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Supplement of

Release of hydrogen peroxide and antioxidants by the coral *Stylophora pistillata* to its external *milieu*

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List of content:

Supplement S1. Changes with time of k_{antiox} and its calculation for missing time points.

Supplement S2. How does coral size and water volume influence the obtained rates?

Supplement S3. Pictures of the experimental setup and of *S. pistillata* coral fragments.

Supplement S4. H₂O₂ release by *S. pistillata* fragments under complete darkness, low light intensity, and high light intensity.

Supplement S5. H₂O₂ release of *S. pistillata* under natural high irradiance.

Supplement S6. A complimentary experiment with decreased stirring speed.

Supplement S7. H₂O₂ and antioxidant activity release kinetics over long incubation experiments.

Supplement S8. Summary of 3-Amino-1,2,4-Triazole experiments.

Supplement S1. Changes with time in k_{antiox} and its calculation for missing time points

In all experiments k_{antiox} values increased with time. The release kinetics could be fitted with a linear or an exponential function. To determine the more appropriate function we fitted both of these functions to data from 15 long incubations (100-240 min) and compared the resulting R^2 values of the fits (Table S1). We found that R^2 values of linear correlations (0.84 ± 0.15) are significantly higher ($p=0.019$, paired T-Test) than those of exponential correlations (0.77 ± 0.13 ; see Table S1).

Table S1. Equation fitting through the antioxidant activity data. Curve-fitting for the increase in k_{antiox} over time for 15 long incubations (100-240min). R^2 values of linear and exponential correlations were calculated and compared. R^2 values of linear correlations are significantly higher ($p=0.019$, paired T-Test) than those of exponential correlations.

Date	Exp. Duration	Decayes assays	Linear fit	Exponential fit
	(min)	(number)	R^2	R^2
22.7.2012	120	7	0.76	0.83
22.7.2012	120	7	0.95	0.89
22.7.2012	120	7	0.94	0.58
23.7.2012	240	13	0.91	0.84
23.7.2012	240	13	0.87	0.71
23.7.2012	240	13	0.84	0.77
25.7.2012	100	6	0.78	0.81
25.7.2012	100	6	0.96	0.92
25.7.2012	100	6	0.96	0.91
25.7.2012	100	6	0.61	0.63
25.7.2012	100	6	0.69	0.64
25.7.2012	100	6	0.46	0.48
8.8.2012	100	6	0.96	0.87
8.8.2012	100	6	0.97	0.88
8.8.2012	100	6	0.98	0.76

Average	0.84	0.77
SD	0.15	0.13
n=	15	15
paired T-Test	p = 0.0195	

The change with time in the antioxidant activity and its curve fitting is important for our understanding of this phenomenon and for practical reasons of calculating missing data points. The later issue of missing data points is of significance for the calculation of H_2O_2 release rates according to equation 5. This calculation is conducted for small time

intervals of 5-10 min, while the antioxidant activity was determined only every 20 min. Hence we had to calculate 1-3 k_{antiox} values (depending on the spacing of the H_2O_2 measurements) to allow frequent calculations of H_2O_2 release rates. For long experiments (with many k_{antiox} assays) we used linear equation to calculate missing k_{antiox} values during the experiment and in its last stages (where data are often missing). This linear equation was used also to calculate k_{antiox} values where For short experiments, including the different stirring speed experiments (when few k_{antiox} assays are available and linearity could not be determined), we calculated k_{antiox} for the missing time intervals by averaging the starting and final values of that time interval and correcting for the elapsed time. This calculation still assumes linear increase in k_{antiox} but for only 20 min increments, and hence it prevents large deviations from the measured values.

Supplement S2. How does coral size and water volume influence the obtained rates?

Normalization of the measured rates to coral related parameters

A clear benefit to our newly described phenomenon can stem from normalization of the rates of H_2O_2 and antioxidant activity release to some parameters of the coral that generate them. However, at current, the “appropriate” parameter of normalization is not yet known since the source of H_2O_2 and antioxidants are not yet fully resolved. Several parameters can be considered such as coral size (surface area, volume, and weight), protein content, zooxanthellae density, tissue or mucus thickness etc. The data set described in the paper was conducted using similar sized coral fragments and similar water volumes, in an attempt generate comparable experiments. It is however possible that some of the variability observed between experiments reflect coral parameters that were not measured.

