

Supplementary document for:

Patterns and determinants of diversity and community structure of Halophilic Archaea
(Class Halobacteria) in Tunisian endorheic salt lakes and sebkhet systems

Afef Najjari, Mostafa S. Elshahed, Ameer Cherif, and Noha H. Youssef

Table S1. Genera possessing/lacking the otsAB system encoding for trehalose biosynthesis. The list is shown both for those genera that have experimental and/or genomic evidence for trehalose accumulation, as well as for those predicted to harbor the system based on their phylogenetic affiliations.

Genera possessing the OtsAB system		Genera lacking the OtsAB system	
Based on experimental/genomic evidences	Predicted based on phylogenetic affiliations	Based on experimental/genomic evidences	Predicted based on phylogenetic affiliations
<i>Haladaptatus</i>	<i>Haloarchaeobius</i>	<i>Haloarcula</i>	<i>Halapricum</i>
<i>Halalkalicoccus</i>	<i>Halorubellus</i>	<i>Halobacterium</i>	<i>Haloarchaeum</i>
<i>Halobiforma</i>	<i>Halorussus</i>	<i>Halobaculum</i>	<i>Halobellus</i>
<i>Halococcus</i>	<i>Natronoarchaeum</i>	<i>Haloferax</i>	<i>Halolamina</i>
<i>Halogranum</i>	<i>Salinarchaeum</i>	<i>Halomicrobium</i>	<i>Halomarina</i>
<i>Halopiger</i>	<i>Salinirubrum</i>	<i>Haloplanus</i>	<i>Halomicroarcula</i>
<i>Halosarcina</i>		<i>Haloquadratum</i>	<i>Halonotius</i>
<i>Halosimplex</i>		<i>Halorhabdus</i>	<i>Halopelagius</i>
<i>Haloterrigena</i>		<i>Halorubrum</i>	<i>Halopenitus</i>
<i>Halovivax</i>		<i>Natronomonas</i>	<i>Halorientalis</i>
<i>Natrialba</i>			<i>Halovenus</i>
<i>Natrinema</i>			<i>Salarchaeum</i>
<i>Natronobacterium</i>			
<i>Natronococcus</i>			
<i>Natronolimnobius</i>			
<i>Natronorubrum</i>			
<i>Halostagnicola</i>			

Table S2. Percentage abundance of different Halobacteria genera encountered in the samples studied. Sample designation follows Table 1.

