Supporting information for:

Hypoxia threatens coral and sea anemone early life stages

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Supplementary materials and methods

Adult culture and spawning

Adult *Nematostella vectensis* sea anemones were collected from a salt marsh in Brigantine, New Jersey in the fall of 2020. Following transport to the University of Pennsylvania, anemones were kept in 12 parts per thousand (ppt) artificial seawater (Spectrum Brands) at 18°C in a dark incubator (Boekel Scientific). Animals were fed twice per week with *Artemia* nauplii (Brine Shrimp Direct), with water changes occurring approximately every 2 weeks for ~3 years to remove waste and maintain seawater normoxia (> 6.5 mg dissolved oxygen (DO) L^{-1}). Spawning was induced using a standard method for *N. vectensis* (Hand and Uhlinger 1992; Fritzenwanker and Technau 2002; Stefanik et al. 2013), which entailed exposing anemones (*n* = 200 adults across four containers) to light and elevated temperatures (24°C) for 14 h, followed by transfer to room temperature (~18–19°C), after which spawning occurred within 1–2 h. As culture containers housed both male and female anemones, gametes were left to fertilize following spawning and the resulting embryos were then transferred to a plastic dish with ~25 mL of new seawater. Embryos were placed at 18°C in the dark, where they were left for 3 days to develop into swimming planulae for use in the experiment. These procedures yielded a single cohort of larvae with mixed parentage.

A captive aquarium population of adult *Galaxea fascicularis* colonies located at Carnegie Science in Baltimore, MD, USA (*n* = 9 females, 10 males; symbiont community unknown) were spawned in an *ex situ* coral spawning system designed as previously described (Craggs et al. 2017, 2020; O'Neil et al. 2021). The colonies were maintained in aquaria under lights with the timing of sunset, sunrise, moon set, moon rise, and photoperiod mimicking those in Cairns, Australia (Craggs et al. 2017). The seawater temperature in the aquaria was maintained at a non-sequential 10-year average for this region derived from published data (Puntin et al. 2022). Spawning occurred over several days in August 2023. On the second day of spawning, bundles containing gametes were collected using a transfer pipette immediately upon release from parent colonies and placed in 50 mL conical tubes. Upon bundle dissociation, the eggs were washed with artificial seawater, and then fertilization was performed by combining eggs and sperm from multiple colonies into a single pool. These procedures yielded a cohort of larvae with mixed parentage, which were kept in an incubator (Percival) at 27°C under lights (25 µmol photons m^{-2} s^{-1}) with a 12-h:12-h day:night cycle until experimentation.

Five days prior to the July 2023 new moon, adult *Porites astreoides* colones (*n* = 20; symbiont community unknown) with a surface area of ~170 cm^2 were collected via hammer and chisel at a depth of ~2–5 m from Bailey's Bay Flats (32°22'13" N, 64°44'27" W), a patch reef off the northern coast of Bermuda. Colonies were transported in coolers containing seawater to the Bermuda Marine Mesocosm Facility (BMMF) at the Bermuda Institute of Ocean Sciences, where they were then placed in individual kitchen jugs set atop egg crate in an outdoor tank (~400 L) with flow-through seawater. The temperature in the tank was controlled by a 300 W chiller (AccuTherm) set to recapitulate ambient temperatures (~27.5–29°C) at Bailey's Bay. To mimic natural irradiance conditions on the reef (Wong et al. 2021), the tank was covered with a

46 screen of black mesh (500 µm), and water levels were maintained at ~50 cm above the colonies
47 during the day. Larvae (endosymbiont community unknown) were collected according to
48 previously developed procedures for this species and facility (Rivera and Goodbody-Gringley
49 2014; Reich et al. 2017; Goodbody-Gringley et al. 2018). Specifically, each night following the
50 collection of the adult colonies, the water level in the holding tank was lowered to a point just
51 below the lips of the containers, and tubes with lightly flowing seawater were placed alongside
52 each colony in the containers. This setup ensured that any planulae released overnight would be
53 carried by flowing seawater down the jug handles and into a mesh (150 µm) container set at the
54 base of each handle. On four separate occasions (i.e., four larval cohorts), larvae collected
55 overnight were pooled across the parental colonies and kept in one of the mesh collection
56 containers in the tank for ~12 h prior to the experiment. The hypoxia experiment was repeated
57 for each of the four cohorts. For all 3 species, the conditions under which larvae were cultured
58 before and after the treatment period represent ambient conditions and match those experienced
59 by the adult populations from which gametes were sourced. As each of these species requires
60 abiotic conditions (e.g., temperature and salinity) not shared with the others, the larvae were not
61 cultured under identical conditions, which may have influenced their hypoxia responses.
62

