 1mm² squares in the corners of the grid. To remain consistent, the cells were only

counted on the lines of the grid if they were on the top or left hand side of the square, and only the distinct dark circular cells were included, as there was still some debris left from the centrifugation. Three replicate counts were carried out and averaged for each of four samples from fragments from both treatments. The total number of zooxanthellae for each fragment was calculated and multiplied by 10,000 (for the number of zooxanthellae per 1mL).

Coral surface area was determined using melted paraffin wax maintained at 65°C (Veal et al., 2010). The skeleton was dried and then weighed, before each fragment was dipped in the paraffin for three seconds (only covering the measured area) and taken out and shaken gently. After leaving them to cool at room temperature, the covered fragments were weighed again. The four fragments from the treatment and control aquaria randomly selected for measuring chlorophyll content and zooxanthellae abundance were used to normalise the results from these experiments to the surface area of the skeletons. The weight of wax was transformed to surface area using a calibration curve ($R^2=0.978$) based on standardised cubes produced from sandpapering *S. pistillata* coral skeletons to known surface areas (similar to Winters et al., 2009).

Statistical Analysis

The effect of temperature on photochemical efficiency was analysed separately for each parameter, Y(II), ETR, qN and F, using a one-way Analysis of Variance (ANOVA). For further analyses of these parameters when a significant difference ($p<0.05$) was detected, Tukey's *post hoc* multiple comparisons tests were performed over data points and between treatments (temperature; *Mini-Tab 16*). Kolmogorov-Smirnoff normality test and Levene's homogeneity of variance test were used to determine whether the assumptions of the parametric ANOVAs were satisfied. If data could not satisfy the test for equal variances, even after log₁₀ transformations, a non-parametric Kruskal-Wallis test was used. Paired t-tests were applied to determine whether there were significant differences in the number of zooxanthellae and the chlorophyll content, between the two temperature treatments. Unless otherwise specified, mean values are presented with \pm S.D.

Results

Photochemical Efficiency

The fragments of *S. pistillata* fragments showed much variation in their photosynthetic capacities when exposed to different temperatures, particularly at 32°C. Table 1 shows the values for each of the four parameters at 531 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the peak of the Rapid Light Curve (RLC) to represent the maximum value of fluorescence so the optimum could be calculated.

There was a significant difference ($F_{5, 23} = 10.76$ $p = 0.001$) between all the experimental temperatures tested using the Imaging-PAM fluorometer (25, 26, 27, 28, 29 and 32°C) for the effective PSII quantum yield, Y(II) at a PAR intensity of 531 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, when an analysis of variance was applied, but the *post-hoc* Tukey test revealed that the means for the samples at all temperatures between 25 and 29°C were statistically similar, and the difference was between these and the mean value of Y(II) at 32°C (Table 1). A similar result was obtained for ETR_{max} , as there were similar means for all temperatures between 25 and 29°C shown by the

post hoc test, but there was a difference of ($F_{4,19}=1.40$ $p=0.001$) for fragments at 32°C. The results were not significantly distinct, but the highest ETR_{max} value (36.5 ± 8.4) was 29°C at a PAR intensity of $531 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, and the highest $Y(\text{II})$ was at 26°C (ambient temperature), although the second greatest value was also at 29°C.

However, both parameters qN and F were both found to be non-normal when the values for 32°C were included, even after logging the data, so the non-parametric Kruskal-Wallis test was used. The results showed that there was no significant difference between the means of qN ($H = 6.08$ d.f.=5 $P= 0.298$), and although the data was normal when only testing the values from 25 to 29°C, there was still no difference. The highest value for qN was at 27°C (0.706 ± 0.06), which differs from that of the effective quantum yield and electron transfer rate, but the lowest value was at 32°C (0.397 ± 0.46), followed by that at 29°C (0.559 ± 0.05). Similarly, fluorescence was tested using Kruskal-Wallis, but there was no significant difference between the temperatures ($H = 4.69$ d.f. = 5 $P = 0.455$), which could be due to the large amount of variation from values at 32°C.

Table 1: Values from RLC data at PAR intensity $531 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the peak of the curve before photoinhibition started to occur. Values are means \pm SE. One Way Analysis of Variance and Tukey's pairwise comparison for parameters $Y(\text{II})$ and ETR_{max} , with $P < 0.05$. A Kruskal-Wallis test comparison was conducted for qN and F , with $p < 0.05$; superscript letters represent similar means; Asterisks represent significant difference $P < 0.05$.