	S1	S2	S3	S11	S12	S4	S10	S5	S6	S15	S18	S29	S7	S42	S40	S13	S14	S26	S28	S27	S17	S16	S19
<i>Halorientalis</i>	56.40	34.78	22.93	6.04	32.41	11.54	16.53	62.11	12.11	25.41	33.64	12.20	32.38	27.53	10.57	17.15	24.90	47.87	36.08	42.21	19.89	0	24.97
<i>Halorubrum</i>	4.21	40.06	2.17	35.11	16.07	0.96	6.16	6.34	8.87	3.94	2.73	31.85	8.19	3.30	14.27	4.51	4.02	7.02	5.16	7.04	3.75	50.93	4.86
<i>Halomicrobium</i>	1.13	0	5.01	7.48	2.11	5.77	3.44	1.46	5.10	8.38	8.26	4.37	4.45	2.86	2.56	4.36	0.96	3.51	4.90	4.43	4.73	3.70	5.46
<i>Natronomonas</i>	5.02	0	3.01	2.30	1.27	1.92	2.27	3.04	3.13	7.26	0.56	0.42	2.85	3.30	9.55	2.47	0.77	1.25	1.57	1.63	2.10	0.93	3.53
<i>Halobellus</i>	0.32	0.31	3.31	2.16	0.52	2.88	6.29	3.85	0.43	2.61	1.78	5.22	1.25	2.86	6.48	1.60	4.41	0.75	3.53	2.75	0.38	0.93	3.20
<i>Halolamina</i>	0.97	0	9.18	15.68	1.56	10.58	4.39	0.38	2.28	5.62	1.27	0.47	1.78	0.22	9.95	2.91	0.19	1.25	3.53	1.68	3.53	0	4.06
<i>Haloplanus</i>	2.76	0	2.93	2.59	0.70	0.96	5.98	0.05	3.51	2.04	2.84	3.03	1.96	0.44	0.51	0.44	2.49	2.51	1.44	1.35	1.20	6.48	3.53
<i>Haloferax</i>	3.89	0	1.01	0	0.18	24.04	1.34	0	15.94	0.31	7.04	1.10	2.49	27.09	11.77	2.62	0.77	0.50	2.61	2.52	1.65	0	2.26
<i>Halorhabdus</i>	0.49	3.42	5.92	1.01	1.56	0.96	2.38	0.60	4.30	6.90	3.49	1.80	1.96	3.08	1.14	0.44	0.77	1.75	0.78	2.15	0.53	0	1.66
<i>Halapricum</i>	3.40	0	3.79	1.01	1.54	0	1.58	1.08	0.21	1.12	2.31	0.68	1.96	0	1.02	1.74	0.19	2.26	0.92	1.91	0.15	0	0.13
<i>Halonotius</i>	0.65	2.48	3.41	2.88	0.20	0	8.31	0.49	1.91	1.99	0.87	12.29	1.42	0.44	0.85	5.52	34.48	0.25	0.72	0.79	2.55	0	1.33
<i>Haloquadratum</i>	0	0	0.53	3.02	0	1.92	0.61	3.20	1.17	0.51	0.82	18.63	0.36	2.64	0.17	3.63	0.77	1.00	0.78	1.35	0	0	2.40
<i>Halobacterium</i>	0.65	0	3.16	1.15	0.24	0	0.37	1.41	0	1.33	0.11	0.06	2.31	1.98	0	0.29	0	1.75	0.72	1.03	1.35	0	2.66
<i>Halovenus</i>	1.13	2.80	1.47	0.14	1.01	0.96	2.30	0.49	1.38	0.46	0.98	1.63	1.07	0.22	0.11	0.15	0.77	1.25	1.18	0.70	0.68	0.93	0.33
<i>Halopelagius</i>	0.65	0	0.53	1.44	0.21	0	0.01	2.87	0.32	1.28	1.11	0.38	2.14	0.22	0.17	0.87	0.57	2.01	3.14	3.13	0	0	1.13
<i>Haloarcula</i>	0	0	2.07	2.59	0.15	0.96	0.21	0	0.43	2.04	0.04	0.17	0.53	1.54	5.46	0.29	0.96	0.50	1.24	0.23	1.20	0	0.07
<i>Halomicroarcula</i>	1.62	0	1.69	0.29	0.31	0.96	2.59	0.38	0.53	1.33	0.95	1.12	0.36	2.64	0.45	0.29	0	0.25	0.65	0.65	0	0	0.27
<i>Halopenitus</i>	0	0	0	5.04	0	0	0.55	0	1.22	4.35	0.58	0	0	1.32	0	0.15	0	0.25	0.20	0.23	0	0	0
<i>Salarchaeum</i>	2.11	0	0.20	0	0	0	1.30	0	1.33	0.31	0.11	0	0	0	0	0	0	0	0.13	0.05	0	0	1.46
<i>Halobaculum</i>	0	0.62	0	1.58	0	0	0.10	0	0.74	0.41	0.89	0.09	0.18	1.10	0.34	0.15	0	0.50	0.92	0.28	0.98	0	1.86
<i>Halomarina</i>	0	0	0	4.03	0	2.88	0	0.05	0	0.05	0.16	0.02	0.53	0	0	0	0	0.25	0.65	0.23	0	0	0.67
<i>Haloarchaeum</i>	0	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Halogramum</i>	0.49	0	1.72	3.31	0.05	8.65	0.17	1.68	11.64	3.63	8.50	0.79	7.83	7.27	5.34	9.74	0.57	4.01	9.61	6.81	8.18	21.30	9.39
<i>Halorussus</i>	1.78	0	1.82	0.14	0.31	4.81	0.10	1.90	2.02	5.67	1.60	0.17	2.85	1.10	2.73	2.62	0	3.01	3.07	2.05	6.01	0	1.26
<i>Halobiforma</i>	2.43	0.93	1.72	0.14	0.14	0	0.15	2.01	1.33	1.89	2.73	0.87	1.60	0.88	5.91	0.15	18.77	2.01	1.90	1.03	0.15	0.93	1.46
<i>Halorubellus</i>	4.86	0	0.48	0	3.72	0	0.76	1.14	0.96	0.36	0.16	0.21	1.96	0	0	0.44	0.77	2.26	0.26	1.45	1.13	0	2.46
<i>Halostagnicola</i>	0	0	0.18	0.14	0	2.88	0	0	0	0.58	0.02	1.60	0	0.85	1.45	0	1.50	1.37	1.35	4.58	0.93	1.80	
<i>Salinarchaeum</i>	3.40	0	1.85	0	0.12	1.92	0.04	0.60	1.22	1.43	1.64	0.38	1.07	0.22	0.11	0	0	1.00	0.85	0.75	0.23	0	0.07
<i>Haloterrigena</i>	0	0	0.05	0	0	0.96	1.39	0	0	0	2.04	0.04	2.14	0	0	1.60	0	2.26	1.90	0.75	1.80	0	1.66
<i>Natronorubrum</i>	0	0	0.73	0	0.03	0.96	0.04	1.14	0.96	0.26	1.95	0.02	1.96	0.44	1.65	0.29	0.19	0.75	1.96	1.35	1.73	0	2.06
<i>Salinirubrum</i>	0	0	1.57	0	0.18	0.96	0.05	0	0.96	1.53	3.20	0.40	1.42	0.88	0.06	1.02	0.38	1.00	0.92	1.12	0.38	0	0.80
<i>Haladaptatus</i>	0	0	0.13	0	0	1.92	0	0	0.80	0.05	0.27	0.06	0.53	0	1.71	1.16	0	0.75	1.63	0.47	0.53	0	1.40
<i>Halovivax</i>	0	0	0.18	0.14	0	1.92	0.01	0	0.85	0.20	1.44	0	0.71	0	1.99	0.15	0	1.00	0.65	0.47	0.98	0	1.93
<i>Natronoarchaeum</i>	0.16	0	0.23	0.29	0	0.96	0.02	0	1.43	0.10	0.02	0.02	0	0.22	0.57	0	0.19	0.25	0.20	0.37	1.20	0	1.07
<i>Natronolimnobiis</i>	0	0	0.08	0	0	2.88	0	0	0.05	0.05	0.47	0	0	1.98	0	0	0	0	0.98	0.23	0.45	0	0.67
<i>Halosimplex</i>	0	0	0.33	0	0.29	0	0.12	0.05	1.01	0.26	0.29	0.02	0.36	1.32	0	0.44	0.19	0	0.20	0.42	0.23	0	0.40

