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submitted by/vorgelegt von

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Effects of elevated temperature and CO_2-concentration on photosynthesis, respiration and calcification of the scleractinian coral *Stylophora pistillata* (Esper 1797)

Auswirkungen von erhöhter Temperatur und CO_2-Konzentration auf Photosynthese, Respiration und Kalzifizierung der Steinkoralle *Stylophora pistillata* (Esper 1797)

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Assertion of originality

I hereby confirm that the submitted study was written independently by me and that no other than the cited sources were used. Furthermore i confirm that i have followed the general principles of scientific work and publishing, as they are constituted in "Leitlinien guter wissenschaftlicher Praxis" of the Carl von Ossietzky University Oldenburg.

Oldenburg, 15.11.2010

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(Moritz David Lürig)

Abstract

Elevated pCO_2 and temperature are the most destinct consequenses of the anthropogenic greenhouse effect and the accompanied change in climate. The resulting implications on water chemistry and temperature affect the ocean surface on a global scale and therewith physiology and metabolism of reef building corals. *Stylophora pistillata*, a hermatypic coral, was exposed in short term trials of 20 h to elevated CO_2 concentration (5mg/L), temperature (30°C) and a combination of both. Reactions in photosynthesis, respiration and calcification were analysed using a modified intermittent flow respirometer.
Results showed significant differences in response to the respective treatments: during the light phase, elevated pCO_2 created a reduction of two thirds in net primary production with regard to control, whereas both elevated temperature alone and the combined treatment evoced respiration. At night, the respiration rate under high temperature did not differ from the control, whereas pCO_2 and the combined treatment produced increased respiration rates that in turn where not signigically different. A diurnal rhythm showing a bell shaped trend with a peak in oxygen flux during the afternoon was observable for the control and all treatments except temperature, where an inversed bell shaped trend was visible. Calcification rate was highest in control, about two thirds reduced in the combined treatment and fluctuating around zero during CO_2 and temperature treatment.
Control measurements and reaction to elevated pCO_2 are in agreement with previously published works, whereas such a high day-respiration rate as a reaction to elevated temperature has not been described yet. This reaction is probably due to insufficient lightning that could not serve the corals phototrophic energy supply. This theory needs to be further investigated. The average day-respiration rate during the combined treatment does not differ significantly from the rates measured during the temperature treatment, whereas both treatments show differnt diurnal patterns. It is assumed, however, that temperature exerts the greater influce while combined with elevated pCO_2. Further investigation in this matter is necessary, on other species as well; to asses the respective influence of temperature and pCO_2 on hermatypic corals.

Contents

1 Introduction

This work focusses on reef building (hermatypic) corals and their physiological reaction to the anthropogenic climate change. Its most prominent impacts are elevated temperature and CO_2 concentrations, that will impair distribution and expansion of tropical coral reefs. All experiment wer conducted at the center for tropical marine ecology ("Zentrum für marine Tropenökologie - ZMT").

It is estimated that 284 000 km^2 of the worlds oceans are covered with coral reefs (Spalding et al. 2001). This relatively small area has a tremendous impact on the oceanic ecosystem. Warm waters, high light irradiation, low turbidity and a shallow ocean basement provide optimal growth conditions that are found on the continental shelf and suboceanic mountain ridges within the 20 °C winter isotherme. These enormous biogenic constructs are the product of billions of single coral animals, which, by the help of endosymbiotic dinoflagellate algae (zooxanthallae), precipitate enormous amounts of calcium carbonate ($CaCO_3$). Some reefs and atolls are the results of a hundreds of thousands years of this process called calcification. The first reefs of coralline origin appeared about 237 million years ago (Stanley & Fautin 2001).
Today, coral reefs are a crucial to the regions where they appear; being not only critical for the local and global network of ecology, but also of importance to the cultural or social frameworks. For millenia, the fishes inhabitating coral reefs have been a primary food source for various cultures, in some cases they are the solitary source of animal proteins. For instance, on islands in the pacific regions, they supply every person with over 100 kilograms of fish every year (Spalding et al. 2001). In the past decades the fishing grounds around the reefs have become attractive for supraregional markets, most notably the Asian market. This major economic activity puts additional pressure on the foodweb of the reefs. Apart from fishery, tourism also harnesses coral reefs and has become a great source of income for the economically underdeveloped parts of the Indo–Pacific realm, being one of the of the most visually impressive habitats of the planet while hosting an unparalled genetic diversity. Beside these economical factors, reefs exert very basic funtions. Physical protection of the tropical

coastline is an intrinsic task of barrier and fringing reefs. Although single coral colonies are fragile in their appearance, their ability to repair and recover their structure is crucial to their success. Reefs expand in highly wave–dynamic environments and gradually build up submerged megastructures around islands or entire coastlines that buffer from the worst storms (Spalding et al. 2001). During those storms, coral rubble is created from broken corals, then turns into sand which piles up and enlargens the beaches. The existence and prospective safety of nations like the Maledives or Seychelles depends on these processes, which themselves are associated to healthy reefs and therefore healthy coral animals.

Hermatypic corals only contribute to this reef expansion only under certain physiological and chemical conditions. If these are altered from the corals' requirements, the animal host easily becomes stressed: coral reefs are affected strongly by both temperature and pCO_2 (Reynaud et al. 2003). The accumulation of anthropogenic CO_2 in the atmosphere intensifies the natural greenhouse effect; leading to a dramatically increased ocean surface temperature Randall & Wood (2007).The stress response of corals to high temperatures manifests in physiological reactions such as damaged photosystems (Fitt et al. 2001; Jones et al. 2000), reduced primary production (Porter & Lewis 1999; Nyström et al. 2001), altered respiration (Porter & Lewis 1999; Fitt et al. 2001; Nyström et al. 2001) and reduced calcification (Abramovitch-Gottlib et al. 2002).
Beside increasing temperature, the anthropogenic greenhouse effect shifts the balance in global carbon fluxes. Since the beginning of the industrial age, enormous amounts of carbon dioxide have been absorbed: it is estimated that from 1800 to 1994 the oceans assimilated 118 ± 19 petagrams of carbon that originated from anthropogenic CO_2 emissions (Sabine et al. 2004). Also, a dramatically increased uptake of 53 ± 9 petagrams between 1980 and 2005 has been calculated (Bindoff & Willebrand 2007). IS92a scenario of the 2007 IPCC report suggests that atmospheric CO_2 will double by the end of the century, with regard to 1990 (Randall & Wood 2007). On this basis Caldeira & Wickett (2003) estimated that the succeeding pH–drop on the surface of the oceans will be

around -0.4. This has severe consequences to marine carbonate chemistry.

As CO_2 enters the ocean, most of it is reacting with carbonate to bicarbonate (equation 1):

(1) $$CO_2 + CO_3^{-2} + H_2O \longrightarrow 2HCO_3^-$$

(2) $$\Omega_{arag} = [Ca^{2+}][CO_3^{-2}]/K^*_{sp}$$

$$\text{with } K^*_{sp} = \text{stoichiometric solubility product}$$

Carbonate and calcium ions are formed to the corals' calcium carbonate skeleton inside the cells of the calicoblastic epithelium with the structure of aragonite. The distribution of the worlds coral reefs is, among other parameters defined by the aragonite saturation state Ω-arag of the surface waters (Kleypas et al. 1999, see eq.2). With increasing CO_2 concentration, the aragonite saturation state is lowered. Hence, the availability of carbonate ions becomes a limitating factor and hinders a reef to expand or to repair itself from the damage suffered. With a reduced calcification rate, it has been observed that photosynthesis is also lowered (Schneider & Erez 2006; Nordemar et al. 2003). Therefore it has been assumed that calcification and photosynthesis are connected intensively (Al-Horani & Al-Moghrabi 2003; Nordemar et al. 2003), which, however, is disputed (Gattuso et al. 2000).

The aim of this study was to investigate how these altering environmental parameters; increasing water temperature and pCO_2, influence fitness and resilience of coral reefs. Therefore, individuals of the hermatypic coral *Stylophora pistillata* (Fig. 1) where exposed to a) water temperature elevated to 30°C , b) a CO_2 concentration of 5 mg/l and c) a combination of both. The physiological response with regard to photosynthetic and respiration rate, as well as calcification rate was recorded and analysed for differences in effect. Furthermore, possible interacting effects induced by the treatments, as well as a dependency of metabolic rates from daytime, were examined.