Temp.	Y(II)	ETR _{MAX}	qN	F
25	0.111 ± 0.028^a	22.1 ± 7.1^a	0.651 ± 0.08	0.276 ± 0.03
26	0.176 ± 0.055^a	37.2 ± 14.2^a	0.619 ± 0.07	0.261 ± 0.02
27	0.160 ± 0.040^a	35.2 ± 10.6^a	0.706 ± 0.06	0.252 ± 0.02
28	0.169 ± 0.047^a	35.7 ± 11.6^a	0.647 ± 0.02	0.250 ± 0.04
29	0.173 ± 0.023^a	36.5 ± 8.4^a	0.559 ± 0.05	0.244 ± 0.02
32	0.014 ± 0.029^b	2.4 ± 4.75^b	0.397 ± 0.46	0.417 ± 0.26
<i>P</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.298</i>	<i>0.455</i>

Figure 2b shows the rate of the Rapid Light Curves at which $Y(\text{II})$ and ETR reached these values at $531 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the different temperatures. There is a significant difference between 32°C and the rest of the experimental temperatures, but the means are statistically similar between 25-29°C for both $Y(\text{II})$ and ETR . Although it is not statistically different, the rate of $Y(\text{II})$ at 27°C was lower than the other means. Figure 3 that the parameters are higher at 31°C for $Y(\text{II})$, ETR and qN than 32°C.

The values for $Y(\text{II})$, ETR and qN produced from the Dual-PAM were correlated against those produced from the Imaging-PAM at temperatures 25 and 32C to determine first whether the data from the two PAM fluorometers could be combined. The data was first ranked before the Spearman's Rank correlation coefficient and regression tests could be applied. The results showed that the Dual-PAM was a significant indicator of the results from the Imaging-PAM for $Y(\text{II})$ at 32°C ($R^2_{adj} = 0.887$ $F_9= 71.47$ $p=0.000$), and at 26°C ($R^2_{adj}=0.973$ $F_9=324.01$ $p=0.000$). In addition, there was also a significant correlation between the Dual-PAM and Imaging-PAM results both at 32°C ($R^2_{adj}=0.626$ $F_9=16.20$ $p=0.004$) and at 26°C. However, the

results for ETR from the Dual-PAM at 32°C were not significant indicators for those from the Imaging-PAM ($R^2_{adj}=0.00$ $F_9=0.11$ $p=0.748$), nor for those at 26°C ($R^2_{adj}=0.50$ $F_9=1.47$ $p=0.260$).

Oxygen Evolution

The time taken for oxygen levels to start increasing after the decline (i.e. to re-start photosynthesising) varied amongst the experiments conducted at the different temperatures, possibly because of evaporation, so the results were taken from the inflexion point at which the oxygen levels of the heated corals exceeded that of the heated control levels and measured for 60 minutes. The readings from the two samples in the heated flasks were averaged and normalised against the non-heated control sample to counteract any confounding effects from evaporation. The rate of oxygen production at each temperature were calculated, and the highest rate was at ambient temperature 26°C ($3.628 \text{ mgL}^{-1}\text{h}^{-1}$), followed by the rates at 25°C ($2.295 \text{ mgL}^{-1}\text{h}^{-1}$) and at 27°C ($1.542 \text{ mgL}^{-1}\text{h}^{-1}$). The rates at the subsequent temperatures: 28°C ($1.093 \text{ mgL}^{-1}\text{h}^{-1}$), 29°C ($1.49 \text{ mgL}^{-1}\text{h}^{-1}$), 30°C ($0.977 \text{ mgL}^{-1}\text{h}^{-1}$), 31°C ($0.929 \text{ mgL}^{-1}\text{h}^{-1}$) and 32°C ($0.667 \text{ mgL}^{-1}\text{h}^{-1}$) gradually decreased with each 1°C rise in temperature.

Effects of Temperature on Zooxanthellae

The paired *t*-test showed that there was a significant difference ($p=0.002$) between fragments at 26°C and at 32°C in density of zooxanthellae, when normalised to surface area of the skeleton of the corals. There was a higher density at 26°C of $1.915 \times 10^5 \pm 2.893 \times 10^4$ zooxanthellae per mL of FSW from the fragments when normalised to unit skeletal surface area, and $1.166 \times 10^5 \pm 4.112 \times 10^5$ zooxanthellae per unit surface area mL^{-1} of FSW at 32°C. There was also a significant difference between the content of the pigment chlorophyll at 26 and 32°C, with $2.420 \pm 1.276 \mu\text{g}$ and $0.805 \pm 0.130 \mu\text{g}$, at 26 and 32°C, respectively.