63 *Symbiosis establishment trials for* Galaxea fascicularis

64 To assay symbiosis establishment in aposymbiotic *G. fascicularis* juveniles, dinoflagellate
65 endosymbionts (family Symbiodiniaceae; community composition unknown) were introduced
66 into culture containers shortly after 84 h, at which time juveniles of this species are likely to be
67 competent for symbiont uptake (Wei et al. 2023). To ensure taxonomic diversity and symbiotic
68 amenability of supplied symbionts, *in hospite* endosymbionts were sourced from fragments of

69 several colonies of the coral *Pocillopora acuta* and polyps of the sea anemone *Exaiptasia*
70 *diaphana* kept in a laboratory aquarium. Specifically, *P. acuta* fragments were waterpikked with
71 sterile seawater and the resulting tissue slurry was collected in a 50 mL conical. Next, two *E.*
72 *diaphana* polyps were added to the tissue slurry and immediately homogenized using a rotostator
73 at 25,000 rpm for 15 sec. The isolation of symbionts from host (i.e., coral and sea anemone) cells
74 was confirmed via brightfield microscopy. The isolated symbionts were pelleted by
75 centrifugation at 1,700 rpm for 5 min, then resuspended in 6 mL of seawater (estimated
76 concentration: ~500,000 cells mL^{-1}), of which 1 mL was added to each culture container. After 6
77 h, water in culture containers was gently stirred to encourage the resuspension of settled
78 symbionts. After an additional 18 h, a water change was performed to wash out free symbiont
79 cells, and the symbiotic juveniles were sampled (n = 15–30 juveniles $treatment^{-1}$) by culture
80 container and stored at -80°C for later analysis of symbiont density.
81
82 *Data and statistical analyses*
83 RStudio with R version 4.2.1 was used for all analyses (RStudio Team 2020). Preliminary
84 analyses suggested a lack of significant differences between the four *P. astreoides* cohorts, so
85 cohort was not included as a variable in the following analyses. First, survival data from larval
86 heat tolerance assays were used to create dose-response curves represented by two-parameter
87 log-logistic functions using the package *drc* (Ritz et al. 2015), and an LD50 was determined for
88 each curve (n = 3 curves $treatment^{-1}$ time $point^{-1}$ $cohort^{-1}$ $species^{-1}$) using the package *chemCal*
89 (Ranke 2022). For photochemical yield data from *P. astreoides* larval heat tolerance assays,
90 dose-response curves represented by log-logistic functions were created also using *drc*, and
91 LD50s were again determined for each curve (n= 3 curves $treatment^{-1}$ time $point^{-1}$ $cohort^{-1}$) using

92 *chemCal*. Next, for data pertaining to all metrics (swimming behavior, settlement rates, size,
93 ash-free dry weight, respiration, photosynthesis, photochemical yield, symbiont density,
94 chlorophyll per symbiont, and LD50s), linear models were constructed relating each metric to
95 the interaction between species, treatment, and/or time (h post-treatment) as appropriate. Group
96 within each experimental treatment was included as a random effect variable in the linear models
97 where appropriate and permissible by the data structure. Each of these linear models was
98 confirmed to meet relevant assumptions (linearity, statistical independence of errors,
99 homoscedasticity of errors, and normality of the error distribution) via visual inspection of
100 diagnostic plots (residuals vs. fitted values, residuals vs. leverage, scale-location, and normal
101 Q-Q, respectively) generated using the "plot(lm)" function. Next, each model was subjected to
102 an analysis of variance (ANOVA) with type II (no interactive effects in model) or III (interactive
103 effects present in model) sums of squares to determine the statistical significance of the model
104 terms. Finally, Tukey's Honest Significant Difference post-hoc tests were used to determine the
105 significance of pairwise comparisons where appropriate. Additional packages used for analysis
106 include: *ggplot2* (Wickham 2016), *emmeans* (Lenth et al. 2023), *ggpubr* (Kassambara 2023), and
107 *lemon* (Edwards 2023). All values are expressed as averages rounded to appropriate significant
108 figures ± standard error of the mean (SEM), and all original data and code are publicly available
109 online (Glass and Barott 2024).

