**Highly conserved evolution of the hypoxic response in metazoans**

**Abstract**

The earliest metazoans evolved in marine environments under low-oxygen conditions (hypoxia)1, currently emerging as a primary stressor for marine biota2-4. However, hypoxia tolerance in marine metazoans and the mechanisms employed during hypoxic events remains enigmatic2,4. By combining physiology, phylogeny, and diurnal transcriptomic alterations in the coral *Stylophora pistillata*, a basal marine metazoan, we report that (i) central genes that are responsive to oxygen and circadian rhythms have a common phylogenetic origin with most being transcriptionally affected by hypoxia;(ii) S. *pistillata* uses the same primary transcriptional response to withstand hypoxia as mammalian cells do (glycolysis, regulation of apoptosis, alteration of mitochondrial dynamics and numbers)5-9, in addition to coral-specific ones. The transcriptional alterations include an acute (12h–1w) and prolonged (1–2w) response, highlighting potential mechanisms involved in hypoxia tolerance. This physiological plasticity could have enabled corals’ survival of mass extinction events10 and might enable their persistence under accelerating, climate change-driven ocean deoxygenation. Here we substantiate the presumed co-evolution of oxygen and circadian rhythm response mechanisms from a single gene11. We suggest that photosynthetically driven oxygenation12 during the Neoproterozoic Oxygenation Event1 eventually created significant diurnal fluctuations of oxygen levels directing the evolutionary drive to link the development of genes responsive to day-night changes and oxygen levels.

**Introduction**

The current decrease in oceanic dissolved oxygen is amongst the most widespread and pressing problems in marine environments3,4.Severe hypoxic “dead zones” of coastal waters are rapidly increasing in prevalence worldwide, raising concern as to how marine biota will be impacted by this stressor2. Life on earth initially evolved under low-oxygen levels (~1% of current atmospheric levels) that started to rise approximately 750 million years ago (Ma)1,12. Metazoans evolved approximately >635 Ma, following oxygenation of the deep ocean that occurred during the Neoproterozoic Oxygenation Event (NOE).While oxygen likely reached current atmospheric levels at that time, it was unstable and characterized by multiple anoxia events imposing metabolic versatility to hypoxia1. Based on laboratory experiments, it is suggested that the earliest common ancestor of all living animals respired under low-oxygen conditions(at least 0.25% of current atmospheric levels)11.Photosynthetically-driven oxygenation and more stable oxygen levels approximately 550 Ma allowed metazoans to increase their tissue complexity1. This enhanced metazoan dependence on oxygen as a terminal electron acceptor.

In animals, responses to low-oxygen levels are mediated by the transcription factor HIF (hypoxia-inducible factor) that consists of two subunits, HIF-α and HIF-β. Under normoxia HIF-α is marked for degradation by prolyl 4-hydroxylases (PHDs)13. Under hypoxia the PHDs are downregulated and HIF-α can associate with HIF-β. The HIF heterodimer directly activates transcription of a conserved core set of gene targets (see Fig. 1A) that enable cells to rapidly survive hypoxia by (i) providing oxygen-independent means of ATP production via glycolysis, (ii) controlling hypoxia-induced apoptosis, (iii) enhancing oxygen and nutrient supply via blood vessel formation, and (iv) adjusting mitochondrial activity and dynamics5-7,9.

According to phylogenic analyses of metazoans, the HIF pathway evolved only once in animals, with HIF-α and HIF-β diverging from the ancestral circadian rhythm sensing genes (which mediate physiological responses from day to night)11. Accordingly, the HIF genes are missing in the first primitive metazoans (sponges and ctenophors). They start to emerge in unicellular eumetazoans (placozoans) and are then present in the first diploblastic eumetazoans (cnidaria, e.g., corals) and all triploblastic eumetazoans. These HIF and circadian rhythm gene families are highly conserved among metazoans11.

Scleractinian corals are a unique model to investigate the evolution of the transition from hypoxia to oxygen respiration due to their co-evolution with photosymbionts14.This co-evolution presented corals with hyperoxic conditions in the daytime (when photosynthesis occurs) and hypoxic conditions at night (when both coral and algae are respiring)15. Hence, we speculate that metabolic versatility to hypoxia is best preserved in these basal eumetazoans. However, their tolerance to hypoxia and the mechanisms employed during hypoxic events remains enigmatic2,4.

