Table S1. Environmental data. Samples were collected at the dates and times indicated (shaded rows indicate night samples). Station numbers correspond to site numbers in Fig. 1.
Numbers indicate averages and standard deviations of three independently measured replicates.

Date	Time	Latitude	Longitude	Station	Salinity (ppt)	Light flux (µmol photons m^{-2} s^{-1})	Depth (m)	Cells mL^{-1}	Bacterial production (ng C L^{-1} h^{-1})	Chl *a* (µg L^{-1})	NO_3^- (µmol L^{-1})	NH_4^+ (µmol L^{-1})	PO_4^{2-} (µmol L^{-1})	SiO_4 (µmol L^{-1})	TIRF (percent of cells)	SARPR (percent of genomes)	LG1 (percent of genomes)
4/11/15	12:04	39.4208	-76.0358	2	0.07	n.d.*	1.85	3050000 ± 818000	40.6 ± 1.6	2.1	61.94 ± 5.3	3.32 ± 0.016	0.202 ± 0.00141	60.93 ± 0.47	17.5 ± 9.05	b.d.**	0.70
4/11/15	23:01	39.4145	-76.043	6	0.07	n.d.	2.32	2520000 ± 138000	23.2 ± 16.5	3.1	63.11 ± 1.59	3.19 ± 0.044	0.208 ± 0.0113	61.79 ± 0.78	4.6 ± 1.99	0.001	10.99
4/12/15	11:30	38.8953	-76.4153	10	6.65	4.98E+02	1.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	26.05 ± 20.9	23.02	1.41
4/12/15	11:30	38.8953	-76.4153	10	8.2	1.37E+02	2.98	2540000 ± 586000	42.4 ± 2.9	15	56.39 ± 1.37	2.49 ± 0.01	b.d.	35.11 ± 0.26	15.64 ± 5.98	11.52	1.22
4/12/15	11:30	38.8953	-76.4153	10	12.9	1.39E+00	8.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	17.64 ± 8.58	88.60	0.90
4/12/15	22:56	38.6802	-76.471	15	8	n.d.	3.1	3270000 ± 522000	39.7 ± 1.1	4.5	55.02 ± 0.34	2.35 ± 0.042	b.d.	29.47 ± 0.81	11.2 ± 4.63	15.70	24.20
4/13/15	11:00	38.1257	-76.2555	17	14.94	9.53E+02	1.72	2300000 ± 476000	19 ± 0.7	4.9	11.25 ± 1.94	0.85 ± 0.27	b.d.	0.25 ± 0.12	14.93 ± 7.92	39.77	0.75
4/13/15	22:59	38.1027	-76.2827	20	15.1	n.d.	1.3	1590000 ± 634000	18.6 ± 2.4	5.6	8.79 ± 2.89	0.89 ± 0.66	b.d.	0.19 ± 0.29	21.57 ± 11.3	39.14	13.31
4/14/15	11:04	37.1317	-76.184	22	20.2	4.18E+02	1.51	2320000 ± 702000	25.9 ± 3.1	4.6	1.80 ± 0.63	1.53 ± 0.32	b.d.	0.33 ± 0.076	38.03 ± 26.26	49.86	1.25
4/14/15	11:04	37.1317	-76.184	22	22.8	5.66E+01	5.43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	25.44 ± 5.8	18.66	0
4/14/15	11:04	37.1317	-76.184	22	27.15	1.47E+01	8.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27.63 ± 24.71	50.13	0.94
4/14/15	23:00	37.2537	-76.1167	26	21.01	n.d.	1.6	2620000 ± 836000	21.5 ± 0.7	3.3	2.86 ± 0.19	1.51 ± 0.41	b.d.	0.35 ± 0.51	21.44 ± 20.26	43.54	14.83
4/15/15	11:05	36.9883	-76.032	29	25.74	7.81E+02	2.27	1690000 ± 326000	20.6 ± 2.3	2.8	2.77 ± 0.075	1.095 ± 0.37	b.d.	0.46 ± 0.10	25.97 ± 30.6	77.09	1.08
4/15/15	11:05	36.9883	-76.032	29	28.8	1.90E+02	5.49	2140000 ± 656000	18 ± 1.5	2.2	3.46 ± 0.085	4.27 ± 1.25	b.d.	0.59 ± 0.031	30.5 ± 27.22	51.47	0.84