2 Materials and methods

2.1 The used coral species

The test subjects were specimens of the pink phenotype of the hermatypic coral *Stylophora pistillata*, purchased at DeJong Marine Life, Spijksesteeg 2a, 4212KG in Spijk, NL. In September 2009 they were transported to the ZMT and directly put into an indermediate reservoir for regeneration and monitoring. After a few days they were transferred to the mesocosm of the ZMT aquaculture. The purchased individuals were of DeJongs own breeding line.

Figure 1 The test subjects: branching coral *Stylophora pistillata* in the mesocosm at the ZMT-aquaculture

2.1.1 Husbandry

Before and during the entire experimental period, the corals were kept in an autonomous circulatory system in the aquaculture of the ZMT. Consisting of a circular tank (PE, Polyplan) with a diameter of 1.50 m and 40 cm in height, it contained a mesocosm with reef-stone. Furthermore a variety of scleractinian- and soft-corals, benthic fauna and coral fishes were kept in the tank and sustain a miniature reef with high water quality (see. section 2.1.2).
Outside the central radiance cone of the light source (Spacelight 1000W, Aquamedic.

SQ3 37W, Osram) the subjects were placed on the limestone (CaCO3) ground substrate. Water pumps (28 W, Eheim) provided sufficient currents inside the tank. The water was purified by a protein skimmer (APF 600, Deltec) and an ultraviolet lamp (UV-C 37W, AquaCristal). Micronutrients (Energy-Elements 1-3, Energy-Elements) were supplemented by a dosage system as well as carbonates by a carbonate-reactor (Jetstream 1, Schuran) to adjust total alkalinity (TA).

2.1.2 Keeping conditions

From September 2009 on the corals were kept in the mesocosm. From then until the end of the experiments at March 31st water parameters were recorded and, if necessary, adjusted. Good water quality with parameters close to conditions in nature and low nutrient concentrations were important to the corals' regeneration after the stressfull experimental trials (see table 1).
Since the mesocosm provided habitat to a variety of calcifying corals that were quite large in numbers, the water's carbonate system fluctuated strongly, dependent from the time of the day: starting with very low pH in the morning, due to increased CO_2 produced by the respirating corals during the night, the consumption of the same increased the buffercapacity and therewith the pH of the system's water. The temperature was affected strongly by the lighters: being low in the morning it increased until the compensation point where the cooling system started operation.
The mesocosm-tank was supplemented with frozen adults (Golden Gate) of Artemia sp. three times a week, live nauplia of Artemia sp. (Poseidon) and food for filter feeders (Liquifry) was given to the system once a day at noon.

Table 1 Conditions in mesocosm tank (average values, each measured at the same daytime. All paramters related to nitrogen- and phosphate chemistry in mg L^{-1}, E[Einstein] = photons m^{-2} s^{-1}). Temperature, salinity and pH were checked before and during experiments

T (°C)	Sal.	pH	TA (dKH)	Irr. (µE)	NH_4^+	PO_4^-	NO_2^-	NO_3^-
26.5	32.6	7.93	8	220 - 440	<0.004	<0.017	<0.026	<0.022

2.2 Experimental setup

The experiments were performed in the ZMT's respiration-lab, which is adjacent to the aquaculture. An intermittent flow repirometer (Forstner 1983) modified by C.v.Dorrien (Zimmermann & Kunzmann 2001) was used (see Fig. 2). The oxygen- (Oxi 340i + Cellox 325, WTW) and temperature-sensor (PT 100 thermal element + signal transformer, Driessen & Kern) were connected to the central control- and measurement unit ("Respi1" mod.: DT9803 + control unit, Data Translation). Within one minute, this control unit transmits approx. 10.000 pairs of associated oxygen/temperature readings from the sensors to the software (DT Measure Foundry v4.07.48: "Intermittent Flow Respiration Measurement 2.1.0.277, Data Translation) on the respirometer computer, which displays and saves them averaged and by exclusion of definite outliners.

The actual water-cycle consisted of the circular, 1301 ml bearing respiration chamber (acryl, manufactured at the ZMT), the smaller closed- or measurement-cycle with the temperature/oxygen sensors and the larger open cycle which connected the closed to a reserve tank. This reservoir stood hydrostatically elevated above the measurement-cycle and contains seawater oxygenised by a diffusor. Both cycles were conncected through a threeway valve (Nortec) that is regulated by the control unit. Whenever the software registrates a value below the adjusted range, the valve is opened and water from the reservoir flushes the small cycle until the saturation reaches the upper threshhold. Furthermore, the valve can be opened manually.

The respiration chamber was placed inside a larger containment filled with freshwater to compensate for greater fluctuations in temperature. An HQI lamp (Ocean Light 150W, Aquamedic), was installed above the tray and thus above the chamber. Heating rods with internal thermostats were placed both in the compensation tray and the reserve tank (50W, Jaeger), which autonomically adjust temperature according to the experimental programm. The water inside the cycle is circulated by a teflon cogwheel pump (manufactured by Ismatec, magnetic coupling by Faulhaber) with 18 cm^3/s without pumping-intervalls to induce a constant flow against the sensors and inside the chamber to disperse the water equally around the coral. The flexible tube itself is made of tygon

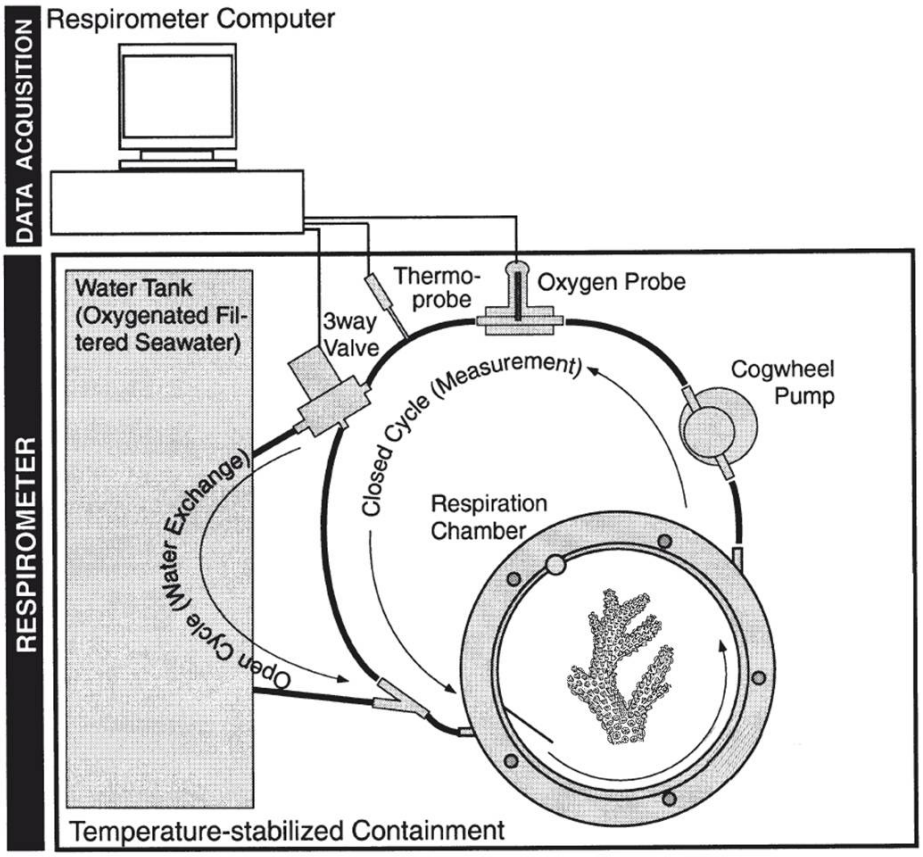

Figure 2 Intermittent flow respirometer (schematic, modified from Zimmermann & Kunzmann (2001))

(r3603, St. Gobain) a resistant and inert material.