This study combines phylogenetic analyses with gene orthology and diurnal expression as well as physiological measurements in the Scleractinian coral, *Stylophora pistillata,* subjected to prolonged hypoxia to examine (i) the evolutionary conservation of the genetic repertoire and primary response of metazoan cells to hypoxia, (ii) species-specific mechanisms promoting tolerance of corals to this stressor, and (iii) the effect of symbiosis on the diurnal hypoxic response.

**Results**

**Oxygen sensing, the HIF pathway, and circadian rhythm genes affected by hypoxia**

First, we determined whether the *S. pistillata* genome contains the PHD-HIF genes and established their evolutionary position (Supplementary Table S1).We followed the expression of these genes in hypoxia versus control (from here on referred to as H*vs*C) (Supplementary Fig. 1) to establish if functional correlation to hypoxia exists.

The evolutionary position of the HIF subunits revealed that both arose from a series of duplications from one ancestral gene that also gave rise to the circadian rhythm genes (Fig.1B). The current HIF gene family tree is composed of six highly conserved groups. Orthologous genes of *S. pistillata* were present in all six groups; in five groups, the expression of at least one gene was significantly affected by hypoxia. Most genes were upregulated, including HIF-α and HIF-β (Fig. 1C**,** and Supplementary Table S2).

The PHD family gene tree indicated that cnidaria have only two copies of PHDs1-3 orthologs (Supplementary Fig. 2). In contrast, the PHD4 tree included a single-copy of the human PHD4, but many copies of cnidaria orthologs, composing 16 large groups (Supplementary Fig. 3A). Hypoxia-induced an immediate significant downregulation of most PHD4 orthologs (Supplementary Fig. 3B): out of 20 orthologs, 14 were downregulated in H*vs*C while only two were upregulated, a highly significant trend based on Fisher’s Exact Test (*p*-value = 0.003, observed odds ratio 6.15, considering all PHD4 down/upregulated groups; Supplementary Table S3?). Next, we identified putative orthologs of direct HIF target genes. Most were DE and upregulated, specifically, one PHD1/2/3 ortholog was upregulated at all time points, and its expression fold change level was the highest among all putative HIF target genes (Supplementary Fig. 3C, Supplementary Fig. 4, Supplementary Table S3).

**Expression patterns/activated pathways in response to hypoxia**

We further expanded our search for evolutionarily conserved versus divergent molecular responses to hypoxia between cnidaria and mammalian cells/species by applying two approaches: (a) examining the expression patterns of additional hypoxia-related orthologous genes (*bottom-up* approach); (b) using functional enrichment analysis (*top-down* approach) (Supplementary Table S4).

The immediate transcriptional response indicated (i) a metabolic shift to glycolysis at most time points, subsiding only after 2weeks (Fig. 2A, B, e.g., PFK, the rate-limiting enzyme for glycolysis and a direct HIF target gene); and (ii) complex apoptotic/cell death response at night (12hours) subsiding at 2weeks (Supplementary Fig. 5A). However, apoptosis/cell death GO-terms were not enriched and all corals survived the 2-week hypoxia treatment. Also, transcription of anti-apoptotic styBcl-2 was upregulated concomitant with downregulation of styCaspase-3, an executioner of apoptosis (Fig. 2A and Supplementary Fig. 5B). During prolonged hypoxia, GO-terms of DNA repair pathways were upregulated, while the ERK signaling pathway and reproduction were downregulated (Fig. 2B).

Species-specific responses indicated immediate upregulation of genes that (i) participate in generating the tubular structure of polyps (the basic unit of the coral colony) (Fig. 2A, 12hours-1week, “tubulogenesis”); and (ii) enhance acquisition of sterols and carbohydrates from the symbiont (Fig. 2A “symbiosis-derived inputs” and Fig. 2B “starch binding”), prevailing over time. Conversely, after 1-2weeks, nematocyst formation (essential for predation) was downregulated (Fig. 2A, B).

