Science Advances
AAAS

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Supplementary Materials for

Microbial rhodopsins are major contributors to the solar energy captured in the sea

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Published 7 August 2019, *Sci. Adv.* **5**, eaaw8855 (2019)
DOI: 10.1126/sciadv.aaw8855

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/8/eaaw8855/DC1)

Data S1 (Microsoft Excel format). Detailed sampling measurements and calculations obtained in the Mediterranean Sea and East Atlantic Ocean from 30 April to 28 May 2014.

Supplementary Materials and Methods

Sample collection and ancillary parameters. Seawater samples were collected along a Mediterranean-Atlantic Ocean transect (Fig. 1) on board the Spanish RV Sarmiento de Gamboa, from April 30th to May 28th, 2014. Sampling dates and station coordinates are given in Data S1. Samples were collected at various depths within the photic zone using a CTD rosette equipped with 12–liter Niskin bottles. For the retinal and Bchl-*a* analyses, 8 liters of seawater were filtered at each depth using a peristaltic pump connected to a sequential filtration system. Previous estimates of PR using proteomics *(29)* and laser flash photolysis *(15)* required the filtration of >100 liters of seawater. To avoid the presence of large planktonic organisms in the samples, the seawater was pre-screened using a 10-µm nylon mesh attached to the inflow of the filtration system. The 3.0-10 µm and the 3.0µm-0.2 µm microbial size fractions were collected using 47 mm, 3µm pore-size polycarbonate filters (Millipore corp., Billerica, MA) and 0.2 µm Sterivex-GV filter units (Millipore), respectively. Filters were stored at -80^{0}C until pigment extraction. For Chl-*a* measurements, 500 ml of seawater were filtered through 25 mm GF/F filters (Whatman) using low vacuum. The filters were frozen at -20 ºC before Chl-*a* was extracted on board in 90% acetone for 24 h in the dark at 4 ºC. Chl-*a* concentrations were estimated by fluorometry in a Turner Designs fluorometer calibrated with pure Chl-*a* (Sigma Chemical)*(41, 42)*. Dissolved inorganic nutrient samples (nitrate, phosphate) were collected from the Niskin bottles in 20 ml acid-washed polyethylene flasks and their concentrations determined on board using standard segmented flow analysis methods with colorimetric detection *(42)*. Autotrophic picoplankton (*Prochlorococcus*, *Synechococcus,* and picoeukaryotes) and heterotrophic prokaryotes were stained with the fluorochrome SYBR green I Molecular Probes (final concentration 1000x dilution of the commercial product) and enumerated by flow-cytometry (Becton–Dickinson FACScalibur)*(43).*

Proteorhodopsin quantification method. X-ray diffraction and molecular results have shown that the structure of all the known rhodopsin photosystems is conserved across the three domains of life and consists of one membrane protein (opsin) and one retinal chromophore *(21, 22)*. We therefore optimized an analytical technique for quantifying retinal oxime *(19, 20,24)*, used as a proxy for rhodopsin in natural marine microbial communities and pure cultures of marine bacteria. For the method implementation, we used a proteorhodopsin (PR) containing member of the genus *Vibrio* (strain AND4), known to use PR to prolong survival under starvation conditions *(5)*. A knockout mutant strain of AND4 (Δ*prd*), was used as a negative control. In this mutant strain (AND4-Δ*prd*), only the PR genes were knocked out, while the retinal synthesis genes are still present. Thus, with no PR proteins in the membrane, any retinal signal in the AND4 -Δ*prd* would be from PR-unbound retinal. The retinal quantification method was further validated with field samples collected at the San Pedro Ocean Time Series Station (SPOT) in the Southern California Bight.

Preparation of marine bacterial culture samples for method optimization. Pure cultures of *Vibrio* sp. AND4-wt (wild type) and AND4-Δ*prd* (knockout mutant) were grown from glycerol stocks (25% final concentration) kept at -80ºC. Bacteria were first inoculated on ZoBell agar plates. Individual colonies were picked and transferred to 40 ml ZoBell liquid media and incubated under full light with gentle agitation at room temperature for 24 hours. 100 µl of the culture were then used to inoculate a second culture of 1-liter ZoBell media and grown overnight for biomass collection. Cells were harvested by filtering 10 ml aliquots of culture onto 47 mm diameter, 0.22 µm Durapore® membrane filters (Millipore) and immediately frozen at -80ºC. Samples for bacterial counts were fixed with 0.2 µm -pore-size filtered formaldehyde (4%, final concentration), stained with acridine orange *(45)*, filtered onto black 0.2 µm pore-size polycarbonate filters and counted by epifluorescence microscopy within 48 h.

Collection of field samples for method implementation. Surface seawater samples for retinal analysis (8 liters) were collected at SPOT (https://dornsife.usc.edu/spot/). The picoplankton fraction was isolated from larger particles using a series of in-line filters. The samples were pre-screened using a nylon 10.0 μm mesh attached to the inflow of the peristaltic filtration system. The 3.0-10.0 μm and the 3.0-0.2 μm microbial size fractions were collected using 47 mm, 3.0 μm pore-size polycarbonate filters (Millipore) and Sterivex-GV filter units (Millipore), respectively. Filters were stored at -80º C until pigment extractions.