For all experiments sea-water taken out of the mesocosm–tank was used in order to provide conditions for the corals that were similar to their husbandry. On the day before the particular experiment, approximately 12 liters were filtered through a 2 µm cellulose filter (Cat. No. 516-0802 type 410, VWR) by the use of a vacuum pump. The filtered water then was stored over night, oxygenated and temperated in an opaque container, for immediate use the next morning. Before the filling of the system, the water was filtered again through a bacterial filter (Acro Pak 1000 0.8/0.2 µm, Pall Corporation) to eliminate bacterial activity in the water.

Table 2 Experimental schedule outlined.

Treatment	Period	Conditions
control	01.02 - 04.02, 31.03	sea water at husbandry conditions
temperature	15.02 - 18.02, 29.03	temperature: 30 °C
CO_2	02.03 - 05.03, 25.03	CO_2 concentration: 5mg/l
CO_2 + temperature	09.03 - 12.03, 30.03	5mg/l CO_2 + 30 °C

control: During the control phase, sea water was taken from the mesocosm, filtered and filled into the circulation system.

temperature: The water was pre-heated over night, filled into the circulatory system prior to the measurements and then raised to 30°C. It proved to be difficult to maintain equally high temperatures in the measuring cycle and the reservetank due to the hysteresis of the heating rods and variability in the rooms temperature.

CO_2: To elevate the CO_2 concentration in the cycle's water, gaseous CO_2 was lead directly into the reservoir. Since TA is not altered by CO_2 inflow (Smith & Key 1975), the designated and actual concentration of CO_2 could be supervised and controlled by pH measurement. TA was determined by the use of a quick test kit (Alkalinity Profi Test, Salifert), pH with a computer system (with a plastic pH electrode, Aquamedic). Then, with the knowledge about TA and pH, the desired concentration was calculated by the use of formula 3:

$$CO_2\ [\text{mg l}^{-1}] = \left(\frac{\text{TA}\ [^\circ\text{dH}]}{2,8}\right) 10^{7,91-\text{pH}} \tag{3}$$

The pH computer system controls the CO_2 inflow by a magnetic valve (M-valve Standard, Aquamedic) that connects a diffusor in the reserve tank with a pressure cylinder containing CO_2. The pH electrode inside the tank detects the current value and, if necessary, opens the valve to the diffusor. Beside further TA testing for checking purposes this system was running autonomously.

2.2.1 Photosynthesis and respiration measurements

The oxygen flux was analysed under the aspect of metabolic quotients, which are used either for animals, plants or symbiotic units. For the latter one has to distinguish between photosynthetic and respiratory quotients of the host and the algae. The experimental setup did not have this ability, hence the appliance of the metabolic quotients was simplified; the whole animal including the zooxanthellae were taken as one unit. According to the terminology of Gattuso & Jaubert (1988) following quotients were examined:

$$P_n \longrightarrow \Delta O_2 \text{ during light phase}$$
$$R_c \longrightarrow \Delta O_2 \text{ during dark phase} \tag{4}$$

The software saved measured oxygen levels in % saturation, as well as temperature in °C; one pair of values per minute. Thus a complete recording of one experiment presented 2 plots over time. To calculate net O_2 changes adjusted from temperature, the saturation values hat to be converted to absolute O_2-concentrations.
The total and relative oxygen-flux was determinded by defining slopes in the plots, substracting the O_2-value at the end from the value at the slope's start and dividing the result by time. With the knowledge of the corals' surface, the oxygen flux per surface and time could be calculated (formula 5,Schneider & Erez (2006)):

$$P_n \text{ or } R_c \ (\mu\text{mol cm}^{-2}\ \text{h}^{-1}) = \frac{\Delta O_2\ (V_{chamber} - V_{coral})}{\Delta T\ S_{coral}} \tag{5}$$

with $V_{chamber}$ = total volume of the closed cycle [L], V_{coral} = displacement volume of the coral [L], ΔO_2 = measured difference of oxygen [µmol] during ΔT = intervall [h], S_{coral} = specific coral surface [cm^2]. (Schneider & Erez 2006)

Bacterial respiration can be a major error soure, therefore cleaning and sterilization was essential for high quality measuring: all water-bearing parts of the

respirometer were cleaned and purified in three ways prior to all measurements: initially, a mechanical cleaning with fresh water and brushes to abrade possible bacterial coating from the inner surfaces. Then all portable components (tubes, sensor compartments, chamber) were submergend in a bath of 70 % ethanol. For those that were locally bound to the respirometer (pump, valve, sensors, reserve) a spraybottle was used. Finally, all components were rinsed thoroughly with millipore water.
After the system was stacked together, the filtered sea-water was filled into the reserve tank and dispersed to all compartments. Then the whole system was checked carefully for air bubbles that disturb the measurement, closed up and the circulation was then initiated by starting the pump.
Before each trial a blank-run of the system was performed without the coral to create an experimental blank. This procedure was repeated after every measurement, to check for bacterial respiration.

2.2.2 Calcification measurements

Calcification and total alkalinity (TA) are linked in a simple stochiometric relationship: TA is being reduced by 2 mEq for each mmol change in $CaCO_3$ (Smith & Key 1975). To determine calcification rate the alkalinity anomaly method was used (see formula 6 and 7.
Unlike the metabolic rates, which were measured in situ, the calcification rate had to be determinded afterwards. Therefore, usually before and after a flushing event, a 25 ml PE-jar was filled completely by opening a valve in the chamber and then stored in a refrigerator at 4°C. By this procedure, pairs of associated samples were created that gave information about how the coral influenced the carbonate system in total, while the measuring cycle was cut off from the large cycle and reservoir.
In the post experiment phase, all samples were analysed for TA using acid–base titration (Ivanenkov 1978, see eq.6): methyl red was combined with sodium hydroxide and dissolved in ethanol, so was methyl blue. Both reagents combined synthesized the indicator. 0.02 M HCl was used as the titrant. An automated

titration stand (Titronic Universal, Schott) was employed to accelerate and standardize the analytic procedure. All samples were measured threefold with 5 ml volume and before every day of measurement, a certified sea-water-standard (25 g/l $CaCO_3$, 1:1000 diluted, Hach) was analysed to ensure quality.

$$\text{TA [mM]} = 1000 \cdot \frac{\text{V HCl [L]} \cdot \text{c HCl [M]}}{\text{V sample [L]}} \tag{6}$$

After measuring TA, net calcification rate was calculated using ΔTA:

$$CaCO_3\ [\text{mmol m}^{-2}\text{h}^{-1}] = -0.5\rho\left(\frac{V_C\ [L]}{S_C\ [m^2]}\right)\left(\frac{\Delta TA\ [mM]}{\Delta t}\right)\left(\frac{[L]\ [mmol]}{[m^3]\ [\mu mol]}\right) \tag{7}$$

with V_C = volume of closed cycle, S_C = coral surface, Δt = time between sampling

The negative factor of 0.5 represents the stoichiometric conversion from molar units of TA to $CaCO_3$. Formula 7 is modified from Langdon & Atkinson (2005). The precision of this analysis is 0.04 mM and the accuracy 0.06 mM.

2.3 Calculation of the corals surface and volume

The approach of avanced geometry (Naumann et al. 2009) was used to determine the corals' surface. All branches of one colony were allocated to simple geometric forms, cylinders, spheres and planes, then measured with a digital caliper. Only living, photosynthetically active tissue was taken into account — the limestone-foot was disregarded. When finished, all single forms where summed up to gain the total surface of the colony. An error of 5 % was determinded by repeated measurements of a single colony.
To determine the total volume of the colonies including their feet, they were immersed into a measuring pitcher to read off the resulting difference in volume from the scale. The accuracy in this method was ± 5ml.

2.4 Statistical analysis

All photosynthesis, respiration and calcification rates were tested for normal distribution with the Shapiro-Wilk test with (α<0.05). This test is recommended for medium n. Because all results of the photosynthesis and respiration, as well as some of the calcification measurements were not normally distributed, non-parametric rank test had to be used. Therefore, significance in homogenity was tested with the Mann–Whitney–Wilcoxon test, which was Boferroni–corrected (α/number of tests). The measured respiration rates were tested for interactions of the treatments with the Kruskal-Wallis test. All statistical tests were performed with "R", v2.12.0.