**Hypoxia affects the mitochondria**

The transcriptional response indicated: (i) immediate antioxidant upregulation, upregulation of genes affecting fusion rather than fission (Fig. 2A and Supplementary Fig. 6A), and downregulation of mitophagy (Fig. 2B), (ii) enhanced oxidative phosphorylation (OXPHOS) and upregulation of distinct subunits of the electron transport chain (ETC) (Fig.2A, B, Supplementary Table X), and (iii) upregulation of pyruvate conversion to lactate and enhanced fatty acid β oxidation (Fig. 2A, B**,** Supplementary Table X, Supplementary Fig. 5B). Most of these transcriptional responses were significant at night, but not at day. Importantly, hypoxia-induced mitochondrial activity at night was accompanied by the upregulation of glutathione transferase activity (Supplementary Table SX). Enhanced mitochondrial biogenesis (Fig. 2B, Supplementary Table SX) was confirmed by the significant increase in mitochondria DNA after 1–2weeks (Supplementary Fig. 6C).

**The transcriptional response to hypoxia varies temporally and diurnally**

Non-metric multidimensional scaling (NMDS) ordination of whole transcriptome (Fig. 3) demonstrated that the hypoxic response was more extensive at night than during the day, at all time-points. Additionally, based on both NMDS and DE results (Supplementary Fig. 7A), the response to hypoxia was gradually reduced with time, including HIF-α levels (Fig. 2A ). We further identified genes and pathways associated with significant differences in hypoxia response between night and day, and between time-points, by calculating interaction effects (Supplementary Tables S5 and S6).

The temporal interactions supported a higher H*vs*C response at 12hours and 1week than at 2weeks (Supplementary Fig. 7B, Supplementary Table SX1) ,( e.g., glycolysis), but also indicated a late-onset transcriptional upregulation and downregulation of certain pathways (e.g., mismatch repair and ERK cascade pathways, respectively).The diurnal interactions supported a general trend of higher H*vs*C response at night than during the day, at all time-points (Supplementary Fig. 7C). This was especially noted for energy production pathways (e.g., glycolysis, OXPHOS and mitochondria activity) (Supplementary Table SX2).

**Effects of hypoxia on physiology of coral and symbionts**

All corals survived 2weeks of severe hypoxic conditions without apparent morphological changes. Calcification rates showed a trend of initial reduction, resuming control levels after 2weeks. Symbiont numbers seemed to increase and chlorophyll-*a* content per symbiont cell to decrease over time. However, these trends were not significant (Supplementary Fig. 8A, B and C, Supplementary Table SX)

**Discussion**

We show that *S*. *pistillata* can endure severe prolonged hypoxia without a significant physiological response, despite significant gene expression alterations. These gene expression patterns and HIF-α expression returning to basal levels16 point to a “two stage” model of short-term (12hours–1week) *vs.* prolonged (1–2week) response, supporting acclimation to hypoxia16. This model includes the same primary mechanisms evoked in mammals in response to hypoxia (Fig. 5A) in addition to coral-specific ones (Fig. 5B).

Controlling mitochondria ROS production6,7,17 and dynamics7,18 are essential to apoptosis regulation and the promotion of cell survival during hypoxia. Under hypoxic conditions mammalian cancer cells have been shown to protect mitochondria by the increased expression of antioxidative proteins, consequently promoting fusion19. Also, diving mammals that experience recurrent hypoxia have a constitutively higher antioxidant capacity than do non-diving mammals20. Similarly, symbiotic corals are well adapted to diurnal fluctuations in ROS production and employ an efficient antioxidant capacity21. Hence, the upregulation of antioxidants and glutathione transferase activity suggest that the control of total ROS accumulation under acute hypoxia might safeguard *S*. *pistillata* from ensuing fission, mitophagy, and apoptosis. Therefore, coral species reported to be resistant to hypoxia2 such as *S*. *pistillata* in this study, might experience either natural diurnal oxygen variations greater than sensitive species, or employ higher antioxidant capacity, rendering them better adapted to severe hypoxia.

The adaptation of corals to hypoxia is corroborated by the fact that contrary to cancer cells22, *S*. *pistillata*-enhanced DNA repair mechanisms that are crucial to maintaining genetic stability, similar to those observed in the hypoxia-tolerant mole rat23. Also, the downregulation of the ERK cascade is opposed to the response of cancer cells that upregulate this pathway, thus maintaining prolonged HIF- α activation that comprises the tissue24. HIF-α “desensitization” in mammalian cells is necessary for adaptation to chronic hypoxia25. One direct HIF target gene is the PHD3 gene, which consequently creates a direct negative feedback loop26. In different cells, the expression of PHD3 is extremely elevated as compared to other hypoxia-responsive genes27, promoting HIF-α “desensitization”25,26. The fact that one coral PHD1/2/3 homolog was upregulated at all time points, and its expression change level was the highest among all putative HIF target genes, suggests that this gene might participate in adaptation to prolonged hypoxia in corals as well.