Release of antioxidant activity by corals with different sizes

A preliminary attempt towards obtaining a normalization factor was done by incubating six coral fragments of different sizes in containers with different water volumes. Following 1 h of incubation the coral water was assayed for antioxidant activity. These results were normalized to a constant water volume (to avoid dilution effect) and were plotted against coral size expressed in volume (Fig. S2). The coral volume was estimated by a simple technique of water displacement. In this technique corals are introduced to a beaker completely filled with seawater and the overflowing water are collected and weighted. This simple technique is rather accurate (given sufficient repetitions) and imposes minimal stress on the corals. In our case, we allowed the corals to recover for one week between this measurement and the incubation experiment. The experiment show a general trend of increase in antioxidant activity with the coral (Fig. S2). However

we found a rather weak linear correlation ($R^2 = 0.44$). Considering these results, we think that that normalization of the observed rates to coral size could introduce large noise to the data.

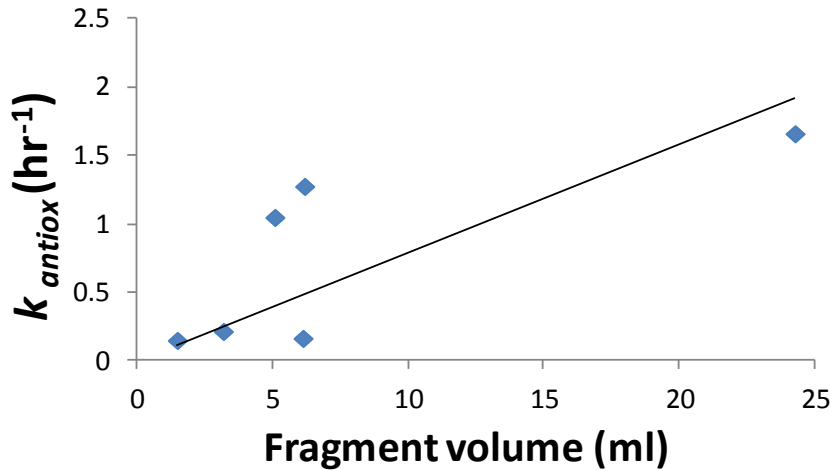


Figure S2. The effect of coral size on antioxidant activity release. Following 1 h of incubation that antioxidant activity in the incubation water was determined for six coral fragments of varying sizes. The coral volumes were examined by the water displacement technique and the antioxidant activities were normalized to a constant water volume.

Recommended optimal coral size for our experiments

Our experiments were conducted using $\sim 20\text{cm}^2$ surface area coral fragments and water volume of 100ml. In order to achieve reliable measurements we recommend keeping this ratio of coral size to water volume. Note that H_2O_2 concentration in the sample is influenced by the coral H_2O_2 releases rates, the antioxidant activity that degrade H_2O_2 simultaneously and the stirring speed in the beaker. In addition, the quality of the measurements is also influenced by the assay accuracy and sensitivity as well as reliable blanks and trustful calibration curve. If one can develop sensitive and accurate H_2O_2 assays, then a smaller ratio of coral size to water volume can be used. We did manage to measured H_2O_2 and antioxidants from much smaller corals ($\sim 2\text{cm}^2$) with a similar water volume, but this required experienced personal. For larger corals, larger water volumes are required and rates are expected to be kept on the same order of magnitude.

Supplement S3. Pictures of the experimental setup and of *S. pistillata* coral fragments

To enable readers to repeat our experiments we included few photos of the experimental setup for measuring H₂O₂ and antioxidant activity release rates.