3 Results

During the two months of this study's experimental period all ten corals of the species *Stylophora pistillata* were exposed to three different stress factors. Their physiological performance with regard to photosynthesis, respiration and calcification was measured. The goal was to check for specific effects of the treatments on the corals physiology during short term measurements of approx. 20h. Before the selected specimens were exposed to the stress treatments, control measurements (= "control") were performed, to learn about their metabolic activity under experimental conditions without treatments. Control experimental conditions differed from keeping conditions in irradiance (80 µE) and sea water, which was filtered and therefore without food particles.
Treatments were elevated CO_2 concentration (5 mg/L, = "CO_2"), elevated temperature (30 °C , ="temp") and both in combination (="CO_2 + temp"/"combined treatment"). To present the oxygen–flux as a function of the surface, which was coral specific; surface and volume of every coral were obtained (Tab. 3, see formula 5 in section 2.2.1).

Table 3 Summary of the used corals of *Stylophora pistillata* and their respective surface and volume. Errors of surface measurement were obtained by repeated measurements of a single coral, error of volume measurement results in the smallest unit of the measuring pitcher

Coral #	Surface in cm^2 (err.: 5%)	Volume in ml (±5ml)
1	148.4	55
2	96.3	45
3	143.4	55
4	125.7	45
5	139.7	55
6	189.9	60
7	106.5	50
8	182.1	65
9	137.9	55
10	140.7	50

3.1 Photosynthesis and respiration measurements

Besides the oxygen flux inside the closed measurement cycle of the intermittent flow respirometer, the water temperature was recorded (see section 2.2.1). Every minute one value for oxygen saturation and for temperature was saved and later processed and interpreted individually.
Fig. 3 shows the average temperature measured during all trials of one treatment. The desired temperature for the control and CO_2 treatment of approx. 26.5 °C was missed by both. 30 °C was barely met by temp and CO_2 + temp. Nevertheless the temperature was adjusted in a way, that one could distinguish between low and high temperature regimes, as aimed for (see Tab. 2, section 2.2).

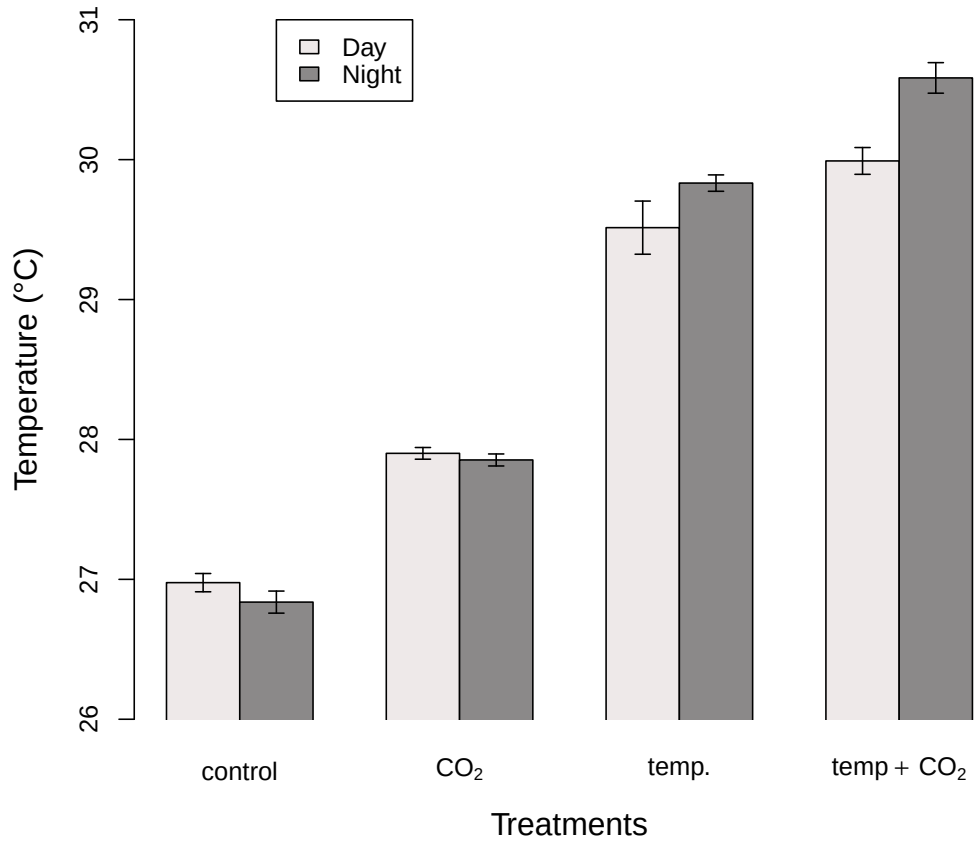

Figure 3 Average temperature measured parallel to oxygen flux for control and treatments during day and night (mean ± Standard deviation (SD). $n_{control}$=84 (Day)/67 (Night), n_{CO_2}=35/49, n_{temp}=24/67, n_{CO_2}=53/59).

Fig. 4A shows the averaged oxygen fluxes for all treatments. The largest oxygen production with a rate of 0.18 µmol cm^{-2} h^{-1} occured during the control measurements, whereas during the CO_2 treatment about one third of this rate was reached. The temp and combined treatments created oxygen consumption rather than production, i.e. -0.01 and -0.11 µmol cm^{-2} h^{-1}, respectively. The photoynthesis rate for the control is considered to be within the regular range of corals that are adapted to artificial keeping conditions. The corals exposed to elevated pCO_2 produced less oxygen and appear to be influenced by this treatment.

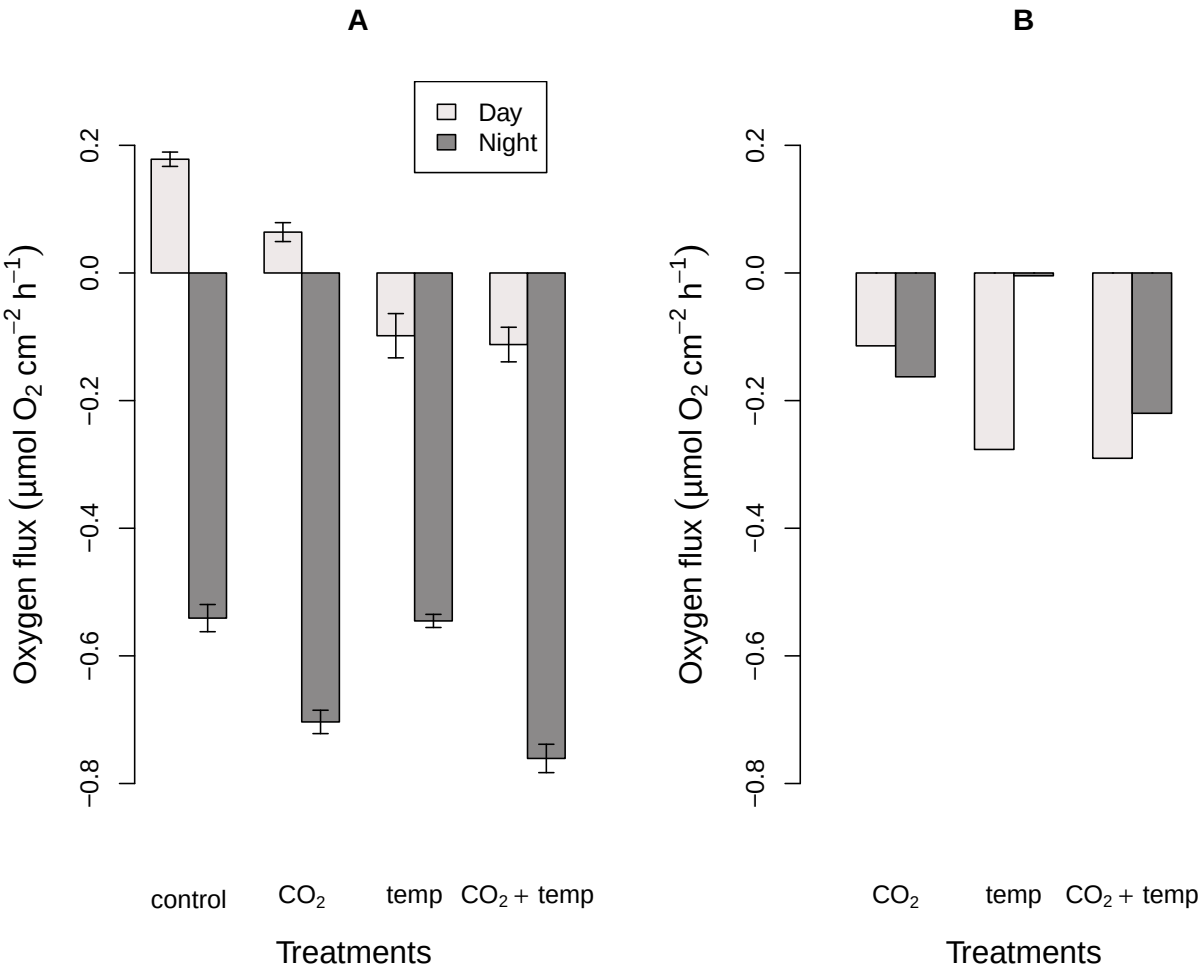

Figure 4 **A:** Oxygen flux created by *Stylophora pistillata* during day and night sorted by treatment (mean ± SD. $n_{control}$=84 (Day)/67 (Night), n_{CO_2}=35/49, n_{temp}=24/67, n_{CO_2}=53/59). **B**: Oxygen flux substracted by mean control flux.