Enhancing oxygen and nutrient supply via formation of blood vessels is also essential for hypoxia survival6. Cnidaria lack blood vessels, and oxygen supply occurs via diffusive gas exchange in the polyps (the basic unit of the coral colony).Tubulogenesis (generating the tubular structure of polyps) genes are ancestral to vascularization (generating the tubular structure of blood vessels)28. It has been observed that under hypoxia corals potentially utilize polyp expansion/elongation to increase tissue surface area and therefore maintain respiratory competence29. The upregulation of genes that participate in tubulogenesis, namely VEGFR30and FGFRb31, suggests that hypoxia enhances polyp expansion/elongation, possibly enhancing overall respiratory capacity.

The immediate metabolic response to hypoxia is indicated by the upregulation of glycolysis6, fatty acid β oxidation, and OXPHOS16. During prolonged hypoxia, the sustained upregulation of these pathways in addition to increased mitochondria numbers/biogenesis8 are highly similar to adaptation in mammalian cells, possibly enhancing overall respiratory capacity8,16. Also, in mammals, enhanced glycogen storage promoted cell survival under hypoxia32 whereas in plants, starch storage was essential to withstand prolonged hypoxia and to activate glycolysis33. Increasing mitochondria biogenesis requires cell membrane components such as sterols. However, corals are sterol auxotrophs, receiving the bulk of them from their symbionts via highly conserved cholesterol binders34,35. In addition to sterols, corals are highly dependent on photosynthetically fixed carbon, translocated from their symbionts and stored by the coral in the form of glycogen36. The upregulation of glycolysis and mitochondria biogenesis, combined with possible accelerated acquisition of symbiont-derived carbon/glycogen and sterols, suggest that acute and prolonged hypoxia imposes a higher demand for symbiosis-derived inputs on corals. During prolonged hypoxia, a decrease in nematocysts formation (negatively affecting external acquisition of sterol/carbon sources via predation) indicates that hypoxia enhances *S. pistillata*s’ dependence on symbiosis-derived inputs (Fig. 5B).These inputs maybe essential to corals and other organisms that are energetically dependent on photosynthetic symbionts to survive prolonged hypoxia. Therefore, additional factors (e.g., thermal stress37) leading to coral bleaching might reduce the ability of corals to withstand prolonged hypoxia. The dramatic effect of this symbiosis on the hypoxic response is evident from total and diurnal gene expression. Both are higher at night, suggesting that oxygen fluctuation due to the symbionts15 results in daytime hypoxia that is less stressful. The fact that corals have expanded their oxygen sensing genes (PHD4 orthologs) as compared to other metazoans, and that the expression of these genes is downregulated under hypoxia, supports the premise that corals benefit from quickly sensing and responding to oxygen variations resulting from photosynthesis/respiration of the endosymbionts.

During prolonged hypoxia, the downregulation of the “reproduction” GO term is similar to the reduced reproduction reported in vertebrates such as fish38 and monkeys39. In corals, the molecular mechanisms controlling reproduction are unknown. However, stress caused by high temperatures has been shown to hinder reproductive development and reduce fecundity40, suggesting that prolonged hypoxia is an additional stressor that reduces corals’ fitness.

By showing that most HIF and circadian rhythm genes are affected by hypoxia, we functionally corroborate their predicted evolution from a single gene11,also explaining the current interactions between oxygen levels and the circadian rhythm genes reported in mammals41,42. Moreover, the high similarity of the gene repertoire and primary response of *S. pistillata*s to that of mammals suggests that adaptability to hypoxia is deeply rooted in metazoan evolution, occurring at the end of the NOE1. In this line, we suggest that the rise of phytoplankton during the NOE12 eventually created significant diurnal fluctuations of oxygen levels, imposing the evolutionary drive to link the development of genes responsive to day-night changes and oxygen levels. This hypoxic adaptability, inherited from the last common ancestor that prevailed under low unstable oxygen, is well-preserved in corals that experience abrupt diurnal oxygen variation, possibly enabling corals’ survival of mass extinction events10 and may assist in their persistence under accelerating, climate change-driven ocean deoxygenation. It is not yet clear how modern corals and other marine biota will respond to the combination of anoxic conditions and thermal stress that is increasing in present times.