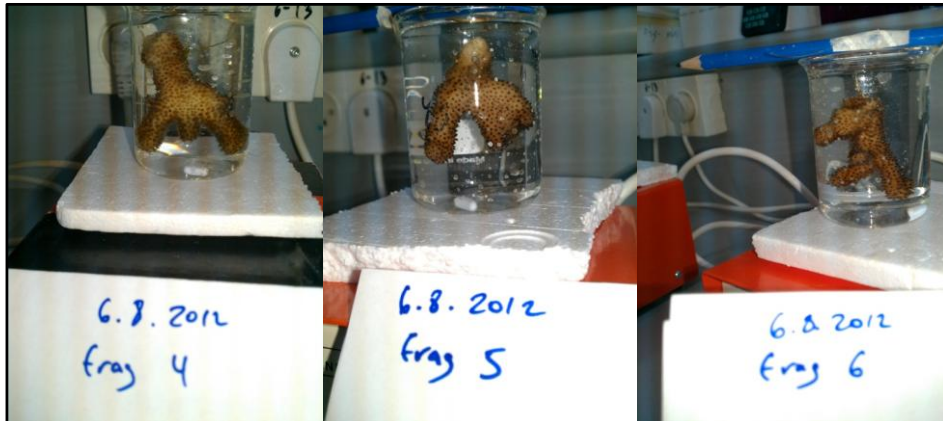


Figure S3 a. Three *Stylophora pistillata* coral fragments are shown suspended in the incubation beakers. The beakers are placed on a stirrer where a 1 cm long magnet bar ensures complete water mixing. The coral polyps were extended and the corals did not show signs of stress.

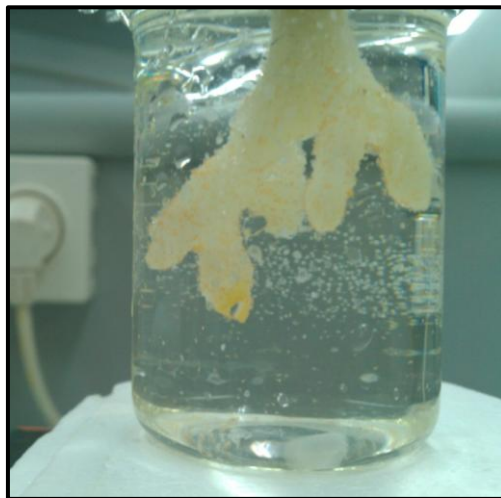


Figure S3 b. Bleached *Stylophora pistillata* coral fragment, pictured during its feeding with a one-day-old *Artemia salina* nauplii.

Supplement S4. H₂O₂ release by *S. pistillata* fragments under complete darkness, low light intensity, and high light intensity.

In general, our experiments were conducted under fluorescence laboratory light of $\sim 10 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$. This low illumination is sufficient for only minimal photosynthesis (or none at all), as it is far below the compensation light intensity of these corals. Several experiments were conducted in complete darkness to allow comparison with our standard low light conditions. In these experiments, the initial H₂O₂ accumulation rates calculated in nmol per min were similar between dark and low light conditions (Fig. S4a). These results suggest that the H₂O₂ released during our standard experiments was not produced via photosynthesis.

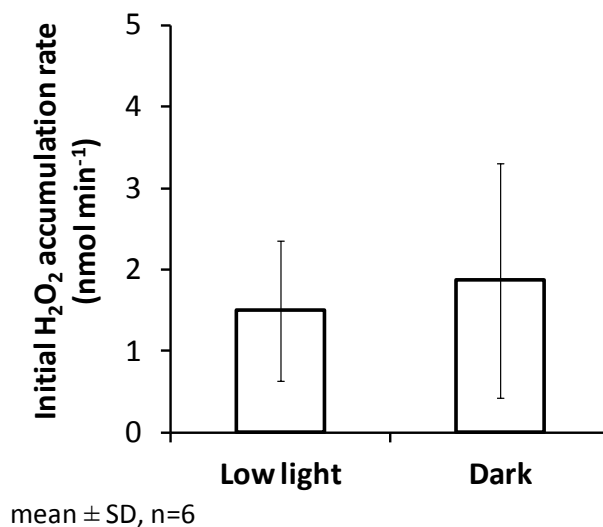


Figure S4a. H₂O₂ release by *S. pistillata* coral fragments at low light intensity and complete darkness. Initial H₂O₂ accumulation rates (calculated in nmol min⁻¹) are compared between experiments conducted at complete darkness (n=6) and low light intensity of $\sim 10 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$ (n=6). Rates are not corrected for H₂O₂ decay since at this stage of the experiment k_{antiox} values are low.

The effect of light on H₂O₂ release by the coral is intriguing since the symbiotic algae, identified as the H₂O₂ source, are strongly influenced by light. To conduct light experiments we had to change the experimental setup and use temperature controlled chambers (metabolic cells). These chambers had a volume of 0.5L and hence larger coral colonies were used ($\sim 100 \text{ cm}^2$). Corals were allowed to acclimate in the dark for 1-2 hrs in the chamber. Before each experimental stage (i.e. light or dark) the incubation water was replaced with a peristaltic pump to wash out the accumulated antioxidants. These washes seem to influence the coral and result in lower H₂O₂ release with time (Fig. S4b). Due to this effect it is more appropriate to compare separately each set of dark and light

treatments (i.e. prior to 130 min and after 140 min). While H_2O_2 levels off after a short while, the initial rate of its accumulation in each of these sets is higher in the light compared to that in the dark. Further research is required to test the effect of light on H_2O_2 release in a more optimized system.

