

FigS1: PCR verification of sync1495- and sync1217- mutant. "1495-" or "1217-" indicates PCR of mutant, "wt" indicates PCR of wild type, "neg" indicates negative control PCR in which no DNA was added. In panel A, lanes 1-3 use primers 1495ckfwd and 1495ckrvs (Table S2) flanking the 399-bp region of recombination such that in wild type a 786-bp product is expected. In the mutant, this 786-bp wild-type fragment is missing. Lanes 4-6 use primers 1495ckfwd and EcoRI CC which is expected to amplify a 650-bp region in the mutant only as the second primer is on the vector. There should be no amplification in the wild-type as it does not contain the integrated plasmid. Lanes 7-9 shows the results of amplification using primers to another genomic gene, sync\_1539 used for a positive control to show that the mutant DNA is amplifiable. In panel B lanes 1-2 use primers to another genomic gene sync1233 used for a positive control to show the mutant DNA is amplifiable. Panel C is a schematic showing annealing locations of primers on both wild type and mutant chromosomes



FigS2: *Synechococcus* CC9311 wild-type and mutant methyl viologen growth assays. Growth rates in both wild type CC9311 ("wt"), sync1495- and sync1217-. Cells/ml from flow cytometry were ln-transformed and a linear regression was performed on each. Points represent ln cells/ml and lines represent linear regression. Error bars represent one standard deviation between three biological replicates.



Fig.S3: *Synechococcus* CC9311 wild-type and mutant mean cell size. Measurements determined by side scatter measurements from flow cytometry. All represent mean cell size over 12 days of three biological replicates, all are from no-Cu control treatments from copper growth assay experiments. Error bars represent one standard deviation between three biological replicates. \* indicates significant difference from wild type (one sample t-test, p<0.01).



Fig.S4 *Synechococcus* CC9311 wild-type and sync1495- gene expression of putative sync\_1495 operon. (a) shows gene expression wild type CC9311 ("wt") and (b) shows expression of sync1495- mutant. Copper stress was 2 hours (pCu10.1) and control conditions (no Cu added). Expression is relative to a housekeeping gene (RPOC) and error bars represent one standard deviation between three biological replicates.

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Fig.S5 Validation of 1495QPCR primers to amplify sync\_1495 in environmental samples. Alignment of cloned nucleotide sequences from 1495QPCR forward and reverse primers tested on SIO pier DNA sample. Grey boxes highlight resulting amino acid substitutions from SNP.



Fig. S6: Correlation test showing no correlation between sync1495 abundance and Clade I or total *Synechococcus* abundance. Error bars represent one standard deviation between three technical QPCR replicates of each sync1495 and RPOC. Clade I abundance numbers and total *Synechococcus* numbers from Tai et al., 2011.



Fig. S7: QPCR showing temporal distribution of sync\_1494 as a percentage of total Clade I *Synechococcus* abundance (as determined by Clade I *rpoc1* abundance) in surface samples off the Scripps Institution of Oceanography pier monthly in both 2005 and 2007. Error bars represent +/- one SEM between 2 replicate years (2005 and 2007).

Fig. S8: Additional measurements of water column from depth profile in Fig.5.



Fig. S8: Additional measurements of water column from depth profile in Fig.5. Nitrate, temperature and oxygen measurements from CTD casts.

Table S1 Primers used in study

Primer	Primer Sequence (5'-3')	Temp (°C)	Size (bp)	Source
RPOC362F-I	TGA AAG GGA TYC CCA GTT ATG T	59	304	Tai et al., 2009
RPOC665R-I	CCC TTA CTI CCA GCA ATC TC			
1495QPCRfwd	TGG ATT TAC CTT CGC TTT GG	59	254	this work
1495QPCRrvs	AGA TCC CAC CAA ATG TCA GC			
1496QPCRfwd	GCCACGTCTCTACAAGGGAA	56	261	this work
1496QPCRrvs	AGCACCGGTTTCTGGATTGA			
1494QPCRfwd	TGAAGCCATTAGTCCAGAGGAG	56	197	this work
1494QPCRrvs	ATCGGGTCAGCAGCAAAAG			
1493QPCRfwd	TTGAGAAGCATTCTGCACCG	56	464	this work
1493QPCRrvs	CAGTTTTGTTGCTGATCCGG			
1495mutfwd	GGAGGCGGATTGATTGCCAG	60	399	this work
1495mutrvs	GGCAAGAACAGGGCTTGCGC			
1495ckfwd	GCCATCAAGAAAACTCGTTC	65	786 (wt)	this work
1495ckrvs	AAGAAGACATCGTGAGTGAG			
EcoRI CC	GCTTATCGATGATAAGCTGTCAAA	58	650 (1495-)	this work
1217mutfwd	TGTTTCTCCTACAAATCTTT	54	500	this work
1217mutrvs	ATATTGACCCAAAGATGGTT			
1217ckfwd	TCACTAGTGATATAGGCAAT	60	706(wt)	this work
1217ckrvs	ATGCTATTGACGTGCTGTAT			
EcoRI CW	ATAGGCGTATCACGAGGCCCT	58	650 (1217-)	this work

Temp. is the annealing temperature used for amplification. Size is the nucleotide length of the PCR product in base pairs (bp).

Location	Habitat Type	Depth (m)	Date collected	Top Hit from reciprocal blastn (nr)	E-value	Alignment Length	
Western Channel	saline water	2	1/28/08	Syne. sp. CC9311, sync_1495 region	5.00E-71	121	
Botany Bay, Australia	Coastal	2	9/15/09	Syne. sp. CC9311, sync_1495 region	3.93E-31	95	
Station 67-155	Open Ocean, DCM	84.5	10/6/07	Syne. sp. CC9311, sync_1495 region	5.00E-29	44	
Station H3	Coastal	5	10/10/07	Syne. sp. CC9311, sync_1495 region	2.00E-27	36	
Palm Island, Townsville, Australia	Coastal	n/a	7/29/09	Syne. sp. CC9311, sync_1495 region	6.00E-52	44	

Table S2: Hits to sync\_1495 in the CAMERA database