Extraction of retinal and bacteriochlorophyll-*a*. Nine different combinations of solvents were tested (Table S1) and methanol (MeOH) was chosen given the best results as an extracting solvent as well as for the LC/MS/MS response for the simultaneous quantification of retinal, retinal oxime and bacteriochlorophyll-*a*. For samples collected with Sterivex cartridge filters; the cartridges were carefully broken apart using pliers and the filters were cut out using an ethanol-cleaned scalpel. All filter types (polycarbonate and Sterivex filters) were transferred into 15-ml Falcon tubes that had been rinsed with MeOH three times and air-dried. Three milliliters of LC-MS grade methanol were added to the filters and left to extract for 30 minutes at -20ºC. After this initial extraction, samples were sonicated at 50% amplitude for 30 seconds on ice and in the dark. 250 μl of 1% butylated hydroxytoluene (BHT) were subsequently added as antioxidant and the samples were extracted at -20º C for 24 hours. To confirm that the pigment extraction was complete, filters were re-extracted for another 24 hours and did not produce any additional retinal signal, indicating that all retinal present in the samples was extracted in the first 24 hours (fig. S2). The extracted material was then centrifuged at 5,000g for 10 min at 4ºC. An aliquot of 500 μl was used for bacteriochlorophyll-*a* determination by HPLC using the analytical conditions previously described *(40)*. The remaining extraction volume was used for retinal oxime quantification. To complete the extraction, 100 μl of 1M hydroxylamine (36 mM final

concentration) were added to the extracted material and illuminated for 2 hours under a >530 nm light source (300 watt halogen projector bulb with Edmund Optics >530 nm long pass filter, average irradiance 180 μmol quanta m^{-2} s^{-1}). Full transformation of *all-trans* retinal into retinal oxime was observed in standards at concentrations to up to 56 μM (Table S2), indicating that the concentration of hydroxylamine used in our extractions was sufficient. The light incubations consisted in placing the extraction Falcon tubes in an ice-water bath maintained at ~4ºC under 2 cm solution of 5% $CuSO_4$. Irradiating with a light source of a wider spectrum (full spectrum visible light; fig. S3) was also tested and did not produce different results than those obtained using the long pass filter. After the illumination step, samples were centrifuged at 5000g for 10 min at 4^0 C and analyzed immediately by LC/MS/MS.

Analytical conditions. The LC/MS/ system used for the pigment quantification consists of a Thermo TSQ Quantum Access electro-spray ionization triple quadruple mass spectrometer, coupled to a Thermo Accela High Speed Liquid Chromatography pump and auto-sampler. For retinoid quantification, the LC system was set up with a Supelco Discovery HS C-18 column (10 cm, 5 μm, 2.1 mm). Mobile phases were (A-acetonitrile, 0.5% acetic acid, B-acetone, C-water (LC-MS grade), 0.5% acetic acid). A 12-minute gradient program (0 min, A-52%, B-16%, C-32%), (1 min, A-30%, B-50%, C-20%), (7 min, A-0%, B-100%, C-0%), (10min, A-52%, B-16%, C-32%); was used with a flow rate set at 250 μL min-1 and a 100 μL sample loop. Retinal oxime standards were prepared by adding hydroxylamine to a standard solution of *all-trans* retinal (Sigma, CAS 116-31-4) in methanol and illuminated for 2 hours as described in the extraction protocol. The mass spectrometer was tuned to retinal oxime and run in 'Selected Reaction Monitoring' mode with a retinal oxime parent of 300 m/z and products of 94.2, 158.6, and 161.5 m/z. By measuring retinal oxime, this method quantifies the total retinal concentrations (on both *all trans* and *cis* isoforms) due to the MS mass-to-charge ratio (m/z) detection of compounds (*46*).

The LC/MS/MS was also setup to detect retinol, however this retinoid form was not detected in any of our samples. The potential presence of additional retinoids other than retinal would have different mass-to-charge ratios (m/z) and would therefore be distinguishable. For instance, retinoic acid has previously been reported in freshwater cyanobacteria (*47*). Yet, if present in any of our samples, retinoic acid would not affect the specific retinal oxime signal since its reaction with hydroxylamine would produce vitamin A hydroxamic acid, which has a different molecular weight and m/z ratio (315.46 g/mol; 316.4 m/z) than retinal oxime (299.46 g/mol; 300.4 m/z). All samples were analyzed randomly and their sample identification was masked to the persons involved in the analyses.

Method Assessment. Optimal methanol extraction time was determined using *Vibrio* sp. strain AND4-wt pure cultures. Cells were harvested from the same culture in triplicates and retinal was quantified after extraction for 2, 12, 24 and 48 hours. Our results indicated that the retinal extraction was complete after 24 hours (fig. S4). To demonstrate the ability of the new LC/MS/MS method to accurately quantify the retinal bound to the protein (as retinal oxime), cultures of the *Vibrio* strain AND4-wt and the knockout mutant AND4-Δ*prd* were grown under the same conditions. Cells were harvested (in triplicate) by filtration and extracted as described above. Our results showed that the number of retinal molecules per cell in AND4-Δ*prd* was about 4-times lower than in AND4-wt (mean± s.d.; 380 ± 25 *vs.* 1460 ± 150, respectively; fig. S5). These results are consistent with the fact that the retinal synthesis genes are still present in the mutant, even though the PR membrane protein is absent *(5)*. Finally, the concentration of retinal oxime measured in the picoplankton samples (3-0.2 μm-size-fraction) in the natural SPOT samples ranged from 7.6 ± 1.9 to 9.0 ± 0.58 pmol per liter (fig. S6). In contrast, the levels of retinal oxime in the 3 μm fraction were < 1 pmol per liter, which is within the uncertainty of our measurements. The potential 11-cis retinal signal from the eye retina of larger marine organisms