Plate 1 illustrates very clearly the morphological effects of an increase in temperature. *Plate 1a* was taken seven days after the fragments had acclimated to the aquarium conditions, when the SST was ambient (i.e. $26 \pm 0.62^\circ\text{C}$). However, there is a clear change in colouration of coral tissue after the aquarium temperature had been gradually raised by 1°C every one and a half days until it was kept stable at 32°C.

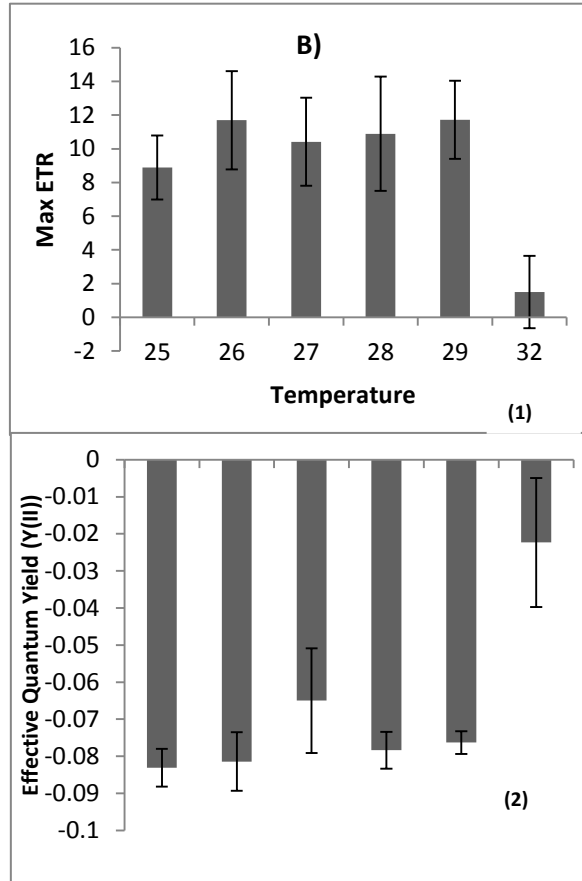
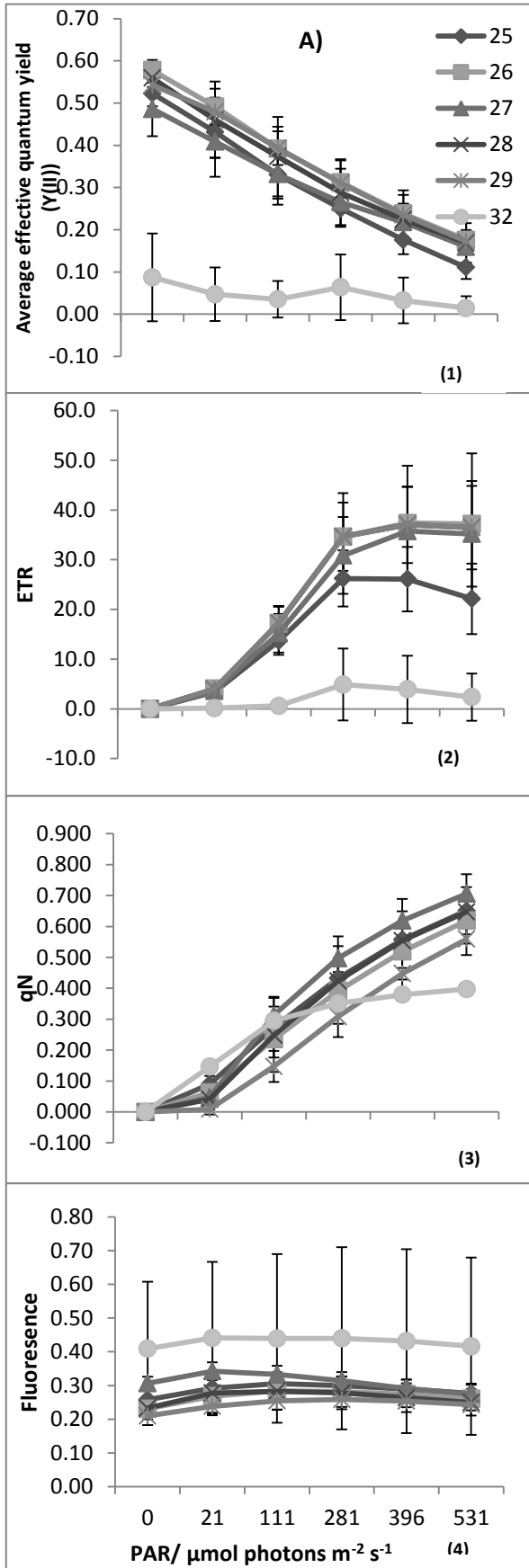