110 **Supplementary tables and figures**

111 **Table S1. Treatment seawater conditions (mean ± SEM).**

Species	**Treatment**	**Time in treatment (h)**	**Temperature (°C)**	**Dissolved oxygen (mg L^{-1})**	**Dissolved oxygen (%)**
Nematostella vectensis	Normoxia	0	20.3 ± 0.1	8.69 ± 0	96.1 ± 0
	Normoxia	6	18 ± 0.1	8.67 ± 0	91.5 ± 0
	Hypoxia	0	20.2 ± 0.1	1.6 ± 0	17.7 ± 0
	Hypoxia	6	18.1 ± 0.1	1.58 ± 0	16.7 ± 0
Galaxea fascicularis	Normoxia	0	26 ± 0.1	7.59 ± 0	93.6 ± 0
	Normoxia	6	27.1 ± 0.1	7.58 ± 0	95.3 ± 0
	Hypoxia	0	25.8 ± 0.1	1.6 ± 0	19.7 ± 0
	Hypoxia	6	27.1 ± 0.1	1.7 ± 0	21.4 ± 0
Porites astreoides	Normoxia	0	27.4 ± 0.2	6.8 ± 0.1	86 ± 1.3
	Normoxia	6	28.1 ± 0.2	6.8 ± 0.1	87.1 ± 1.3
	Hypoxia	0	27.5 ± 0.2	1.6 ± 0	20.3 ± 0
	Hypoxia	6	28.1 ± 0.2	1.8 ± 0.2	23.1 ± 2.5

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113 **Table S2. Statistical testing information.**

Metric	**Degrees of freedom**	***F* value (ANOVA)**	***P* value (ANOVA)**	**Pairwise *p* values (Tukey's HSD)**
Swimming (%)	2	Species*treatment: 4.9246	Species*treatment: 0.02	Treatment: < 0.05 for all species
Settlement (%)	2	Species*treatment: 9.472	Species*treatment: 0.001	Treatment: 0.749 (*N. vectensis*; < 0.001 (others)
Size (larval length)	6	Species*treatment: 2.427	Species*treatment: 0.024	Treatment: > 0.05 (*N. vectensis*); < 0.05 (others)
Size (juvenile polyp diameter)	6	Species*treatment: 2.427	Species*treatment: 0.024	Treatment: < 0.05 (all)
Ash-free dry weight	2	Species*treatment: 5.2247	Species*treatment: 0.007	Treatment: > 0.05 (*N. vectensis*, *G. fascicularis* juveniles); <

				0.05 (*G. fascicularis* larvae and *P. astreoides*)
Respiration	2	Species*treatment: 23.2474	Species*treatment: < 0.001	Treatment: > 0.05 (*N. vectensis*); < 0.05 (*G. fascicularis* and *P. astreoides*)
Photosynthesis	1	Treatment: 50.5192	Treatment: < 0.001	Treatment: < 0.001 (all time points)
Endosymbiont density (*P. astreoides*)	1	Treatment: 52.7837	Treatment: < 0.001	Treatment: < 0.001 (all time points)
Endosymbiont density (*G. fascicularis*)	1	Treatment*Hours post-treatment: 24.5	Treatment: < 0.001	Treatment: > 0.05 (larvae); < 0.001(juveniles)
Photochemical yield (F_v/F_m)	1	Treatment: 16.1945	Treatment: < 0.001	Treatment: < 0.05 (all time

				points)
Chlorophyll	1	Treatment*Hours post-treatment: 36.3112	Treatment*Hours post-treatment: < 0.001	Treatment: 0.2622 (0 h); 0.001 (12); 0.002 (36); 0.3025 (84)
Heat tolerance (survival)	1	Treatment: 4.3941	Treatment: 0.037	Treatment: > 0.05 (*N. vectensis* and *P. astreoides*); < 0.05 (*G. fascicularis*)
Heat tolerance (F_v/F_m)	2	Treatment*hours post-treatment*hours at 36°C: 3.6097	Treatment*hours post-treatment*hours at 36°C: 0.031	Treatment: 0.078 (0 h); 0.004 (12); 0.035 (36)