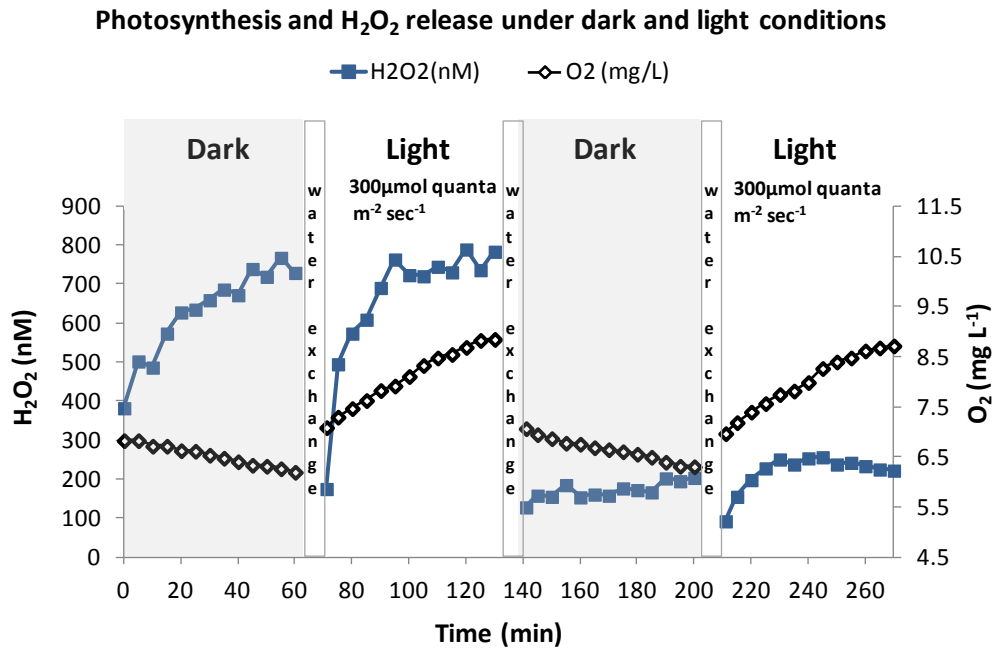


Figure S4b. H_2O_2 release by *S. pistillata* colony in the dark and in the light. The experiment was performed in a sealed metabolic cell with stirring and oxygen concentrations were determined. Prior to each stage the water in the chamber was replaced by a peristaltic pump to wash out antioxidants. Light was supplied by halogen lamps at intensity of $300 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

Supplement S5. H_2O_2 release from *S. pistillata* under high natural irradiance.

The experiments reported in the manuscript are restricted to low light conditions, where photosynthesis is negligible. Since the symbiotic algae are the source of the released H_2O_2 (Fig. 4), it is highly feasible that upon illumination and the commencement of photosynthesis corals will release more H_2O_2 . We are currently investigating these issues and have been establishing a different experimental setup to examine the effect of light on H_2O_2 release dynamics. This setup involves a flow-through system in a water table where constant water exchange enables long experiments under natural irradiance and constant seawater temperature. Analytically it is rather challenging to obtain an appropriate water exchange rate that does not wash out the coral produced H_2O_2 nor allow too much antioxidants to accumulate. Such an experiment was run with a large coral fragment of $\sim 100 \text{ cm}^2$, in a 600 mL glass beaker, with water flow rate of

13.5 mL/min and moderate stirring speed, in Dec 2013. The coral was placed over night in the experimental conditions for acclimation. Shown are H_2O_2 measurements and calculated H_2O_2 release rates (which take into consideration dilution and decays; Fig. S5). This experiment indeed shows higher H_2O_2 concentrations and release rates at noon, when high solar irradiation was measured. We have not measured the coral photosynthesis rate in this experiment, and can relate at current only light and H_2O_2 release. However, this is a promising first step in studying the link between H_2O_2 release dynamics and photosynthesis.