should be negligible in the microbial size fractions analyzed in this study since our samples were pre-screened with a 10.0 μm pore-size mesh, and further filtered through a 3.0 μm and a 0.2 μm pore-size filter. Similarly, while the presence of algal or fungal retinal cannot be discarded in natural samples (*23*), it should be negligible in the picoplankton size fraction (0.2 to 3.0 μm). We found no detectable retinal in the particulate fraction collected on the 3.0-10.0 μm pore size filters collected along the Mediterranean Sea during our study. Retinal was only detected within the 0.2 to 3.0 μm size-fraction samples, which contains almost exclusively prokaryotic cells. Furthermore, most of the retinal in our study was found in oligotrophic, nutrient-poor waters where the abundance of large marine organisms feeding from phytoplankton is expected to be very low. Any additional retinal sources present in our samples but unknown to date, cannot be accounted for at this early stage of environmental retinal research.

Depth-integrated energy capture calculations. Average daily energy absorbed per pigment was calculated using pigment concentrations and radiometer data (Data S1). Specifically, the downwelling spectral diffuse attenuation coefficient per wavelength ($K_d(\lambda)$) was calculated from spectral downwelling irradiance data (PUV-2500 UV Radiometers, Biospherical Instruments, San Diego, CA, USA) using the best fit in the log-linear range. Stations with missing or bad data ($r^2<0.95$) were assigned the average $K_d(\lambda)$ value from stations within the same biogeochemical region (oligotrophic (N=1), coastal-influenced (N=2), open ocean (N=2)). Peak daily surface irradiance was calculated using Reference Solar Spectral Irradiance data (ASTM G-173, http://rredc.nrel.gov/solar/spectra/am1.5/astmg173/astmg173.html) after *(42)* integrated over pigment specific wavelength bands (Table S3). Average daily downward irradiance was then calculated using peak daily irradiance and a solar radiation approximation equation and was converted to scalar using a constant factor of 1.2 *(48)*. *In situ* average daily downward irradiance per depth ($\mathcal{E}_d(z)$) was calculated as

$$\mathcal{E}_d(z) = \mathcal{E}_d(0^-)e^{\overline{-K_d(\lambda_{peak})z}} \quad \text{eq. (S1)}$$

where $\mathcal{E}_d(0^-)$ is the subsurface average daily downward irradiance assuming an average surface reflectance of 4% *(49)*. Average daily PAR, here defined as 400-700 nm, per depth was also estimated. Finally, average daily energy absorbed per pigment i ($\mathcal{E}_i(z)$) was calculated for each sample depth after *(16)* and *(50)* as

$$\mathcal{E}_i(z) = RC * dz * V_{max} * \frac{\mathcal{E}_d(z)}{K_m + \mathcal{E}_d(z)} \quad \text{eq. (S2)}$$

where RC is the concentration of reaction centers calculated assuming that each RC contains 1 molecule of PR, 34 of Bchl-*a* and 300 of Chl-*a*, respectively (RC m^{-3}; Table S4). For PR, only 80% of the retinal signal was used to estimate energy capture. This was done to account for the presence of heliorhodopsins, which have slow photocycles (>1s) and are therefore likely to be incompatible with energy capture functions *(10)*. The only data available so far indicates that heliorhodopsin photosystems could account for ~20% of the rhodopsins found in surface waters *(10)*. dz is the depth over which the light dose is calculated (dz=1m), V_{max} is the maximum photon flux per reaction center (photons s^{-1} RC^{-1}, Table S4), K_m is the half-saturation constant of the photon flux versus irradiance curve (J m^{-2} s^{-1}, Table S4), and $\mathcal{E}_d(z)$ is the pigment specific light dose (J m^{-2} s^{-1}). $\mathcal{E}_i(z)$ was then integrated over depth to get average daily energy absorbed per pigment for the entire water column.

For most pigments, the radiometer bins did not match up exactly with the peak absorbance wavelength and so the closest bin was chosen (Table S3). We acknowledge that this will result in a slightly deeper or shallower profile due to slight differences in $K_d(\lambda)$. In addition, a second bacteriochlorophyll peak exists at ~875 nm however the radiometer (PUV-2500 UV) only quantifies wavelengths between 305 and 710 nm and so we were not able to calculate the energy absorbed by this second peak. However, the maximum absorbance of this second peak is approximately 20% of the primary peak and the width is ~ 1/3 the width of the primary peak such that we do not anticipate that the exclusion of this secondary peak will significantly impact our results.

Cellular daily energy capture estimates. The daily energy captured per cell was calculated using the parameters and assumptions, slightly modified from *(16)* (Table S5). These calculations also assume a hyperbolic response to light which, in contrast to using a linear response to light criterion, allow accounting for the effect of light saturation at high light intensities *(16)*. To estimate the number of PR molecules per cell, we assumed that 75% of the total heterotrophic bacteria contain PRs in our samples *(6, 8, 9)* and that 2.5% contain Bchl-*a (51)*. Chl-*a* containing cells were estimated as the addition of the *Prochlorococcus*, *Synechococcus* and picoeukaryote counts measured with flow cytometry. Our analytical method for the determination of retinal concentrations in natural microbial communities does not differentiate between the different types of rhodopsins proton pumping PRs *vs*. sensory rhodopsins. Therefore, to be able to account for any retinal signal originating from heliorhodopsin, which could represent ~20% of all rhodopsins in the photic zone *(10)*, 80% of the retinal signal quantified in seawater was used in these calculations.