The average oxygen flux in temp and combined treatment is negative, which indicates that these treatments influenced the corals strongly. Furthermore, the O_2 production was stopped or lower than the possibly increased respiration.
During the dark-phases, control and temperature treatment resulted in similar consumption rates at about -0.54 µmol cm^{-2} h^{-1}, respectively. CO_2 and combined treatment showed little lower rates at -0.7 and -0.76 µmol cm^{-2} h^{-1}, respectively. The respiration rate measured in the control trials was elevated and resides at the niveau of the rates measured during the temperature treatment. Both, CO_2 and combined treatment created high respiration rates that, however, reside at the same level.
To check for interactive effects, photosynthesis and respiration rate of the control were substracted from the rates of the treatments, respectively. Fig. 4B shows mean respiration rates set off against control. The bars show the relative level to the control photosynthesis and respiration rate. Neither for light- nor dark-phase any linear additive effects are obvious. The adjusted rate during the light-phase of the combined treatment is smaller than the added rates of CO_2 and temperature, respectively. Respiration rate during the dark phase for temperature shows almost no difference to the control rate. The rates of CO_2 and combined rate are visibly elevated. However, the residual rate for the combined treatment is larger than the added residuals of CO_2 and temperature.
The respiration rates differed significantly between the three treatments; both during day and night (Tab. 4).

Table 4 p-values of Wilcoxcon-Mann-Whitney test for oxygen flux created by *Stylophora pistillata*. Confidence level is 0.9917 (Bonferroni-corrected); not significant results are highlighted

		Day			
		control	CO_2	temp	CO_2 & temp
Night	control	—	9.635e-08	2.444e-11	4.912e-15
	CO_2	5.072e-05	—	4.332e-06	1.587e-06
	temp	0.6356	5.726e-11	—	0.3415
	CO_2 & temp	7.404e-11	0.09446	9.981e-14	—

During the day all treatments except for temperature vs. the combined treatment showed significant difference; at night, however, all treatments except control vs. temperature as well as CO_2 vs. the combined treatment.

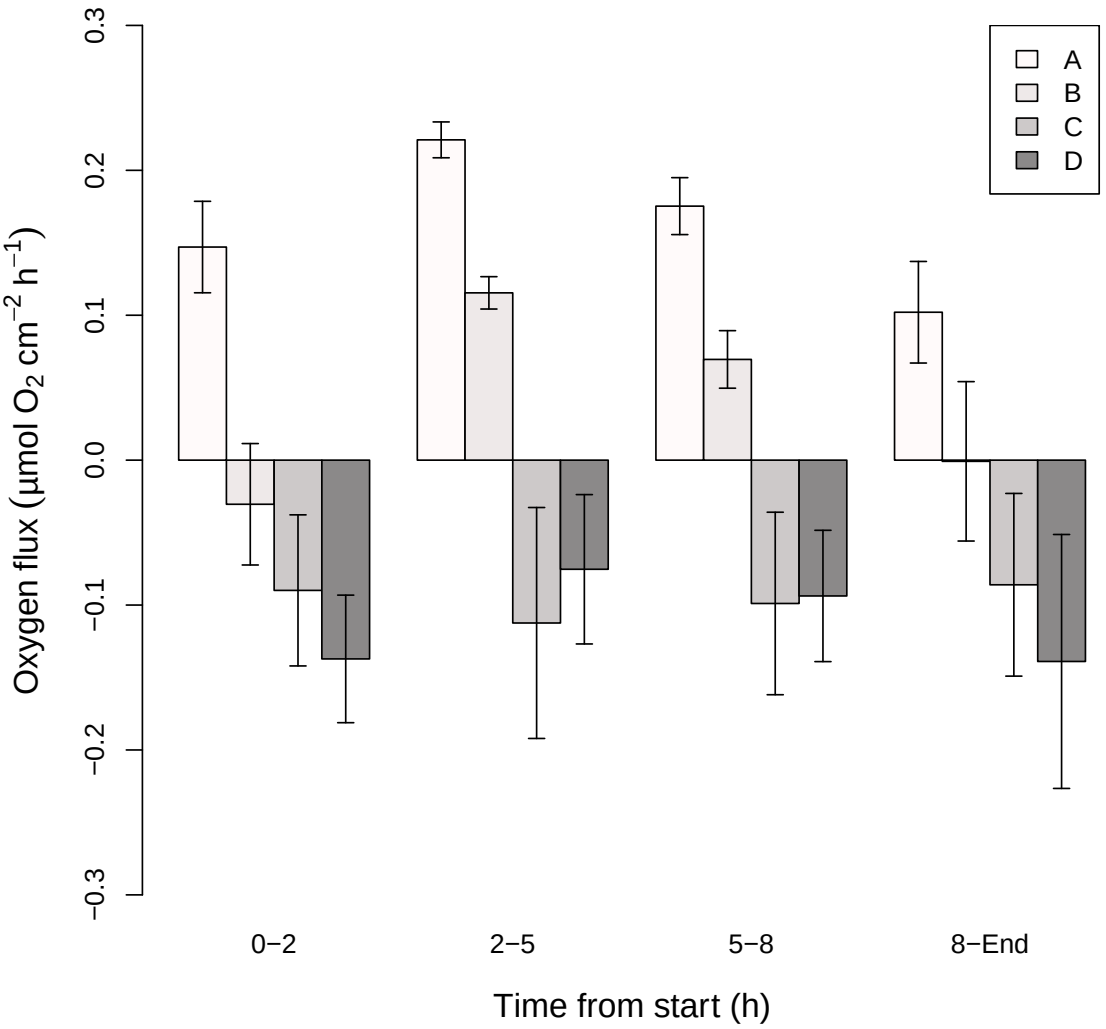

Figure 5 Mean oxygen flux created by *Stylophora pistillata* sorted by diurnal intercepts (hours from start of measurement, mean + SD. n-values see Tab. 10, App.). A = control, B = CO_2, C = temp, D = CO_2+temp