**Methods**

**Experimental paradigm**

S. pistillata fragments were maintained under normoxia (control=C) or constant severe hypoxia (H) for two weeks. We analyzed the diurnal (midnight (zt18) and midday (zt6); from here on referred to as night and day, respectively) and temporal (12 hour, 1 week, and 2 weeks) gene expression combined with physiological measurements. Canonical pathways and physiological parameters that are changed under the hypoxia treatment were identified by comparing hypoxia versus control at each sampling time point (from here on referred to as HvsC) (Supplementary Fig. 1).

**Experimental design, coral collection, maintenance, and sampling**

Corals were collected under the Israel Nature and National Parks Protection Authority permit number 2018/41994. Coral fragments (2 cm long) from the three *Stylophora pistillata* colonies (26 fragments per colony)were collected at a depth of 4–5 meters from the coral nursery of the Inter-University Institute (IUI) at the Gulf of Eilat Red Sea (28.6929°N, 34.7299°E) in February 2019 and immediately transported to a controlled environment aquarium system at the Leon H. Charny School of Marine Sciences in the University of Haifa, Israel. The fragments were glued onto pin-like bases and allowed to recover for 2 weeks under simulated ambient conditions (light parameters, temperature, salinity, pH, calcium, magnesium, phosphate and nitrate), replicating the IUI coral nursery conditions that were tested biweekly and adjusted to standards as needed. Water changes of 10% were performed on a weekly basis using artificial seawater (Red Sea Salt, Red Sea Ltd.) and all corals were fed identical food twice a week (Microvore, BrightwellR aquatics, United States).

After an acclimation period of 2 weeks in the same aquarium system, fragments were divided evenly between two identical 20 L aquaria placed in the same aquarium system used for acclimation, and acclimated again for 5 days. The aquaria were designed with airtight lids containing holes fitted for nitrogen bubbling, a water pump, a multi-parameter measuring device, and a water inlet/outlet. A water pump was used to ensure constant water circulation and to maintain homogenous dissolved oxygen (O2) levels. Dissolved O2 concentrations were measured every 15 minutes with a multi-parameter measuring device (WTW Multi 3420, Xylem analytics,Germany). Reduced dissolved O2 concentrations (hypoxia) in the experimental aquarium were generated by sparging the water with nitrogen43and kept at a level of 0.34 mg/L (±0.16 mg/L)that equals or is less than the minimum level of 0.5 mg/L observed in water-quality surveys2. For RNA extractions, three fragments (one from each colony) from each treatment (hypoxia and control) were sampled at five time points: three time points at midnight (zeitgeber time zt18), after 12 hours, 1 week, and 2 weeks of incubation in hypoxia; and two time points at noon (zeitgeber time zt6), after 1 week and 2 weeks of incubation in hypoxia. The fragments were snap-frozen with liquid nitrogen, and stored at -80°C. For DNA extractions, the same fragments sampled for RNA after 1week and 2weeks were used (n=6 for control and hypoxia, zt18 and zt6 combined). Additionally, four fragments (three from different colonies and one randomly chosen) from each treatment (control and hypoxia) were snap-frozen and used for DNA extractions (n=10 in total for DNA extractions). For calcification rates,four fragments (three from different colonies and one randomly chosen) were sampled at the same time of day from each of the control and hypoxia treated aquariums after 1week, 11 days, and 2 weeks of incubation in hypoxia (Supplementary Fig. 1).

**Calcification rates**

Calcification rate was determined using the total alkalinity (TA) anomaly method with some modification, as described44. In brief, each fragment was sealed in a specimen cup filled with 0.22 mm of filtered artificial seawater collected from the aquarium system holding the fragments. For hypoxia, the filtered artificial seawater was sparged with nitrogen43to achieve hypoxic O2 levels before sealing the cups. The cups were kept in their respective aquarium systems for 1.5 h. Water samples were collected for TA at the beginning and the end of the incubation and were filtered again to remove debris and measured with an automatic alkalinity titrator (855 Robotic Titrosampler, Metrohm, Switzerland). The calcification rates [µmol CaCO3 cm-2 h-1] were then calculated using the equation proposed45:

Calcification rate =

where ∆TA [µmol g-1] is the difference in alkalinity between the beginning and the end of the incubation period; V is the recorded volume of the artificial seawater in the respective

incubated cup [ml]; 1.028 is the density of seawater in the northern Gulf of Eilat [g ml-1]; and SA is the surface area of the coral (cm2) estimated as described46; T is the duration of the incubation [hours].