The energy stored per mole photon absorbed (E_0) was estimated as follows. For **PR,** a maximum of one mole proton is transported per mole photon absorbed *(14, 37)*. From the mole protons moving per mole ADP phosphorylated ratio of the ATP synthase of 3.33 mole proton per mole ATP from structural biology *(37)*, 0.3 mole ADP are phosphorylated per mole photon absorbed. With a free energy of hydrolysis of ATP *in vivo* of 55 kJ per mole *(52)* the energy yield (E_0) is 16.5 kJ per mole photon.

For aerobic anoxygenic phototrophs (AAP) using **Bchl-*a***, a maximum of one mole electron completes a cycle in cyclic electron flow per mole photon absorbed *(37)*. From the proton:electron ratio of two in cyclic electron flow in AAP, 2.0 mole protons are moved per mole photon absorbed *(37)*. From the proton:ATP ratio of the ATP synthase of 3.33 mole photon per mole ATP *(37)*, 0.6 ADP are phosphorylated per mole photon absorbed. With a free energy of hydrolysis in vivo of 55 kJ per mole the energy yield (E_0) is 33 kJ per mole photon absorbed.

For linear electron flow in oxygenic photosynthesis using **Chl-*a*,** the photon yield of PSII is up to 0.8 mole electron per mole photon absorbed by PSII antennae, and the photon yield of PSI is up to 1.0 mole electron per mole photon absorbed by PSI antennae directed to linear electron flow *(37)*. The transfer of one mole electron from H_2O to $NADP^+$ requires 2.25 mole absorbed photons, or 0.44 mole electron per mole photon absorbed. With 109 kJ required per mole electron from H_2O to $NADP^+$ reduced by electrons from H_2O *(52)*, the energy stored is 48 kJ per mole photon absorbed. Three mole protons are moved per mole electron in linear electron flow, or 1.32 mole protons per mole photon absorbed *(37)*. With the proton:ATP of the ATP synthase of 4.67 mol proton per mole ATP from structural biology this yields an ADP phosphorylated per mole photon absorbed of 0.28 mole ATP per mole photon absorbed *(37)*. With a free energy of ATP hydrolysis in vivo of 55 kJ per mole the energy yield is 15.5 kJ per mole photon absorbed. Production of 0.22 mole NADPH and 0.28 mole ATP stores 63.5 kJ per mole photon absorbed. Then, in attempting to provide a value for energy stored per mole photon absorbed for linear plus cyclic

electron flow in vivo, a starting point is the 3 mole ATP and 2 mole NADPH required per mole carbon dioxide assimilated into carbohydrate, assuming no photorespiration. The 'no photorespiration' assumption requires, in air-equilibrium seawater, a carbon dioxide concentrating mechanism (CCM) that requires not less than 0.5 mole ATP per mole carbon dioxide assimilated *(53)*, so the ATP and NADPH requirement for assimilating one mole carbon dioxide is 3.5 mole ATP and 2 mole NADPH. Since linear electron flow yields 2.55 mole ATP per 2 mole NADPH (requiring 9 mole photon absorbed by PSII and by PSI antennae directed to linear electron flow), an additional 0.95 mole ATP is required. Assuming that the additional ATP comes from cyclic electron flow with 4 mole protons moved per mole photon absorbed by PSI antennae associated with cyclic electron flow, as in cyanobacteria, this additional 0.95 mole ATP requires 0.95 mole photon absorbed *(37, 53)*. The total photon requirement for the 3.5 mole ATP and 2 mole NADPH is 9 plus 0.95 or 9.95 mole photon absorbed. The 3.5 mole ATP (3.5 x 55 = 192.5 kJ) and 2 mole NADPH (4 x 109 = 436 kJ) are equivalent to 628.5 kJ per 9.95 photons, or an E_0 of 62.9 kJ per mole photon absorbed.

Effect of PR photocycle on energy capture estimates. Although the blue light-absorbing PRs were originally thought to have slow photocycles (>100 ms)*(15)* the data available today suggests that the specific PR photocycle (either of blue or green-light absorbing PR) depends on the environment where they were isolated from *(44)*. Apart from heliorhodopsin, most PRs from the surface ocean have been characterized as proton pumping PRs with fast photocycles *(6)*, while polar and deep ocean PRs show slower photocycles (>100 ms *(44)*). Therefore, once a possible heliorhodopsin signal of 20% was removed, we did our PR-based energy capture estimates (Fig. 3, Fig. 5) using a 10 ms photocycle typical of proton pumping PRs found in non-polar surface waters. We further tested whether or not our energy estimates could be influenced by the contributions of other rhodopsins with longer photocycles (>10 ms) at intermediate depths within

the water column. To explore this, we compared the amount of energy potentially captured per cell and per day at the different sampled depths (fig. S7). We found that the majority (>95%) of the light potentially captured per cell and per day at each station is actually captured within the surface layers (above 55 m), suggesting that any retinal quantified at deeper layers would have a negligible contribution to the total estimates even using a photocycle of 10 ms for all depths. Whether or not the low amounts of energy captured below 55 m can be used for energetic purposes or not is a question that future studies will need to address.