Fig. 2: A) Rapid Light Curves (RLC) of *Stylophora pistillata* fragments at different temperatures systematically increasing from 25 to 29°C, and at 32°C as a heat stress comparison, using an Imaging-PAM M-series Chlorophyll Fluorometer, increasing in PAR intensity at 20 second intervals after 20 minute dark acclimation. An area of interest (AOI) was defined in the centre of the fragment. (1) Mean effective quantum yield, Y(II), (2) ETR, (3) qN, and (4) fluorescence (F). **B)** The average rate of (1) ETR and (2) Y(II) of the Rapid Light Curves at each temperature measured from 25 to 29°C, and compared to a known “stressful” temperature (Fine et al., 2013) at a PAR intensity of 531 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ using an Imaging-PAM Fluorometer. Mean \pm SD (n=4).

Plate 1: Change in coral colouration of a fragment from one of the six *Stylophora pistillata* colonies sampled at A) 25°C, and at 32°C for B) one day, C) five days, and D) five days, with the measured length included. Coral health was qualitatively assessed for colour using the CoralWatch Coral Health Chart and matching the lightest and darkest colours on the fragment to the chart. The darker colours represent the highest amounts of zooxanthellae. © E. Vaughan



Discussion

Photochemical Efficiency

The comparison of the three states that the light energy absorbed by chlorophyll molecules within the zooxanthellae could be converted into, between $Y(II)$, qN , and fluorescence can be used to determine the performance of photosynthesis within the corals at different temperatures, as the efficiency of photosynthesis can be affected by thermal changes (Maxwell & Johnson, 2000; Zartler, 2012). Therefore, the temperature at which $Y(II)$ is greatest represents the greatest performance of photosynthesis in the fragments. The temperatures claimed to be the optimum range for coral reef formation (25-29°C) (Vaughan & Wells, 1943) and the upper thermal threshold for corals in the GoE (32° C) (Fine et al., 2013) were also the same temperatures used to look at photochemical efficiency in the present study. Although

this was not the original intention at the start of this investigation, it was interesting to compare the current results to these studies to determine whether they support or conflict with them.

The results from the Imaging-PAM suggested that photochemical efficiency is at its highest at 29°C (Table 1), although there were no statistical differences. It is not clear why the rate of Y(II) is lower at 27°C in Fig.2.B.1, which could possibly be due to some other biological or cellular factor. Previous studies have emphasised that results of photophysiology need to be interpreted with caution, as they are not always synonymous with traditional photosynthesis-measuring techniques (Geel et al., 1997). The RLC of Y(II) (Fig.2.A.1) decreased in value with increasing PAR, because fluorescence decreases with exposure time when more electrons are converted into photochemical energy in PSII, as shown by the Kautsky effect (Zartler, 2012), but the highest value at the PAR intensity where the chlorophyll becomes saturated still represents the most efficient temperature (Ralph et al., 2005). The difference in the electron transport rates in PSII was due to very low values at 32°C. The means between 25-29°C were statistically similar, but the highest ETR_{max} value occurred at 29°C (Table 1, Fig.2.A.2). These similar results are not surprising, as ETR is calculated from Y(II).

There were no statistical differences between the RLCs for qN, which could be due to the large amount of variation around the data for qN at 32°C (Fig. 2.A.3), as it signifies a wide variation in the dissipation of heat energy. Some thermally sensitive zooxanthellae may have less capacity to dissipate excess energy from other more thermally-resistant types that have lower excitation pressures (Iglesias-Prieto et al., 2004). There were also no differences found between the fluorescence levels at temperatures 25-29°C and at 32°C. Although the data was normal when only the temperatures between 25-29°C were compared, there was still no significant difference, so the amount of fluorescence was statistically similar at all temperatures.

The malfunction of the Imaging-PAM provided an opportunity to determine whether the results obtained from two types of PAM fluorometers showed similar relative patterns. The inconsistencies of correlations between the results produced from the Dual-PAM and those from the Imaging-PAM at both 32°C and 26°C mean that there is too much difference between them, therefore the data from the Dual-PAM at 30 and 31°C cannot be incorporated into the analysis.