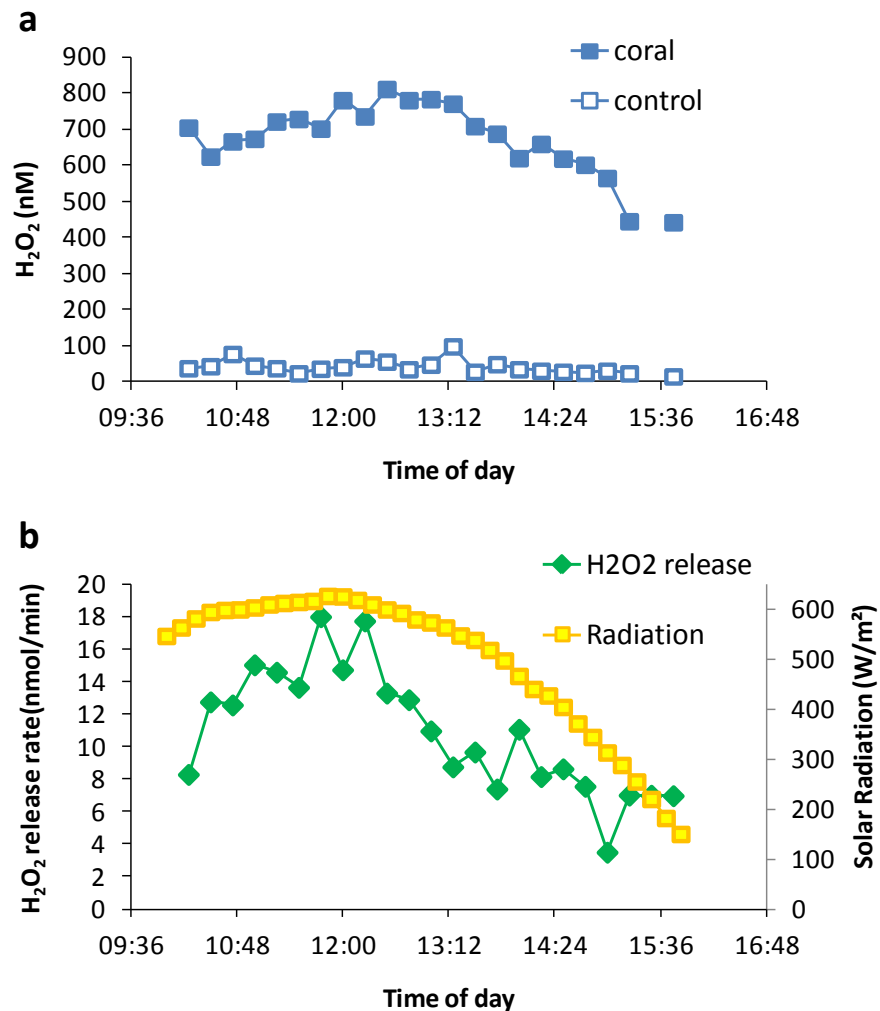


Figure S5. Diurnal variation in H_2O_2 release by *S. pistillata*. (A) Changing H_2O_2 concentration over 5 h experiment at midday in the coral surrounding water and in a control experiment (water alone). (b) Calculated H_2O_2 release rates and solar radiation measurements. The experiment was conducted in a water table under natural daily cycle using flow through system. Water temperature remained constant.

Supplement S6. A complimentary experiment with decreased stirring speed

An experiment with reversed stirring regime (compared to those in Fig. 5) was conducted to test the effect of reducing flow speed on H₂O₂ and antioxidants release. At the beginning of the experiment, fast flow speed was applied and after 60 min the flow was reduced dramatically to slow speed. H₂O₂ concentrations increased initially and then dropped immediately when the flow was reduced (Fig. S6a). Antioxidant activity on the other hand continued accumulating throughout the experiment (Fig. S6a). From these parameters we calculated the amount of H₂O₂ released during each time interval. We then present the cumulative H₂O₂ released (Fig. A6b). It is apparent from the slopes, that the rate of change, which is the rate of H₂O₂ accumulation, is faster when stirring speed is rapid.