Supplementary Figures

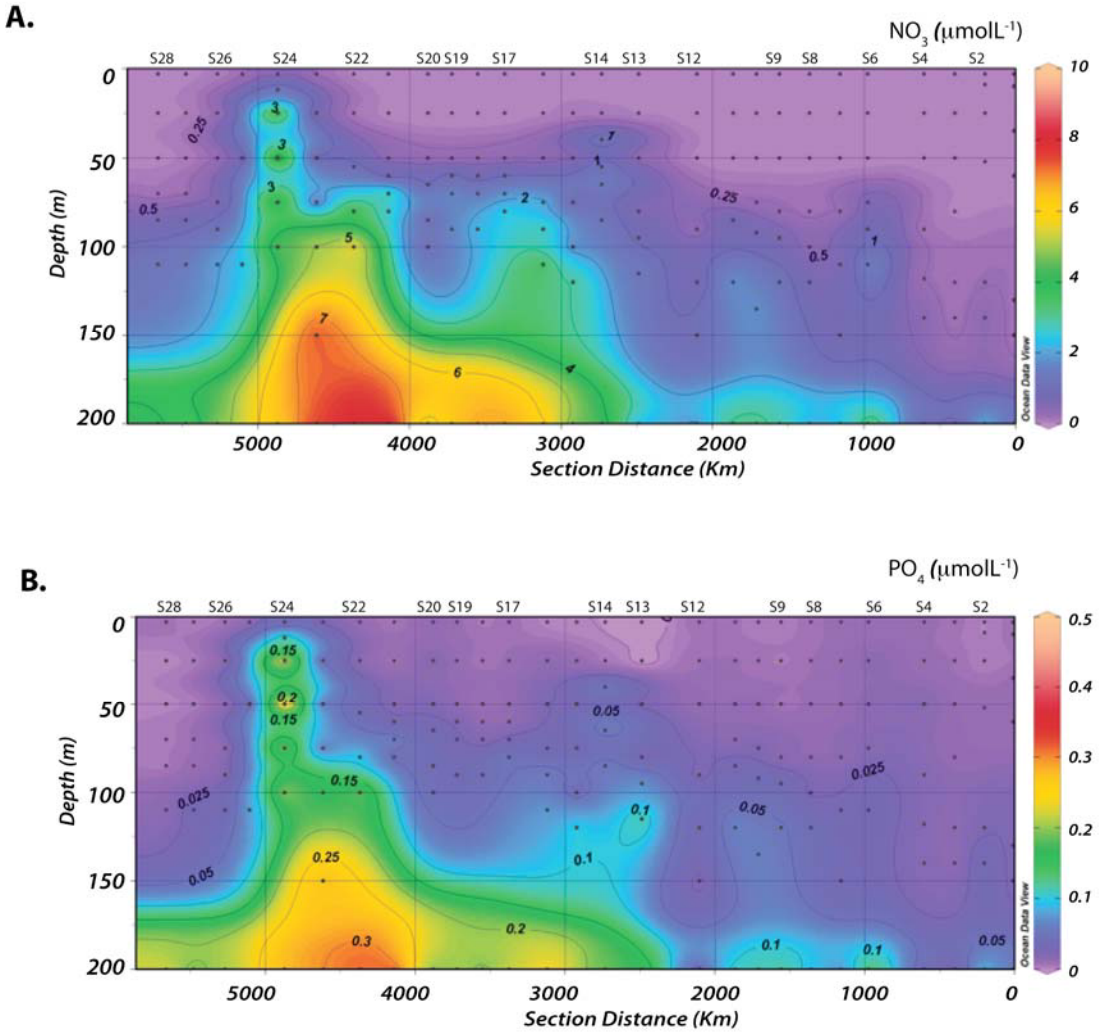

Fig. S1. Inorganic nutrient concentrations measured along the Mediterranean Sea and East Atlantic Ocean. A) Nitrate (NO_3) and **B)** Phosphate (PO_4).