**Protein extraction, zooxanthellae isolation, and chlorophyll-*a* quantification.**

After measuring calcification rates, the samefragments were frozen in liquid nitrogen and kept at -80°C. Coral tissue was removed by an airbrush connected to a reservoir of phosphate buffer saline (PBS) solution filtered through a 0.22 mm filter, and the skeletons were kept for further analysis. The extracted tissue was mechanically disrupted using an electrical homogenizer (HOG-160-1/2, MRC-labs, Israel) for 3 × 10 s. The density of zooxanthellae in the homogenate was determined from three replicate counts of the homogenized seawater slurry using a haemocytometer (Improved Neubauer, BOECO Germany) under a microscope and normalized to coral surface area46. The homogenate was centrifuged at5000 g for 5 min at 4°Cto separate the debris and the zooxanthellae cells from the coral host tissue. A protease inhibitor cocktail (cat. G652A, Promega, United States) was added to the supernatant with the host tissue and sonicated for 3 × 30 s (Ultrasonic cell crusher,MRC-labs, Israel). The protein concentration of the coral host was determined using a fluorometric BCA protein kit (Pierce BCA, United States) following the manufacturer protocol. A PerkinElmer (2300 EnSpireR, United States) plate reader was used to determine the total protein concentration with a 540 nm wavelength emission. To measure the chlorophyll-*a* (chl-*a*) concentrations of the zooxanthellae, 1 ml of 90% cold acetone was added to the pellet. The pellet was homogenized and incubated overnight at 4°C in the dark. The solution was filtered into a glass cuvette through a 0.22 mm syringe filter. A NanoDrop (Thermo-Fisher, United States) was used for spectrophotometric measurements at wavelengths of 630, 647, 664, and 691 nm, and the light absorbance results were used to calculate thechl-a concentration based on the following equation47:

Chl - *a*[μg ml-1]= –(0.3319 \* ABS630)– (1.7485\*ABS647) +(11.9442 \* ABS664)– (1.4306 \* ABS697)

**Photochemical efficiency**

Photochemical efficiency of the algal symbiont was assessed with quantum yield of chlorophyll-*a* fluorescence measured with an underwater diving-PAM fluorometer (Walz, Effeltrich, Germany). Measurements of the maximum (Fv/Fm) quantum yield of photosystem II were taken at 2 days, 1 week, 11 days, and 2 weeks of incubation under hypoxia. Five coral fragments from each treatment were randomly chosen (representing all three colonies) and were measured after 2 hoursof the dark period. All measurements were conducted in triplicates per each coral fragment in order to minimize variations.

**DNA extraction**

Total DNA from frozen coral fragments (n=10) was isolated using the Wizard® SV genomic DNA purification system tissue protocol (Promege, WI, United States) according to the manufacturer’s instructions. DNA concentration and quality were evaluated using the NanoDrop 2000c (Thermo Scientific, United States).

**Quantitative real-time PCR**

Changes in the amount of mitochondrial DNA were determined relative to the DNA of a nuclear single-copy gene (that has two copies/ diploid genome)48. qRT–PCR primers were designed using the software Primer Express 3.0 (Applied Biosystems).   
Primers were planned for the*S*. *pistillata*mitochondrial gene COX1 (AB\_441231) (F-CCTCTTGCTAGTATTCAAGCACAC;R-TTTATTAAAAGACACACCCGGGGCTCG) and the nuclear Timeless gene (XM\_022932799) (F-CCAGAAGATGAAGAGATGGCAGCAG; R-TAATACTTCAAAAGCCAGCAGTATGG).

Transcript levels were determined by qRT–PCR using the 7500 Fast Real-Time PCR system (Applied Biosystems, CA, USA).Thermal cycling was 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 100 s, then a final extension at 60°C for 1 min. Triplicate reactions were carried out in 20 μL volumes consisting of: 1× FastStart SYBR Green Master Mix (Applied Biosystems, CA, USA), 0.25 μM of each forward/reverse primer set, 20ng DNA, and PCR grade water. The difference between CT values of COX1 and Timeless in each sample was used as a measure of relative abundance of the mitochondrial genome48.