The respiration rate was not constant during the measurements, as an arrangement into chronological intercepts; 0–2, 2–5, 5–8 hours and 8 hours until dark, shows (Fig. 5). At noon during the first two hours of measurement — the ex-

periments startet at 11.00— the the oxygen fluxes were lower than the average fluxes calculated for the whole day (see 4); except for the CO_2 treatment which created oxygen consumption in contrast to an average positive day oxygen flux. In the early afternoon, during the second intercept from two to five hours, the measured respective oxygen flux was well above the diurnal mean rates. In this intercept the highest oxygen production was measured for control and CO_2 treatment. Also, the oxygen consumption rate of the corals in the combined treatment was lowest. The temperature treatment created the highest oxygen consumption rate during this period.
The third intercept at the time around late afternoon generated production and consumption rates that best resemble the average diurnal rates. The second highest rates of oxygen production for control and CO_2 treatment occurred during this time. Furthermore, the combined treatment produced the second lowest rate of oxygen consumption. In contrast, the temperature treatment created the second hightest consumption rate of the day in this intercept.
At the time shortly before the light turned off, consumption rates of temperature and combined treatment were similar to those measured in the morning. While the control trials had their minimum in this period, the oxygen flux of the CO_2 treatment was around zero.
The diurnal variation in oxygen fluxes shows a bell shaped trend with its peak between 2 and 5 hours for control, CO_2 and combined treatment. In the control the flux is completely positive, whereas during the CO_2 treatment the fluxes are only postive in the afternoon. In control and CO_2 treatment the oxygen production peaks then, too. The combined treatment shows solely negative oxygen fluxes, whose magnitude is smallest in the afternoon, too. The temperature treatment, however, has an inversely shaped bell trend with its most negative values at noon and afternoon. In this treatment during all periods of the day the oxygen fluxes are negative.
While in the beginning and also at the end of the measurements almost no treatments show significant differences, in the time of two until eight hours after the start the majority of treatments create significant different effects (Tab. 5). A low quantity of measured rates that fall in the first and last period resulting in

high SDs is believed to be the reason (see Tab. 10 App.). Allthough differences between almost every treatment during first and last period are not significant, results show a trend that all treatments but the control lead to oxygen consumption in the corals. This occurs for both treatments around the same level.
A Kruskall-Wallis rank sum test with the respiration rates and the regarding residuals resulted in the acceptance of the null hypothesis; there is no significant difference between the effects of the treatments (chi-squared = 6.9092, df = 3, p-value = 0.07485).

Table 5 p-values of Wilcoxcon-Mann-Whitney test for oxygen flux created by *Stylophora pistillata* in diurnal intervalls (hours from start of measurement). Confidence level is 0.9917 (Bonferroni-corrected); not significant results are highlighted

		0-2 h			
		control	CO_2	temp	CO_2 & temp
2-5 h	control	—	0.004718	0.03810	0.00045
	CO_2	0.001428	—	0.6429	0.1246
	temp	0.000158	0.02345	—	0.5495
	CO_2 & temp	0.004005	0.001301	0.9014	—
		5-8 h			
8 h - End	control	—	0.005793	0.01337	0.000231
	CO_2	0.1377	—	0.003062	0.001807
	temp	0.02677	0.6485	—	0.6968
	CO_2 & temp	0.02042	0.6485	0.7104	—

3.2 Calcification measurements

Tab. 6 und Fig. 6 show averaged calcification rates for all treatments, calculated by formula 7 (see section 2.2.2). With 6.53 mmol $CaCO_3$ m^{-2} h^{-1} the control rate is considered low and furthermore strongly fluctuating, as the large SD shows. However, considered the fact that in the oxygen measurements under control conditions the photosynthesis rate was highest, calcification rate under equal conditions was expectedly also the highest, since both processes are probably linked (see section **??**).

During the CO_2 and temperature treatment the corals precipitated 0.64 and 0.33 mmol $CaCO_3$ m^{-2} h^{-1}, respectively. Because oxygen flux during high temperature was negative, the very low calcification rate under this treatment is comprehensible; the signifcantly lowered photosynthesis during high pCO_2 is also in agreement with the associated low calcification rate. However, with both measured average rates having an SD larger than the measured value, assessing these readings is to be done with caution.

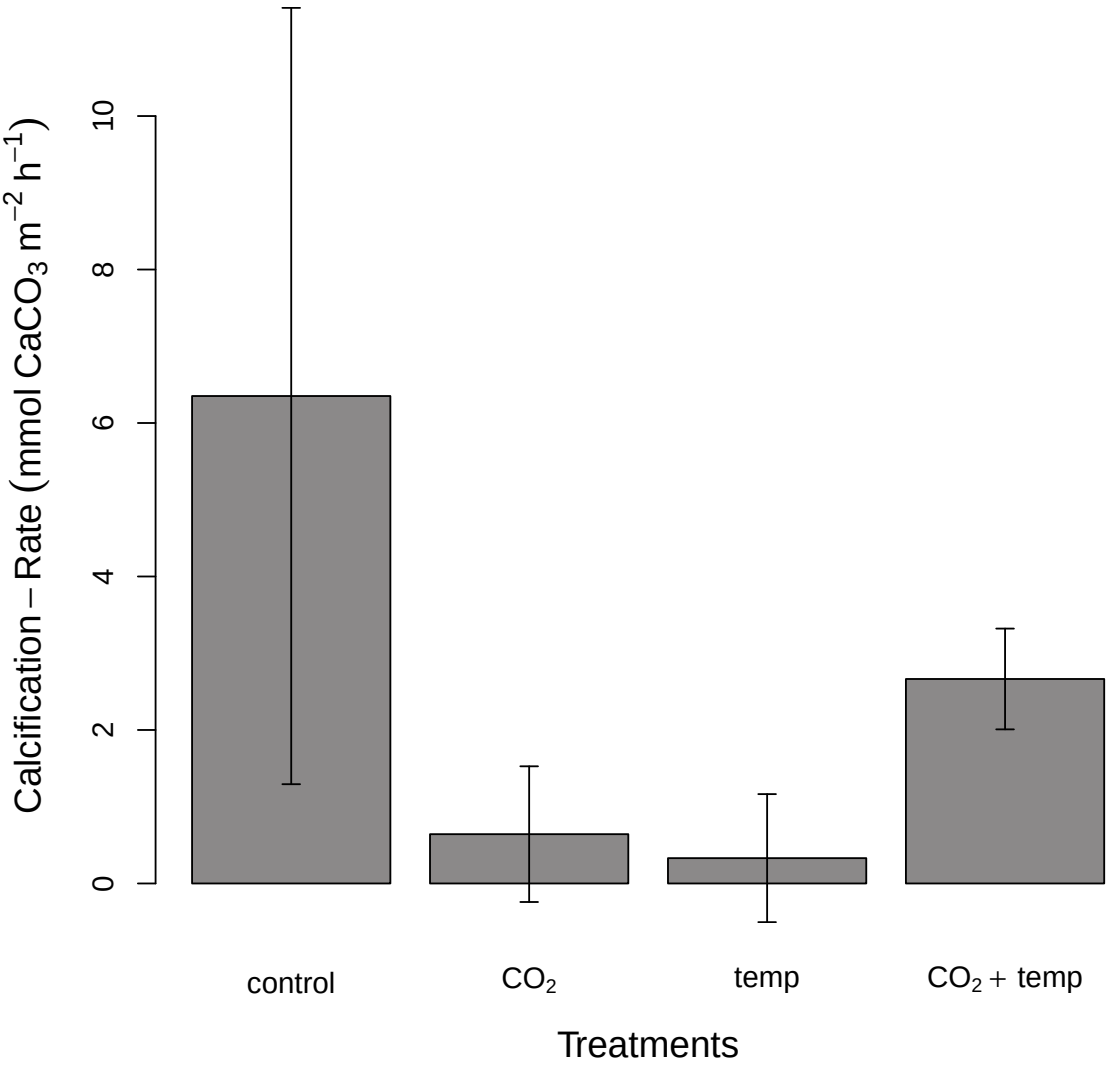

Figure 6 Mean calcification of *Stylophora pistillata* sorted by treatment (mean + SD, $n_{control}$=12, n_{CO_2}=8, n_{temp}=9, n_{CO_2}=10)

The calcification rate that occurs during the combined treatment is with 2.67 mmol $CaCO_3$ m^{-2} h^{-1} also low, but higher than the respective single treatments. This finding does not coincide with the assosiated oxygen flux, which was negative.

Table 6 Calcification rate (in mmol $CaCO_3$ m^{-2} h^{-1}, ± is SD, p=p-value of Shapiro-Wilk test with α<0.05)

	control	CO_2	temp	CO_2 & temp
mean	6.35±5.06	0.64±0.88	0.33±0.83	2.67±0.66
min	-27.08	-5.04	-3.74	0.51
max	42.87	4.21	2.82	5.58
p	0.02068	0.06501	0.1721	0.2059

Beside from control vs. CO_2 vs combined treatment calcification rates do not differ significantly (Tab. 7), which is is due to the high SD.