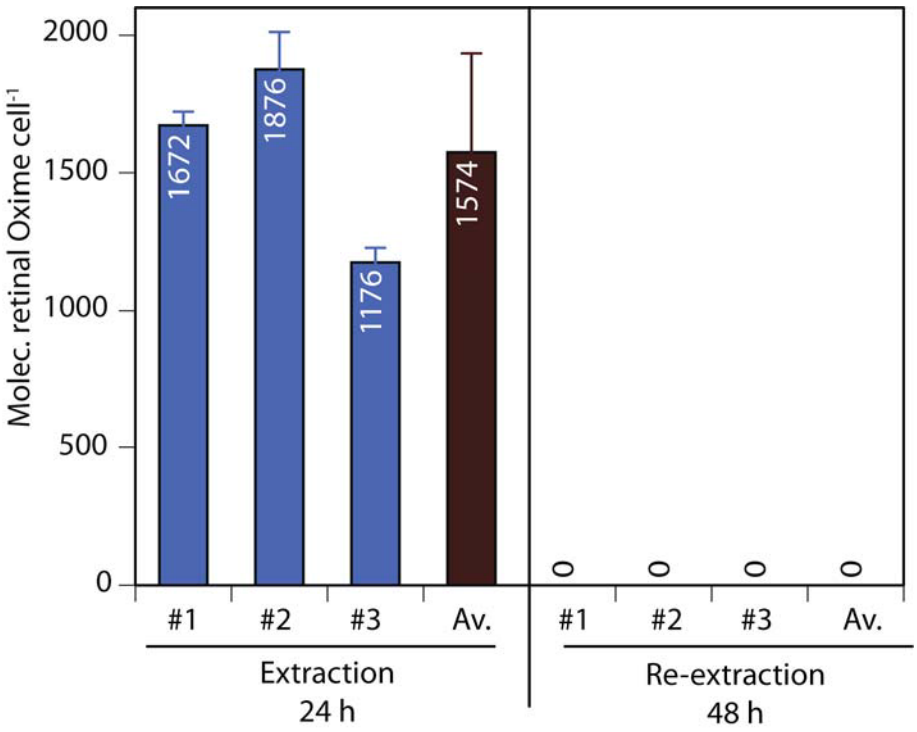

Fig. S2. Retinal oxime reextraction test. Molecules of retinal oxime per cell obtained in filters containing *Vibrio* AND4 after 24 hours of extraction in methanol (MeOH) as in our original method (left panel) compared to a 24 hours MeOH re-extraction of the same filters (48 hours total extraction time). Blue columns show the retinal oxime concentration measured in each individual filter and the error bars of those columns represent the standard deviation of triplicate injections analyzed by LC/MS/MS (instrumental variability). Black columns show the average concentration for the replicate filters analyzed and their error bars represent variability within treatment. This experiment shows that the retinal pigment is fully extracted after 24 hours in MeOH.

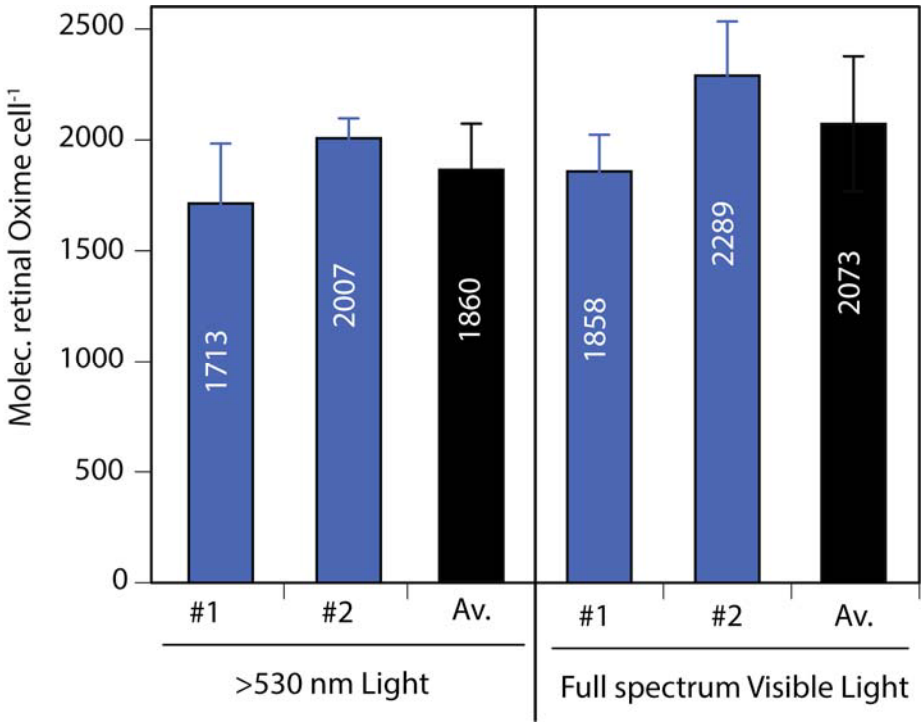

Fig. S3. Concentrations of retinal oxime in *Vibrio* sp. AND4 extracted using hydroxylamine under different light conditions. The left panel shows the retinal oxime concentrations obtained doing the hydroxylamine incubation with methanol (MeOH) in the light using a long pass filter (>530nm). The right panel shows the retinal oxime concentrations obtained doing the hydroxylamine incubation with MeOH using full spectrum visible light. Blue columns show the retinal oxime concentrations of each individual filter and the error bars of those columns represent the standard deviation of triplicate injections analyzed by LC/MS/MS (instrumental variability). Black columns show the average concentration for the replicate filters analyzed and their error bars represent variability within treatment. These results show that the exposure to full visible light did not produce significantly different results compared to those obtained with the long pass light filter (>530 nm).

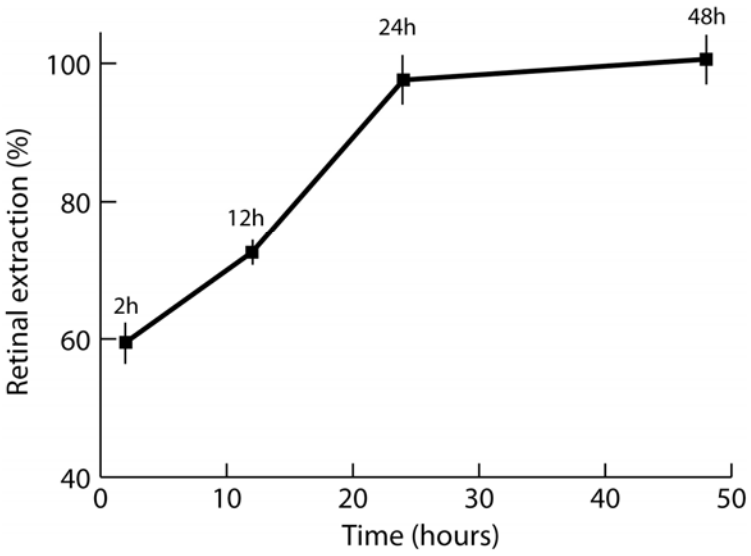

Fig. S4. Retinal extraction time optimization in *Vibrio* sp. AND4. The efficiency of methanol extraction is reported as a percentage of the amount extracted at 48 hours. About 98 ± 6 of the retinal was extracted after 24 hours.