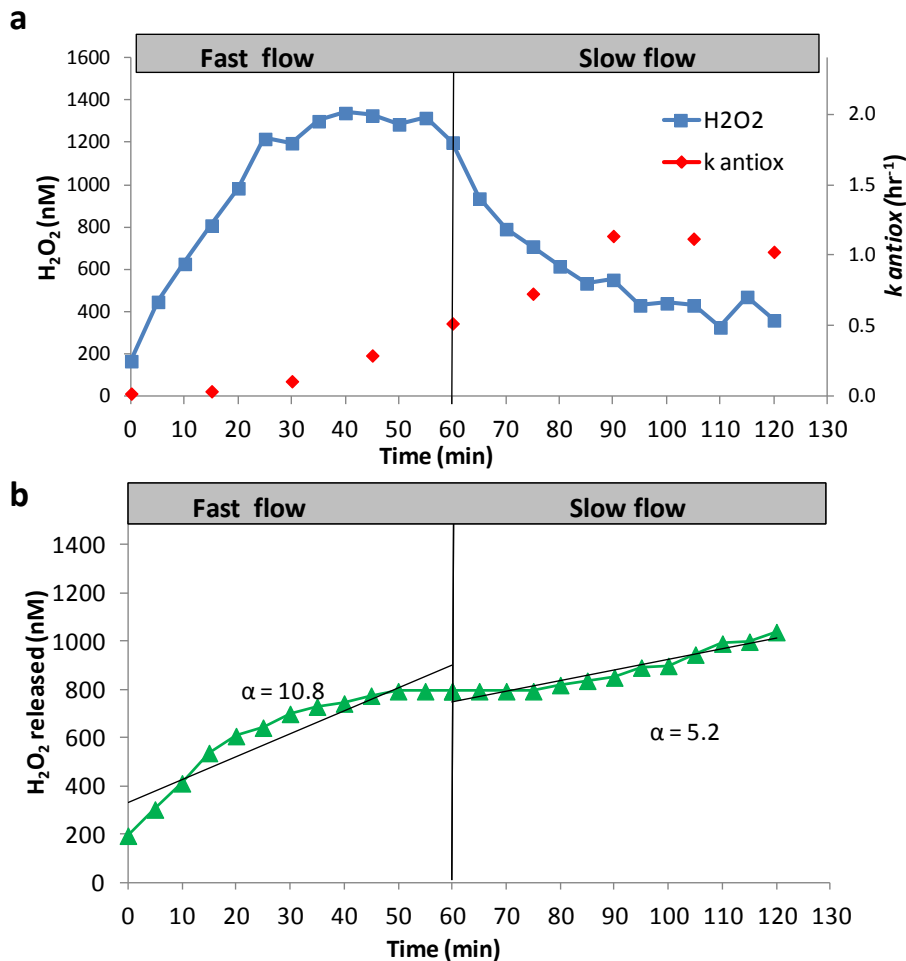


Figure A6. The effects of reversed flow regime on H₂O₂ and antioxidants release. (a) Changes with time in H₂O₂ concentrations and antioxidant activity obtained at fast (0-60 min) and slow (60-120min) stirring speeds. (b) Cumulative H₂O₂ that was released to the water by the coral. The slopes plotted through the data indicate the rate of H₂O₂ release in each of the stirring speeds.

Supplement S7. H₂O₂ and antioxidant activity release kinetics over long incubations.