Table 7 p-values of Wilcoxcon-Mann-Whitney test for calcification rates sorted by treatment. Confidence level is 0.9917 (Bonferroni-corrected); not significant results are highlighted

	control	CO_2	temp	CO_2 & temp
control	—	0.002098	0.01199	0.003322
CO_2		—	0.5303	0.4557
temp			—	0.8763

4 Discussion

The knowledge on implications of global climate change on the most sensible coral reef ecosystem and on single coral species has increased strongly in the years of the new millennium. This process, however, is a necessity, because the relation between atmosphere and ocean surface is complex and changes are difficult to predict. On all accounts it is understood that an increase of atmospheric CO_2 leads to elevated temperature and pCO_2 in the ocean surface. This study simulated the altered environmental paramters under laboratory conditions and recorded the metabolic response of the hermatypic coral *Stylophora pistillata*.

4.1 Respiration measurements

To detect possible changes in photosynthesis, respiration and calcification rates induced by temperature and CO_2 treatments, the coral's physiological activity under normal aquaria conditions had to be determined. The resulting oxygen flux is in excellent agreement with previous experiments of Houlbrèque & Tambutte (2003) and Reynaud et al. (2003). The control rates of this study are also in agreement with, but lower than shown in Moya et al. (2006). In the present experiments the same species under equal experimental conditions were tested, if not stated otherwise (see Tab. 8). It is assumed that the corals were not pre–stressed and keeping as well as experimental conditions were ideal.
The dissociation of the recorded daily oxygen fluxes into several intercepts show a bell shaped profile for the control: the first two hours include production rates slightly below the daily average, whereas during the time from two to five and five to eight hours after the start the production rates peak and are elevated well above the measured average. The last recordings before the end show a declined photosynthetic rate. This observation goes along with Levy et al. (2004) who described in a previous study a "hysteresis effect" in photosynthetic entities: it has been previously observed that photosynthetic rates of phytoplankton, macroalgae, and higher plants decline in the afternoon.

Table 8 **A:** Oxygen flux of *S. pistillata* in present study (in µmol O_2 cm^{-2} h^{-1}, ± is SD). **B:** Data of oxygen fluxes from different published sources. Conditions were equivalent to present study if not stated otherwise (oxygen flux (Day/Night) in µmol O_2 cm^{-2} h^{-1}, C=control conditions, I=irradiance in µE, S=salinity)

A	Phase	control	CO_2	temp	CO_2 & temp
	Day	0.18	0.07	-0.01	-0.11
		±0.01	±0.02	±0.04	±0.03
	Night	-0.54	-0.71	-0.55	-0.76
		±0.02	±0.18	±0.01	±0.02

B	Coral species	Conditions	Oxygen flux	Reference
	S. pistillata	C, I=80	0.2 - 0.3 / -0.29	(Houlbrèque & Tambutte 2003)
	S. pistillata	C (low CO_2), I=380, S=38,	0.17 / -0.25	(Reynaud et al. 2003)
	S. pistillata	C, I=100 - 200	0.46 - 0.56	(Moya et al. 2006)
	S. pistillata	c_{CO_2}=0.9mg L^{-1}, I=380, S=38	0.14 / -0.29	(Reynaud et al. 2003)
	A. eurystoma	c_{CO_2}=1.32mg L^{-1}, I=350, S=40	0.48 / -0.6	(Schneider & Erez 2006)
	var. species	c_{CO_2}=1.1 mg L^{-1}, I=220, S=38	0.52 / -0.6	(Leclercq et al. 2002)
	S. pistillata	T=28.2°C , I=380, S=38	0.3 / -0.28	(Reynaud et al. 2003)
	S. pistillata	T=31°C , I=0 - 1500, S=35	0.74 / -0.57	(Hoegh-Guldberg & Smith 1989)
	T. mesenterina	T=31°C , I=300 - 500	1.27 / -0.9	(Faxneld et al. 2010)
	S. pistillata	T=28.3°C c_{CO_2}=0.9mg L^{-1}, I=380, S=38	0.2 / -0.32	(Reynaud et al. 2003)

This phenomenon is called the "afternoon depression". Levy et al. (2004), in contrast, detected a "morning depression"; oxygen production was clearly higher in the afternoon than in the morning. The present study is in perfect agreement with their findings; the highest production was detected between 13.00 and 15.00, the second highest from 15.00 to 19.00. In the morning, after 12 hours of darkness, the photosystems are not capable of employing the sudden photon flux as efficient as in the afternoon. By then, the zooxanthellae have become light acclimated and increase their photosyntesis (Levy et al. 2004).

During the light period, elevated CO_2 concentration resulted in a significant reduction in oxygen production; considerably more than pictured in previous studies (Schneider & Erez 2006; Leclercq et al. 2002). The measured oxygen flux at high pCO_2 in Reynaud et al. (2003) was closer to the results of this study. With regard to these disagreements in oxygen flux one has to consider the difference in various parameters, that vary from the current setup. In all references cited, that included a high CO_2-programme, the irradiance was higher; between 2.75 and 4.75 times than in the present study (80 µE). Previous experiments showed that scleractinian corals strongly depend on light availability, adapt to the respective intensity and produce more oxygen the higher irradiance is; up to a critical threshold (Mass et al. 2007). Salinity was higher in the cited experiments (38 & 40 vs. 32.5); also influencing the resulting oxygen flux (Ferrier-Pages et al. 1999; Faxneld et al. 2010). The possible connection between higher salinity and photosynthesis, however, needs to be further analysed. The greatest difference of this study to previous ones was the very high CO_2 concentration, which was between 3.8 and 5.6 times higher. An enrichment with this magnitude has been performed for the first time and shows an explicit reduction in oxygen production for *S. pistillata*. This effect is possibly due to a dramatically reduced Ω_{arag} (see formula 2), section 1.3), that detains the coral from a normal calcification rate. Schneider & Erez (2006) explain in their work that calcification may enhance photosynthesis against the common conception that this process occurs only in the other direction. Therefore, low calcification could explain low photosynthesis (see section 4.2). A CO_2 concentration at

this high level (three to four times the anticipated ocean surface concentration in 2100, IS92a IPCC scenario (Randall & Wood 2007)) combined with a very low irradiance puts the trend, that the present study shows, in agreement with fomerly performed experiments: a significantly lowered photosynthesis and elevated respiration under high CO_2 concentration.
The diurnal rhythm of *S. pistillata* during CO_2 enrichment displays a bell shaped trend like the control, with a negative oxygen flux in the morning, a peak in the afternoon and a net flux around zero during the end of the light period. In the morning, the difference to the control is biggest with 0.177 µmol cm^{-2}(see Tab. 9, App.). This span is declining as the experiment progresses and levels off at a difference of approx. 0.1 µmol cm^{-2} (0.106, 0.105 and 0.103 µmol cm^{-2} during the subsequent periods). To adjust a CO_2 concentration of 5 mg L^{-1}, the cycle's water had to be gassed to a pH of about 7.65 (see formula 3, section 2.2). When the coral was put into the water, the coelenteron is filled with low ph water which hinders calcification directly at the calcification site by a dramatically lowered Ω_{arag}. As stated above, a reduced calcification may also result in a distinct reduction of photosynthesis. At the basal plate of the calcicoblastic epithelium, the $Ca^{2+}/2H^{+}$-ATPase pump increases pH and therewith Ω_{arag} (Al-Horani & Al-Moghrabi 2003; McConnaughey & Whelan 1997). This process takes the first two hours; by afternoon the pH is adjusted and photosynthesis has acclimated at a lower, but stable level.