	Salinity	Light flux	Cells mL^{-1}	Bacterial prod'n	Chl *a*	NO_3^-	NH_4^+	SiO_4^{2-}	TIRF	SAR PR	LG1	Total qPCR	TIRF: qPCR ratio
Salinity (ppt)	1	NA	0.29	0.05	-0.54	**-0.91***	**-0.93***	**-0.94***	**0.94***	0.8	-0.5	0.67	0.54
Light flux (µmol photons m^{-2} s^{-1})	0.06	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cells mL^{-1}	**-0.44***	**-0.61***	1	0.69	-0.67	0.06	-0.07	0.14	0.02	-0.02	0.07	0	0.07
Bacterial production (ng C L^{-1} h^{-1})	**-0.81***	-0.28	**0.55***	1	-0.21	0.34	0.11	0.34	0.02	-0.36	0.45	-0.23	0.22
Chl *a* (µg L^{-1})	-0.34	-0.19	-0.02	**0.37***	1	0.28	0.3	-0.15	-0.32	-0.11	0.64	0.1	-0.57
NO_3^- (µmol L^{-1})	**-0.87***	-0.15	**0.38***	**0.76***	0.27	1	**0.93***	**0.94***	**-0.89***	**-0.94***	0.52	**-0.81***	-0.34
NH_4^+ (µmol L^{-1})	-0.14	0.06	-0.2	0.21	0.31	0.28	1	**0.93***	**-0.9***	**-0.87***	0.5	-0.75	-0.42
SiO_4^{2-} (µmol L^{-1})	**-0.86***	-0.23	**0.37***	**0.72***	0.07	**0.94***	0.25	1	**-0.98***	**-0.97***	0.11	**-0.99***	0.22
TIRF (percent of cells)	**0.76***	0.14	-0.7	**-0.71***	-0.5	-0.69	0.01	-0.57	1	0.73	-0.43	0.63	0.64
SARPR (percent of genomes)	**0.7***	0.27	**-0.97***	**-0.86***	-0.49	**-0.81***	-0.14	**-0.74***	**0.62***	1	-0.3	**0.95***	0.01
LG1 (percent of genomes)	-0.13	0.36	-0.46	-0.01	0.39	-0.18	-0.1	-0.25	0.17	0.18	1	0.01	-0.61
Total qPCR (percent of genomes)	**0.7***	0.28	**-0.97***	**-0.86***	-0.48	**-0.81***	-0.14	**-0.74***	**0.62***	**1***	0.19	1	-0.19
TIRF: qPCR ratio	-0.4	-0.32	0.57	**0.81***	**0.84***	**0.81***	0.18	**0.85***	-0.18	**-0.79***	-0.18	**-0.8***	1

*indicates $p \leq 0.05$.

Table S2. Correlations between environmental parameters and rhodopsin abundances during the day (white boxes) and night (shaded boxes)**.** Pearson's R values were calculated based on normalized data. Units of measurement are indicated in the left-hand column. TIRF:qPCR ratio is the ratio of the percentage of cells with active rhodopsins, as quantified by TIRF microscopy, to the percentage of genomes with detectable rhodopsins as quantified by qPCR (SARPR + LG1). Phosphate was excluded from this analysis because only three sites had concentrations above the phosphate detection limit; light flux (photosynthetically active radiation, 400-700 nm) was not measured at night. Data in this table is also depicted in Fig. 3.

Figure S1.