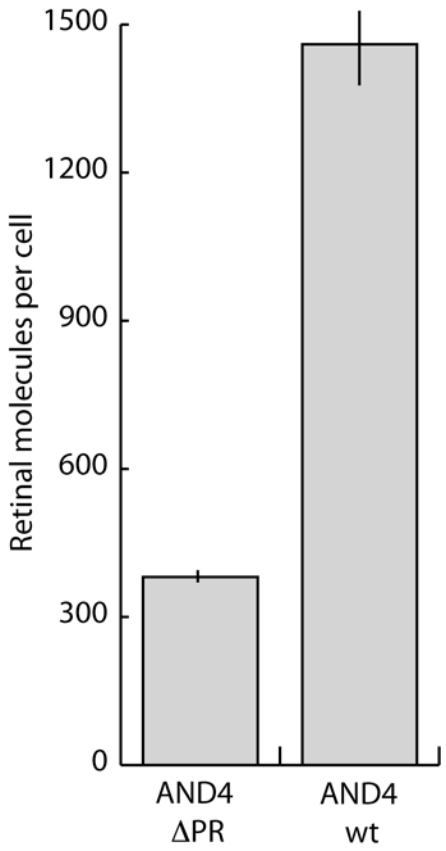

Fig. S5. Number of retinal molecules (as retinal oxime) measured in the *Vibrio* sp. AND4 wild-type (wt) and in the knockout mutant strain (Δ*prd*). Our analysis shows that the wild type strain contains about 4-times more retinal molecules per cell than the mutant.

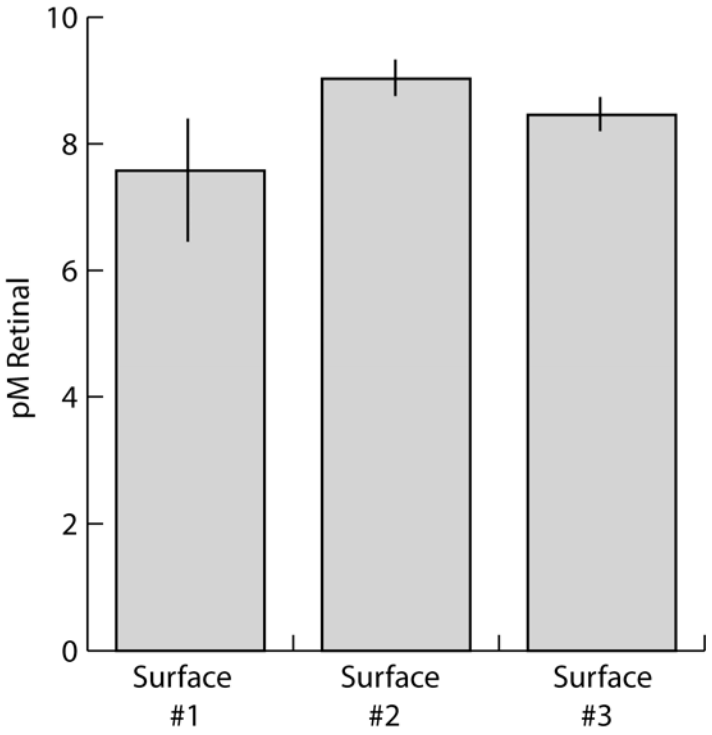

Fig. S6. Concentration of rhodopsin-bound retinal (as retinal oxime) measured in surface waters collected in January 2015 at station SPOT. The concentration of retinal oxime ranged from 7.6 to 9.0 pM.

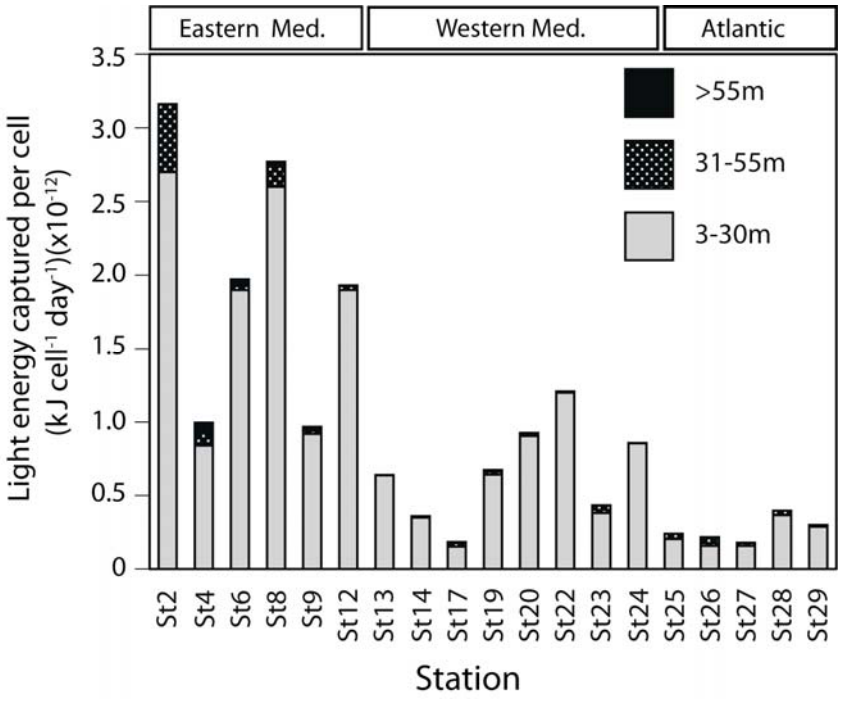

Fig. S7. Contribution of the potential energy captured by PRs at different depths. The potential energy yields calculated for samples collected above 55 m contributed to more than 95% of the total energy measured at different depths. This trend was observed at all sampling locations.