Oxygen Evolution

Fragments at 26°C had the highest rate of O₂ production (3.628 mgL⁻¹h⁻¹), and the rates subsequently decreased progressively with each 1°C rise in temperature. This indicates that 26°C is the optimum temperature for the production of oxygen over time, which is lower than that in the photochemical efficiency experiments (27-29°C). However, these results still fit the suggestion made by Vaughan & Wells (1943) that the general optimum temperature range for coral reef formation is between 25-29°C. Although this present study has refined this range for the corals in the GoE, it suggests that there may be other unknown underlying biochemical processes to also drive oxygen production, so more research is needed before it can be linked more closely with photochemical efficiency to gain an overall rate of photosynthesis (Geel et al., 1997).

Effects on Zooxanthellae

In comparison with the significant reductions in both zooxanthellae density and chlorophyll content at 32°C, and with *Plate c& d*, it can be assumed that the variation in all four parameters of photosynthetic performance at 32°C may be because some samples bleached. For example, the highest amount of fluorescence at 32°C supports this (*Fig. 2.A. 1*), as the smaller number of zooxanthellae in the tissue could mean less light energy is converted into chemical energy for photosynthesis and is mainly reflected off the exposed calcareous skeleton (Gates et al., 1992). It was observed that fragments from colonies 3 and 6 bleached to a greater extent than the others at 32°C, from both the visual signs of bleaching, and because there was a huge amount of variation between the fragments used from different colonies at this high temperature during the experiments. Therefore further studies should also consider the potential intra-colonial variation of *Symbiodinium* between different colonies to determine whether zooxanthellae diversity plays an even more specific role in the resilience of corals of the same species.

Conclusion

There was no single temperature that provides the optimum conditions for all of the parameters collectively, sometimes due to similar means or large amounts of variation, but the results from the photochemical efficiency experiments suggest that the optimum range of temperatures for the highest performance of photosynthesis is 27 to 29°C, which narrows the general range proposed by Vaughan & Wells (1943) but still supports it, as does the highest rate of oxygen production at 26°C. Despite the amount of variation, the significant differences 32°C in all of the tested parameters in the coral fragments at were clear, so this study supports Fine et al. (2013) in that 32°C is a stressful temperature for corals in the GoE.

These results should be linked to ecosystem function and health to determine their ecological relevance, such as the effects of thermal stress and/ or optimum conditions on communities and coral cover (Kotb, 2004; Loya, 2004, 2007). To give a more realistic interpretation of the health of the reefs, and their optimal temperature(s), this experiment could be expanded by looking at the effect of temperature of the rate of calcification as an indicator of coral growth rate (De'ath et al., 2009) because it is thought that this will increase in warmer waters in the GoE (Fine et al., 2013). In addition, the effects of ocean acidification should also be tested in conjunction with temperature, as was done by Reynaud et al. (2003), because coral sensitivity to thermal stress may be increased by decreased pH levels (Anthony et al., 2008). The large number of potential knowledge gaps in the literature highlighted in this study alone clearly shows that studying the optimum temperature of corals could provide so much useful and critical information when attempting to find out how reefs will respond to the actual predicted environmental changes.

The temperature summer maximum (in August) has been increasing at an annual rate of ~0.04°C. Current forecast models suggest that the predicted threshold temperatures of coral species will not be reached in the GoE until after the end of the 21st Century. The comparison of the results of this study to the predictions of future SSTs in the GoE (Fine et al., 2013), it indicates that because the temperature is increasing at an annual rate of 0.04°C yr⁻¹, the local average summer maxima will reach ~28°C by 2030, and ~29°C by 2050. This indicates that between now and the middle of this century, the photosynthetic rate, and hence the health of the coral

reefs, will be at their optimum. Therefore, this is the time to maximise strict protection and management plans to minimise damage and stress from other factors that otherwise threaten these resilient organisms, and hence the diverse reef ecosystems that they form (Kotb, 2004; Gladstone et al., 2013).

In conclusion, this study aimed to provide the optimum conditions at which *Stylophora pistillata* can carry out photosynthesis the most efficiently and hence enhance coral health. Further studies should either combine these results (or repeat the experiment to increase the number of replicates) for the optimum temperatures for photosynthetic rates with more detailed prediction models or forecast methods to determine an even more specific time when the when the range of SSTs in the GoE will be reached in the next century. This will provide more precise estimations as to when management or conservation schemes should implicate these conservation plans to protect these corals when they are thriving.

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