**Figure legends**

**Fig. 1. Functional and protein-structure conservation of HIFs in metazoa and cnidaria. A.** Schematic representation of the HIF pathway in animals. **B.** Left: Metazoan phylogenetic species-tree representing evolution of major species clades. Representative species are symbolled A/S/N/H to denote: *Acropora*, *Stylophora*, *Nematostella*, and human genes; Right: Gene tree representation of the HIF gene family in metazoa and fungi, with ~50 metazoa species represented. Leaf colors match the species-tree clade colors. Grey boxes represent six conserved groups of human *vs.*cnidaria orthologs. **C.** A heatmap representation of *S*. *pistillata* gene H*vs*C log2 fold changes, in each time point (values lower than -1 or higher than 1 are color-coded as maximum values). *S. pistillata* genes have the same order as in the gene tree, where Ids in red/green correspond to significantly down/upregulated genes, respectively.

**Fig. 2. Functional enrichment of pathways associated with the transcriptional response to hypoxia in *S*. *pistillata* in each of the five time points.A.** Heatmap representing the expression patterns of additional hypoxia-related orthologous genes DE (adjusted *p*-value<0.05) in addition to coral-specific ones (*bottom-up* approach). Colors represent log2fold change expression differences between H*vs*C, in each gene. **B.** Selected functional groups of genes significantly overrepresented among H*vs*C DE genes (*top-down* approach). Enrichment log2 fold changes represent the fraction of DE genes in foreground *vs.* background (see Methods). Positive/negative bars correspond to up/downregulated genes. Only overrepresentations with adjusted *p*-values <0.05 are shown. Numbers at the bars represent the count of DE genes for the category at the specified time point.

**Fig. 3. Diurnal and time-dependent changes of the transcriptional response to hypoxia in *S. pistillata*.** Non-metric multidimensional scaling analysis (NMDS)-based ordination presenting dissimilarity between transcriptomes of *S. pistillata* placed under ambient conditions (control) or constant hypoxia. Corals were sampled at midnight and midday, after 12 hours, 1 week, and 2 weeks from the onset of hypoxia (n=3).Bray-Curtis distance matrix using log10FPKM-based Bray-Curtis distances.

**Fig. 4.Suggested cellular responses of *S*. *pistillata* to acute *vs.* prolonged hypoxia at night A.** As compared to mammals**. B.** Coral-specific. Molecular responses are based on significantly enriched GO and KEGG terms, on specific DE gene expressions, and on relative mitochondria DNA numbers.

**Supplementary Fig. 1.Experiment setup and work flow. A.** Graphic summary of coral collection and the experiment set up in the aquaria. **B**. Graphic summary of the sampling strategy and procedures per aquarium. Numbers in the grey boxes indicate the numbers of fragments sampled. The timeline depicts the collection and measurement timing.

**Supplementary Fig. 2. PHD1/2/3 gene tree.** Left: Metazoa phylogenetic species-tree representing evolution of major species clades. Representative species are symbolled A/S/N/H, to denote: *Acropora*, *Stylophora*, *Nematostella*, and human genes; Right: Gene tree representation of the PHDs gene family in metazoa. Leaf colors match the species-tree clades colors. Grey boxes represent two branches of Deuterostomia PHDs homologous genes in Cnidaria.

**Supplementary Fig. 3. Functional and protein-structure conservation of P4H-TM (PHD4)** **gene family in metazoa and cnidaria A.** Gene tree representation of the PHD4 gene family. This family includes the human P4H-TM (PHD4). Left: Metazoan phylogenetic species-tree representing evolution of major species clades. Representative species are symbolled A/S/N/H to denote: *Acropora*, *Stylophora*, *Nematostella*, and human genes; Right: Gene tree representation of the PHD4 gene family in metazoa and fungi, with ~50 metazoa species represented. Leaf colors match the species-tree clade colors. Grey boxes indicate *S. pistillata* orthologs.. **B.** A heatmap representation of *S*.*pistillata* genes H*vs*C log2 fold changes of PHD4 orthologs. in each time point (values lower than -1 or higher than 1 are color-coded as maximum values). *S. pistillata* genes have the same order as in the gene tree, where Ids in red/green correspond to significantly down/upregulated genes, respectively. *S. pistillata* genes have the same order as in the gene tree. Connecting lines in red/green correspond to significantly down/upregulated genes, respectively. **C.** The numbers of *S. pistillata* putative orthologs of direct HIF target genes differentially expressed in H*vs*C at each of the five time points sampled.