Supplementary Tables

Table S1. Summary of treatments used to optimize the extraction and detection of retinal, retinal oxime, and Bchl-*a*. Known standard concentrations of the pigments were used to test their specific recovery after incubation with the solvent. Asterisk indicates that 1 ml of intermediate standard and 4 ml of solvent(s) was dried under N_2 and reconstituted in 5 ml solvent(s). Methanol (MeOH) was ultimately chosen as the extraction solvent for giving the best results for on three pigments simultaneously.

Extraction treatment	Retinal Oxime		Retinal		Bacteriochlorophyll-*a*	
	Average LC-MS counts	% compared to MeOH	Average LC-MS counts	% compared to MeOH	Average LC-MS counts	% compared to MeOH
MeOH	6,322,016	100	27,875,505	100	31,653,882	100
MeOH/dryness/MeOH*	42,767	1	9,673,700	34	8,393,430	27
Acetone	2,398,850	38	27,300,223	97	16,896,828	53
Acetone/dryness/MeOH*	80,261	1	5,717,937	20	12,211,853	39
Acetone/dryness/IPA:DCM*	815,719	13	8,145,677	29	15,162,641	48
Acetone/MePH	3,149,818	50	14,743,772	52	Not resolved	0
Acetonitrile	7,711,176	122	7,154,565	25	35,327,271	112
Dichloromethane	1,184,607	19	32,237,101	115	2,3031,700	73
2-propanol/DCM	2,401,420	38	34,793,693	124	10,262,836	32

Table S2. Transformation of retinal into retinal oxime using all-*trans* retinal standards. This table shows that retinal (up to 55.7 µM, which was the maximum concentration tested) was fully transformed into retinal oxime after 2 hours incubation with 36 mM hydroxylamine.

Retinal Concentration (µM)	Retinal LC-MS counts		Retinal Oxime LC-MS counts	
	Before Hydroxylamine	After Hydroxylamine	Before Hydroxylamine	After Hydroxylamine
0	0	0	0	0
12.62	318,099	0	0	554,284
25.45	505,072	0	0	1,071,392
37.81	692,510	0	0	1,595,276
55.7	1,067,557	0	0	2,238,064

Table S3. Light wavelength characteristics.

	Peak abs. (nm)	Lower range (50% abs.) (nm)	Upper range (50% abs.) (nm)	$K_d(\lambda)$ (nm)	Reference
PR (blue)	490	440	540	490	*(15)*
PR (green)	530	475	585	555	*(15)*
Chl-*a* (peak 1)	435	410	460	443	*(54)*
Chl-*a* (peak 2)	676	670	682	670	*(54)*
Bchl-*a* (peak 1)	470	410	530	490	*(55)*

Table S4. Parameters used to calculate the light energy captured shown in Figs. 3 and 5.

	V_{max} (photons s^{-1} RC^{-1})	K_m (J m^{-2} s^{-1})	Molecules per reaction center	Reference
PR	100	659.2	1	*(16, 50)*
Chl-*a*	46.7	11.0	300	*(16, 34)*
Bchl-*a*	43.5	48.6	34	*(16, 51)*

Table S5. Parameters used to calculate the cellular daily energy yield per photosystem shown in Fig. 5 and data S1. The terms in the equation can be found in the table above. *T* represents time and 12 hours (43,200 seconds) were assumed for one day.

Factor	Units	PR	Bchl-*a*	Chl-*a*
Photosynthetic units	per photosystem	1*(16)*	34*(16, 51)*	300*(16, 34, 37)*
Photosynthetic units	per cell	Obtained from the field data		
Energy per photon (E_0)	kJ mol^{-1}	16.5*(37)*	33*(37)*	62.9*(37)*
Photocycle	ms	10*(2, 6)*	11*(16)*	7*(16)*
Maximum rate (Vm)	e^- or H^+ RC^{-1} s^{-1}	100	87*(16)*	140*(16)*
Half-saturation (Km)	µmol photons m^{-2} s^{-1}	2700*(16, 50)*	191*(16)*	40*(16)*
Light intensity	µmol photons m^{-2} s^{-1}	Obtained from the field data		

Cellular daily energy yield $= \frac{kJ}{cell*day}$ (kJ $cell^{-1}$ day^{-1})$= \frac{RC}{cell} * V * E^{o} * \frac{1}{T}$

$$V = \frac{e^- or\ H^+}{RC*s} = \frac{Vmax*I}{(Km+I)}$$

Captions for data

Data S1. Detailed sampling measurements and calculations obtained in the Mediterranean Sea and East Atlantic Ocean from 30 April to 28 May 2014. Chl-*a* containing cells were estimated as the addition of the *Prochlorococcus*, *Synechococcus* and picoeukaryote counts measured with flow cytometry. The wavelengths considered for the light intensity data are specified in Table S4. The calculations for "molecules per cell" and "cellular energy yield" used the parameters in Table S5 and assumed that 75% of the total heterotrophic bacteria contained PRs (supplementary materials and methods) and 2.5% contained Bchl-*a (51)*. To account for any retinal signal originating from heliorhodopsin, which could represent ~20% of all rhodopsins in the photic zone *(10)*, 80% of the quantified retinal signal was used in these calculations. For the energy calculations, we also assumed a 12 h daylight period. According to DNA sequence data and the information on their spectral tuning and kinetics *(6, 44)*, most PRs from surface waters have fast photocycles typical of H^+ pumps. Therefore, a photocycle of 10 ms was used for these calculations.