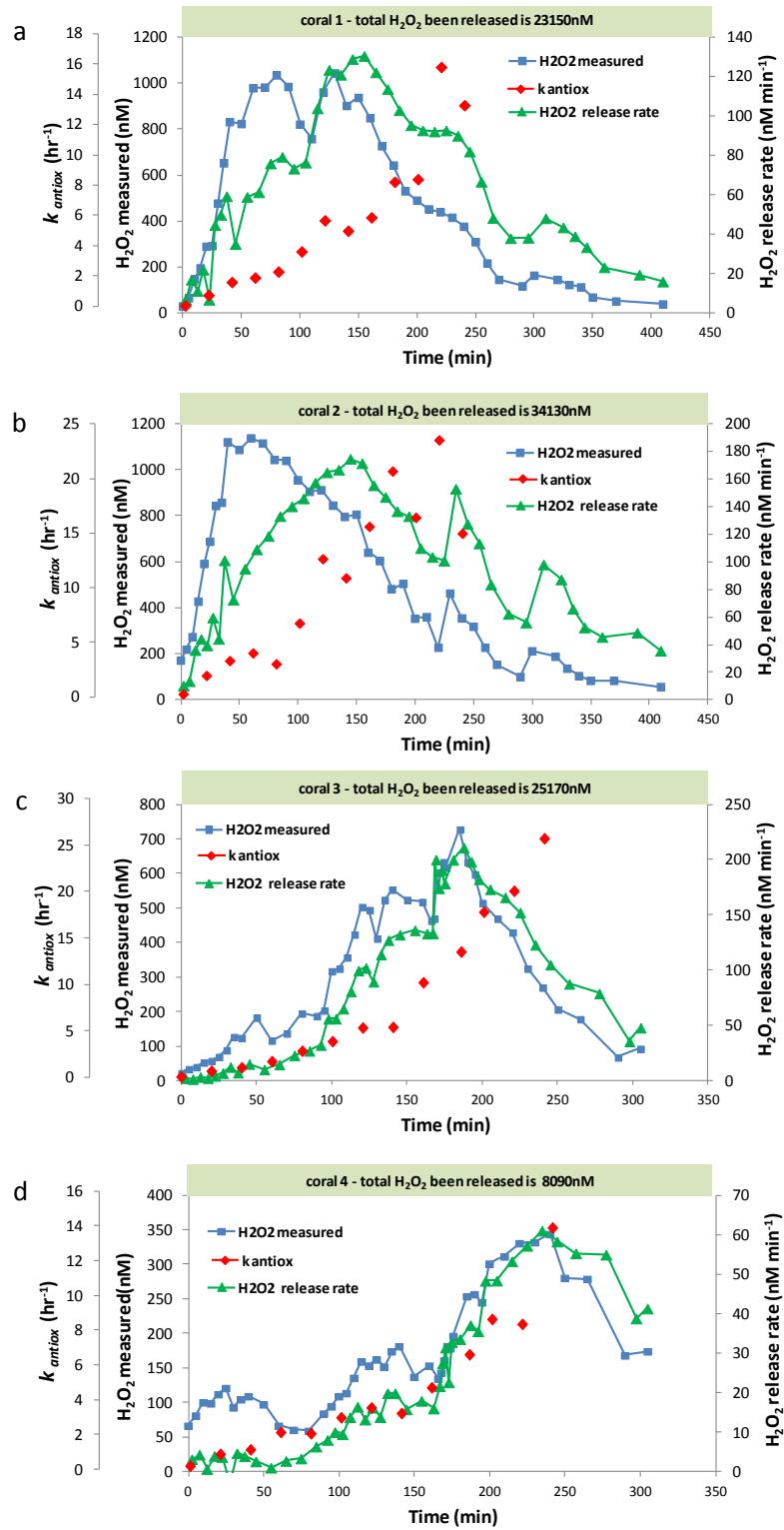


Figure S7. H₂O₂ and antioxidant activity release kinetics over long incubation experiments of four individual coral fragments (a-d) showing comparable patterns of antioxidant activity accumulation (red diamonds) and changing H₂O₂ accumulation (blue squares) and release rate (green triangle) as showed also in Fig. 3. The total H₂O₂ amount been released by the corals (indicated in the title) were summed using the frequent H₂O₂ release rates calculations.

Supplement S8. Summary of 3-Amino-1,2,4-Triazole experiments.

Table S8. A summary of experimental conditions used to inhibit Catalase activity with 3-Amino-1,2,4-Triazole. 3-Amino-1,2,4-Triazole have been used to inhibit catalase activity in various tissues, (including cell suspensions, leaves and whole sea anemones) and various organisms (from mammals to bacteria). Incubations performed at tens of millimolars, inhibited 95-75% of the antioxidant activity. In our experiment, 3-Amino-1,2,4-Triazole inhibited the antioxidant activity a low concentration of 0.1 millimolar with high efficiency of 98%.

Citation	Organism and Tissue type	3-Amino-1:2:4-Triazole concentration and time of incubation	Catalase activity retained
Margoliash and Novogrodsk, Biochem. J. Mar., 68, 468–475, 1958.	Mammals- rat-kidney suspension	20mM 80 min	10%
Tephly et al., JPET, 134, 77-82, 1961.	Mammals- crystalline beef liver	24mM 30 min	5%
Cohen and Somerson, J. Bacteriol., 98, 543-546, 1969.	Bacteria- <i>Mycoplasma pneumoniae</i>	50 mM 60 min	25%
Havir, Plant Physiol. 99, 533-537, 1992.	Plants- Leaves of <i>Nicotiana sylvestris</i>	20mM 13 hours	15%
Merle et al., Free Radical Biology & Medicine, 42, 236–246, 2007.	Cnidaria- Whole sea anemones	0.5 mM 6 days	25%