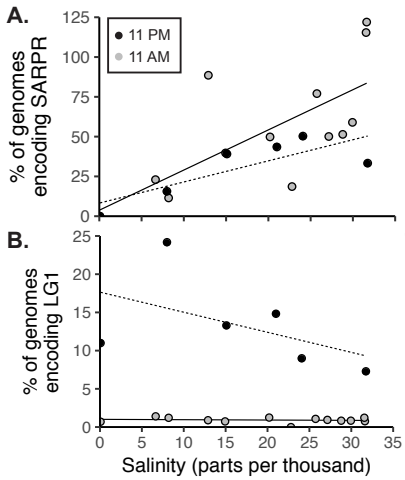

Figure S1. Abundance of rhodopsin genes (qPCR). The percentage of genomes encoding rhodopsin genes were estimated using the ratio of the copy numbers of rhodopsin and 16S genes and assuming that there are, on average, 1.9 16S copies per genome. (A) SAR11-type proteorhodopsin abundance increases with salinity in both day (gray symbols) and night (black symbols) samples. Pearson's R value in the daytime is 0.7, with p-value < 0.05. (B) Actinorhodopsin abundance decreases with salinity in the night samples (black symbols) and is consistently low in daytime samples (gray symbols). The correlation with salinity is not significant at either time of day.

Figure S2.

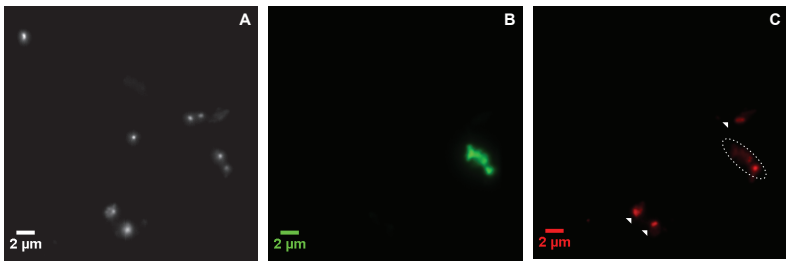

Figure S2. TIRF microscopy images of a Chesapeake Bay sample. The sample was stained with 4',6-diamidino-2-phenylindole (DAPI), a DNA stain. The same field of view was sequentially excited with a (A) 405-nm laser, illuminating DAPI-stained cells, (B) 641-nm laser, illuminating Chl *a*-producing cells, and (C) 561-nm laser, illuminating rhodopsin-producing cells (indicated with white arrowheads). Cells that fluoresce after excitation at both 561 nm and 641 nm (indicated by dashed line) were not counted as rhodopsin-producing cells.

Figure S3.

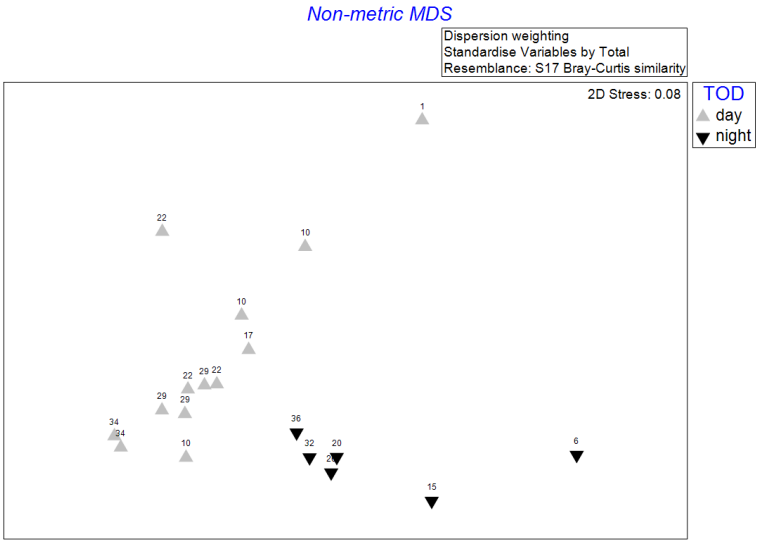

Figure S3. NMDS comparison of biological parameters in daytime and nighttime samples. Samples were compared to each other on the basis of rhodopsin abundance as quantified by TIRF, SARPR-qPCR, and LG1-qPCR. Data were log-transformed and a Bray-Curtis similarity matrix was calculated, then non-metric multidimensional scaling analysis was done using PRIMER v. 7 (1). Day and night samples (gray and black triangles, respectively) clearly group separately, and were analyzed separately in all subsequent data analysis. Numbers indicate the site number (see Fig. 1).

Reference

1. Clarke K, Gorley R. 2015. PRIMER-E v. 7. PRIMER-E, Plymouth, UK.