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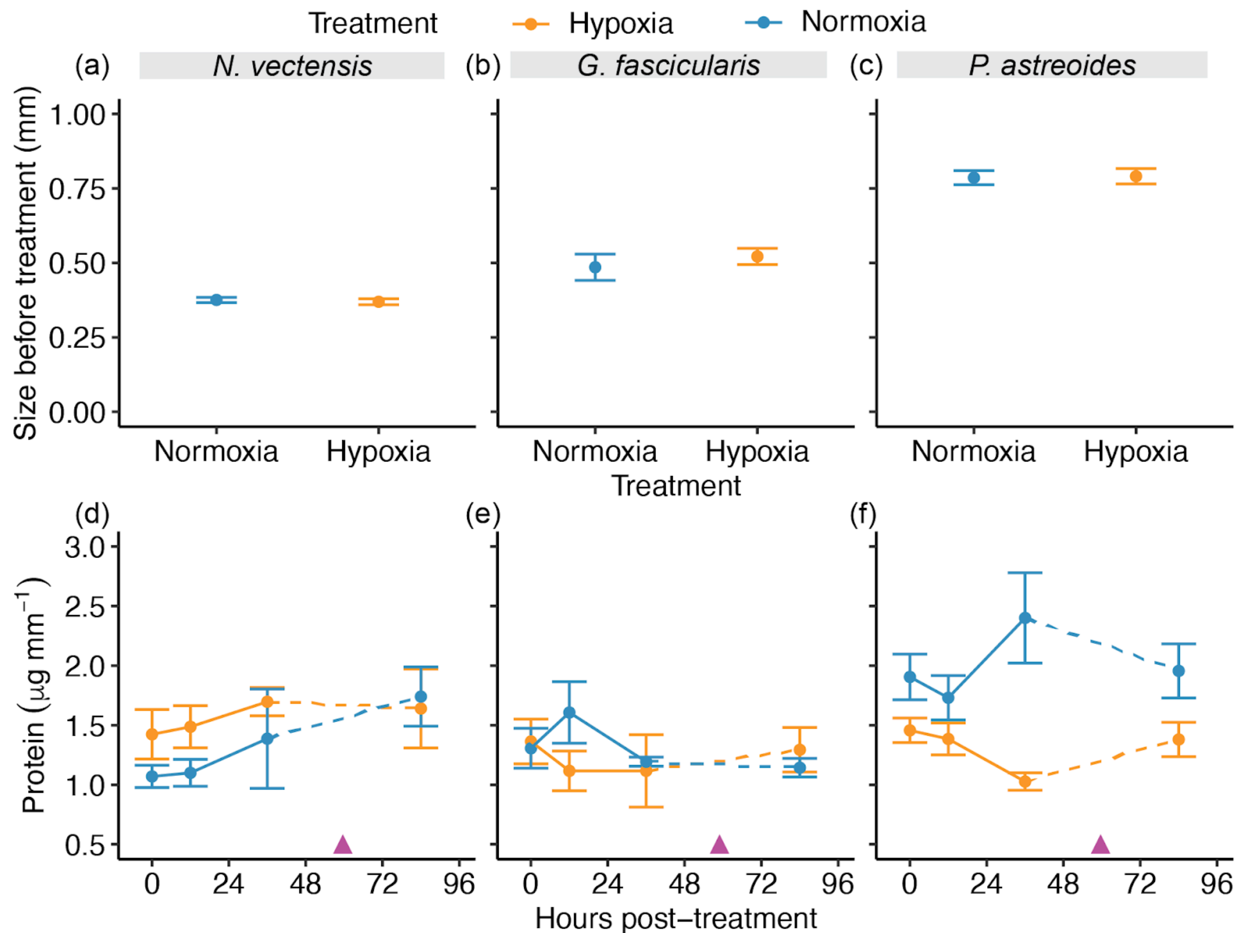

117 **Figure S1. Pre-treatment larval size and effects of hypoxia on protein.** (a–c) Size (larval
118 length in mm) of *Nematostella vectensis* (left), *Galaxea fascicularis* (middle), and *Porites*
119 *astreoides* (right) larvae in the hypoxia (yellow) and normoxia (blue) treatment groups (n =
120 20–30 larvae treatment^{-1} time point^{-1} cohort^{-1} species^{-1}) prior to the start of the experiment. (d–f)
121 Protein (normalized to size; μg mm^{-1}) of *N. vectensis* (left), *G. fascicularis* (middle), and *P.*
122 *astreoides* (right) larvae and juveniles (n = 20–30 larvae treatment^{-1} time point^{-1} cohort^{-1}
123 species^{-1}) over time following the hypoxia (yellow) and normoxia (blue) treatments. In (d–f),
124 purple arrowheads indicate the approximate timing of settlement, points with error bars depict
125 means ± SEM, and asterisks indicate statistical significance ($p < 0.05$) of pairwise comparisons
126 (hypoxia vs. normoxia).

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