**Supplementary Fig. 4. H*vs*C response of putative *S. pistillata* direct HIF target genes.** The heatmap includes significantly H*vs*C *S. Pistillata* DE genes homologous to human HIF target gene. H*vs*C response is represented as Log2FoldChange.

**Supplementary Fig. 5. Expression of apoptosis and cell death related genes. A.** The heatmap represent putative apoptosis and cell death DE genes H*vs*C response at night over time, as H*vs*C Log2FoldChange. **B.** DE (*p*<0.05) of anti-apoptotic styBcl-2 gene and executioner of apoptosis styCaspase-3.

**Supplementary Fig. 6. Schematic representation of putative mitochondrial response to hypoxia in *S. pistillata*.** The transcriptional changes of coral genes (A and B) are shown on schematic figures adapted from mammalian cells 6,7,9.**A.** *S. pistillata* genes mapped to the mitochondrial morphology fusion and fission pathway are significantly overrepresented among H*vs*C DE genes. Major interactions of the pathway in humans are represented here, where *S. pistillata* orthologs are shown in yellow/blue to indicate significant H*vs*C up/downregulation. **B.** Crosstalk between mitochondria and HIF. Details are in part A of this figure. **C.** Quantitative analysis of mitochondrial Cox1 gene DNA normalized to nuclear Timeless gene DNA. Results are expressed in boxplots of independent DNA extractions from distinct fragments (n=10) incubated in control (grey) or subjected to hypoxia (red) (see Fig. S1B). Values were compared by the aligned rank transform test with significant effects for treatment (DF=1, F=20.6, *p*<0.0001). Asterisks indicate significant differences.

**Supplementary Fig. 7. Diurnal and time-dependent changes of the transcriptional response to hypoxia in *S. pistillata*. A.** Total counts of H*vs*C up/downregulated DE genes between comparable timepoints (same daytime, same week). **B.** Heatmap representing genes exhibiting time-dependent changes of the intensity of response to hypoxia. For each time point, log2 fold change of expression differences between H*vs*C are color-coded as blue-black-yellow gradient. Represented genes that have significant Time *vs.* Treatment interactions using DESeq2 are color-coded in orange-black-purple scale shown on the left-side of the heatmap. Interaction log2FC values are color-coded as orange-black-purple gradient. Log2FC values lower than -2 or higher than 2 are color-coded as maximum values. Corals were sampled at midnight and midday, after 12 hours, 1 week, and 2 weeks from the onset of hypoxia (n=3). **C.** Heatmap representing genes exhibiting diurnal-dependent changes of the intensity of response to hypoxia. Details are as in part B of this figure and represented genes have significant Time *vs.* Treatment interactions using DESeq2.

**Supplementary Fig. 8. Effects of hypoxia on physiology of coral and symbionts. A.**

Calcification rates.Calcification rates were determined using the total alkalinity (TA) anomaly (methods). For each time point (7, 11, 14 days), calcification rates were calculated for distinct fragments incubated in control (grey) or subjected to hypoxia (red) (n=4 per time point and treatment). ANOVA analysis of a fitted linear model revealed no significant differences in calcification rates among sampling time points (DF=2, F=0276, *p* =0.762), treatment (DF=1, F=1.174, *P*=0.295) or their interaction (DF=2, F=1.826, *p* =0.193).**B.** Chlorophyll-*a* (chl-*a*) concentration per zooxanthellae cell.Chl-*a* concentrations were determined using acetone extraction (see Methods). For each time point (2, 7, 14 days), Chl-*a* concentrations were calculated for distinct fragments incubated in control (grey) or subjected to hypoxia (red) (n=4 per time point and treatment). Values were compared by Two-way ANOVA test with no significant effects for time (DF=2, F=3.043, *p*=0.0777), for treatment (DF=1, F=2.036, *p*=0.1741) or their interaction (DF=2, F=0.187, *p*=0.8312). **C.** For each time point (7, 11, 14 days), Zooxanthellae cell counts were calculated for distinct fragments incubated in control (grey) or subjected to hypoxia (red) (n=4 per time point & treatment). Two-way ANOVA test revealed no significant differences in zooxanthellae cell counts among sampling time points (DF=2, F=3.200, *p*=0.0717), between treatment (DF=1, F=1.276, *p*=0.2776) or their interaction (DF=1, F=1.068, *p*=0.3700).

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