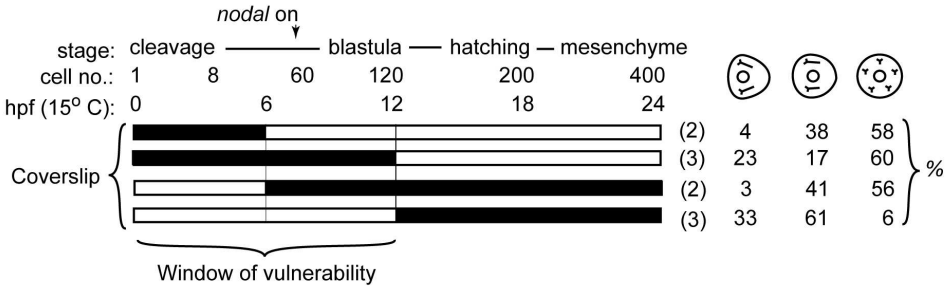
Supplemental Table 1: Effects of hypoxia and seawater acidification on the development of oral-aboral polarity

			Phenotype (48 hrs post-fertilization)		
Buffer	**Oxygen***	**pH**	**Normal**	**Intermediate**	**Radialized**
1 mM Tris	~10 ppm (normoxic)	8.0	50	0	0
		7.5	46	3	1
		7.0	41	9	1
		6.5	15	26	9
	≤1 ppm (hypoxic)	8.0	2	13	33
		7.5	0	12	38
		7.0	0	5	45
		6.5	0	7	43
1 mM Bicarbonate	~10 ppm	8.0	49	1	0
		7.5	46	3	1
		7.0	34	11	5
		6.5	23	13	14
	≤1 ppm	8.0	2	37	12
		7.5	1	28	21
		7.0	1	19	30
		6.5	2	9	39

*To create hypoxic conditions, oxygen was displaced from artificial seawater using nitrogen, until the dissolved oxygen level was 1 ppm (as assayed using an Ocean Optics FOXY sensor and fluorometer). Several thousand fertilized eggs were then aliquoted to dishes of either hypoxic or normoxic seawater, buffered with either 1 mM Tris or 1 mM bicarbonate to the indicated pH. The hypoxic culture dishes were placed in a plexiglass gas chamber, which was flushed with nitrogen and sealed until hatched blastula stage (20 hrs), at which point the dishes were removed from the chamber. Both sets of embryos were then washed into normal artificial seawater and allowed to develop to prism stage under normoxic conditions, and scored for radialization by morphological critera as described in the legend to Fig. 1. Embryos scored as "intermediate" were rounder than normal but retained some bilaterality, such as an asymmetrically displaced gut and a single pair of spicule rudiments (which were typically parallel rather than convergent toward the aboral vertex). Fifty larvae were counted per dish.



Supplemental Figure 1. Experimental determination of the developmental window of vulnerability to cover glass induced radialization. Approximately 1000 eggs were arrayed onto protamine sulfate-treated recessed glass bottoms of MatTek dishes, fertilized, and then covered for the indicated intervals (black bars) with a glass coverslip (see Coffman et al., 2004 for detailed protocol). The embryos were then developed to prism stage (~48 hrs post fertilization) and scored for radialization, as described in the legend to Figure 1. The numbers in parentheses indicate the number of experiments that were carried out for each treatment. Fifty embryos were scored per experiment, and the numbers combined for the reported percentages. Untreated controls developed normally.

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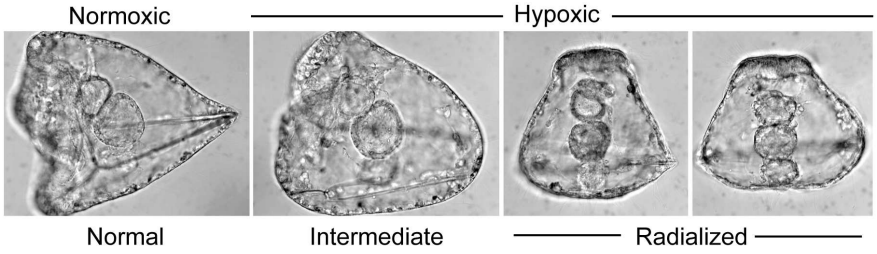

Supplemental Figure 2. Examples of five day old larvae produced by embryos developed in bicarbonate-buffered artificial seawater (pH 8) under normoxic or hypoxic conditions until hatching (see Supplemental Table 1).

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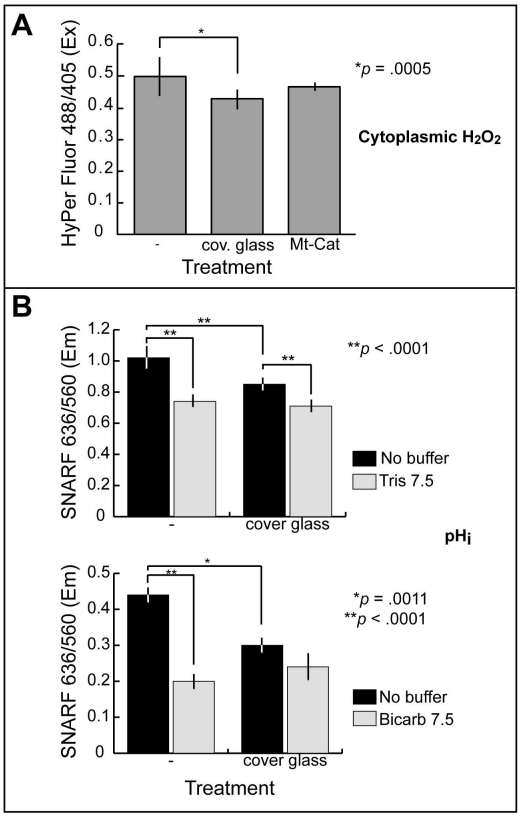

Supplemental Figure 3. Ratiometric measurements of relative intracellular H_2O_2 and pH in confocally imaged embryos subjected to the indicated treatments after preloading with (A) HyPer-cyto (Evrogen) mRNA or (B) carboxy-SNARF-1 AM acetate (Invitrogen), a ratiometric pH indicator whose fluorescence emission maximum shifts from ~640 to ~580 nm with acidification. Ten embryos were imaged per treatment. The data for (A) were obtained as described in Fig. 1B. The data for (B) were obtained by exciting the embryos at 488 nm and collecting fluorescence at 560-615 and 636-754 nm. Statistics are as described for Fig. 1, except the Tukey-Kramer HSD test was used instead of Dunnett's for the comparisons depicted in panel (B).

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