Temperature elevation to slightly below 30 °C resulted in a significant change in oxygen flux during the day; changing to oxygen consumption. This reaction is in disagreement with previous studies that used *S. pistillata* in a similar setup (Reynaud et al. 2003; Hoegh-Guldberg & Smith 1989; Faxneld et al. 2010). However, dark respiration almost remained constant and is in agreement with Reynaud et al. (2003), Langdon (2003) and Nordemar et al. (2003). Possibly, the lamp above the respiration chamber was to small to create sufficent irradiance for the corals (between 3.75 and 18.75 times less than in the papers cited), resulting in increased energy demand and thus a reaction with increased respiration while accessing stored energy reserves (Faxneld et al. 2010). During the

measurements the coral expelled high amounts of mucus and showed indications of slight bleaching. Hoegh-Guldberg & Smith (1989) observed the same process and explained it with low numbers of zooxanthellae, which would indicate a high expulsion rate of the endosymbionts by the coral host. Nordemar et al. (2003) agree with this explanation and suggest that damaged photosystems are a reason for reduced primary production under high temperature regimes, too. With a higher irradiance, this could have been compensated for by the intact systems (Fitt et al. 2001).
The daily variation of oxygen flux during the temperature treatment shows a converse trend to control and other treatments. In the morning, the respiration rate is above the average, whereas during noon and afternoon oxygen consumption peaks and then declines towards the end of the light period. One explanation could be that the previously mentioned "hysteresis effect" is a disadvantage at high temperatures. The photosystems of the present specimens of *S. pistillata* were adapted to a circadian rhythm with highest photosystem efficiency during noon and afternoon. Under the given keeping conditions, the photosystems do not have to adapt to progressively increasing temperatures, because the mesocosm is cooled with high effort and does not excede 27°C. Therefore, the zooxanthellae adjust their photosystems to a circadian rhythm with a constant high photon flux (up to 440 µE in the mesocosm, see Tab. 1, section 2.3) at medium temperatures. Because of low irradiance and high temperature (highest at noon/afternoon) in the given experimental setting, the photosystems are disturbed and possibly damaged (Jones & Hoegh-Guldberg 1998). Towards the end of the light phase, the coral seems to have at least partly acclimated to the negative conditions, as the decreasing respiration rate shows.

The combined treatment with elevated CO_2 concentration and temperature showed significant effects; resulting in oxygen consumption at the same level of the temperature treatment. This reaction of *S. pistillata* is not in agreement with the study of Reynaud et al. (2003), who measured no significant change in oxygen production compared to control. The day oxygen consumption during the combined treatment is not significantly different from the consumption during the

temperature treatment (see Fig. 4B and Tab. 4, section 3.1). This might indicate that elevated temperature induces a greater impact on the corals physiology than high CO_2 levels. The results stated above and the possible implications confirm this theory. The dark phase respiration rate in the treatment, however, is increased by approx. one third which is in agreement with Reynaud et al. (2003).
During the combined treatment, respiration rates fluctuated in a bell shaped pattern - similar to control and CO_2 treatment: high respiration rates in the morning and evening, lowered rates during noon and afternoon. This is in contrast to the diurnal pattern of solely elevated temperature.

4.2 Calcification measurements

Generally, the calcification rates measured in the study are low: the rate measured for the control was 6.35 ± 5.06 which is in agreement with Langdon & Atkinson (2005) and Schneider et al. (2009), if regressed for the light intensity used in this study. However, the values are ranging from -27.1 to 42.9 and therefore result in a standard deviation almost as large as the measured rate itself. For these previous studies, specimens of *Porites compresa*, *Montipora verucosa* as well as *Acropora eurystoma* and *Favia favus* were used under similar conditions, only irradiance was higher.
Calcification rates for CO_2 and temperature treatment are very low, fluctuating around zero. This reaction goes along with Leclercq et al. (2002) and Schneider & Erez (2006), who observed calcification rates around or below zero for a pH smaller than 7.7 (for species and conditions see Tab. 8). A deacrease of this magnitude in calcification during elevated temperature has not been observed yet, allthough hermatypic corals are known to drastically reduce the precipitation of $CaCO_3$ beyond a thermal optimum (Coles & Jokiel 1978; Marshall & Clode 2004).
In the combined treatment the corals calcified with an average rate of 2.67 ± 0.66. Allthough being lower, this rates coincides with (Reynaud et al. 2003), who measured a decline of approx. one third in during CO_2 + temperature

treatment. In this study, the reduction of about two thirds my be induced by the combination of the treatments and low irradiance, which was about twice as high in Reynaud et al. (2003).

Samples for calcification measurements were taken during the running experiment and stored for analysis, which was performend directly after the last day of the experimental phase. Thus, the samples of the first days have been in the refrigerator for two months at 4°C in the dark, however, without previously being fixed. This long period of storage might have altered the measured rate by chemical precipitation (USGS 2006) and led to the large error. Furthermore, a low n of the calcification samples with only 1-3 or less per experiment further increases uncertainty of the measured values. This has to be considered while evaluating these results.

4.3 Conclusions & Outlook

Stylophora pistillata shows reactions in photosynthetic and respiration rates, that differ in amplitude for the employed treatments. Those reactions manifest in a differentiated oxygen flux, showing a diurnal rythm. The much described "afternoon nap" was not observed, however, a "morning depression" was very well recognizable (Levy et al. 2004). It is believed that this phenomenon is connected to internal adjustments in the photosystem of host and algae to a circadian rhythm. Observations of Langdon & Atkinson (2005) and Nordemar et al. (2003) support this theory as well as Levy et al. (2004). In future experiments, calcification should be tested on this pattern, too.
The CO_2 treatment evoced a rection that fit well within the already documented reactions of *S. pistillata* to elevated pCO_2. New in this study was the magnitude of the CO_2 concentration — a similar experiment with comparable CO_2 partial pressure was not discovered. Calcification during high pCO_2 was very low, but possibly because of procedural errors. Therefore, the results should be considered with caution. However, Schneider & Erez (2006) formulated a theory after which photosynthesis and calcification are connected bidirectional, which would confirm the observations of this study. Generally the findings reveal,

that *S. pistillata* is resistant against low pH and environments that are undersaturated with regard to aragonite. To fully understand the physiological processes inside the calcifying systems and to what extend reduced Ω_{arag} influences them, further experiments with gradual levels of CO_2 concentration and the support of a micro–sensor–array should be conducted.
For high temperature treatments, a respiration rate during the day with this amplitude has previously not been observed for *S. pistillata*. However, oxygen consumption in the dark phase coincides with previous studies. This moves the focus on the lightening: because it was too weak, it could not serve the corals elevated energy demand (Nordemar et al. 2003). Therefore respiration increases — to the hightest rates during noon and afternoon. Allthough the observated reactions support this theory; future investigations, that employ the method of fluorence measurements, should investigate changes in net and gross production. Furthermore, additional measurements to isolate the temperature limits, under which photosynthesis and calcification maximize, should be conducted; to make a relative assessment of the coral's photophysiology under optimal and adverse conditions.
The combined treatment showed an effect that has neither been observed by any previous studies nor goes along with the results from the other treatments. Also, the reason for the relatively high calcification rate remains uncertain. The oxygen flux during the day indicates influence by the high temperature, night respiration is around the same level of the CO_2 treatment. The diurnal rhythm in respiration, however, agrees with the other treatments; with lowest rates during noon and afternoon. The "morning depression" is still detectable while the coral is being stressed. Statistical analysises state that temperature and combined treatment induce similar reactions; suggesting that the effect of temperature on the coral is bigger than elevated CO_2 concentrations.

For future experiments it is proposed, to quantify biomass of the used coral specimens in order to gather information about cell specific density. Also counting the zooxanthellae would provide information about the distribution of the endosymbionts inside the host. Focus should be put on different irradiance

levels to approach the stated problems with regard to photo-physiology and -chemistry. As mentioned, fluorescence measurements (e.g. pulse amplitude modulation - PAM) should be performed parallel to micro-sensor measurements, that are able to identify fluxes inside the organism and make observations on the cytological level. The present and the proposed investigations on coral physiology should be performed on other important reef building corals, too. Furthermore, to transpose these findings of laboratory experiments on natural conditions, in situ studies have to be conducted. This should provide further insight about how coral reefs cope with a changing environment.

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Appendix

Table 9 Oxygen flux for the respective diurnal period (mean in µmol O_2 cm^{-2} h^{-1}, ± is SD)

period	control	CO_2	temp	CO_2 + temp
0-2 h	0,147	-0,03	-0,09	-0,137
	± 0,032	± 0,042	± 0,052	± 0,044
2-5 h	0,221	0,115	-0,112	-0,075
	± 0,012	± 0,011	± 0,08	± 0,052
5-8 h	0,175	0,07	-0,099	-0,094
	± 0,02	± 0,02	± 0,063	± 0,045
8h - End	0,102	-0,001	-0,086	-0,139
	± 0,035	± 0,055	± 0,063	± 0,088

Table 10 *n* for the respective diurnal period

period	control	temp	CO_2	temp + CO_2
0-2 h	13	6	2	12
2-5 h	31	15	7	17
5-8 h	29	10	8	16
8h - End	11	4	7	7