<i>Natronococcus</i>	0	0	0.13	0	0	0.96	0.16	1.57	0.37	0.46	0.11	0	1.07	0	0.11	0.44	0	0.50	0.52	0.33	0.23	0	0.73
<i>Halosarcina</i>	0.16	0	0	0	0	0	0	0	7.07	0	0.04	0.02	0	0	0	0.15	0	0	0	0.09	0	0	0.13
<i>Natrinema</i>	0	0	0.05	0	0	0	0	0	0.37	0.66	1.00	0	0.36	0	0.40	0.44	0	0.25	0.13	0.33	0.30	0.93	1.33
<i>Halalkalicoccus</i>	0	0	0.05	0	0	0	0	0	0	0	0	0	0.18	0	0	0	0	0	0.52	0.23	0.08	0	0.40
<i>Haloarchaeobius</i>	0	0	0.05	0	0	0	0	0	0	0.05	0.02	0	0.18	0	0	0.15	0	0.25	0.13	0	0.23	0	0
<i>Halococcus</i>	0	0	0.25	0	0.02	0	0	0.05	0.48	0	0.02	0	0	0	0	0	0	0.25	0	0.09	0	0	0
<i>Halopiger</i>	0	0	0.18	0	0	0	0	0	0	0	0.09	0	0	0	0	0	0	0.25	0	0.09	0.08	0	0.07
<i>Natralba</i>	0	0	0	0	0	0	0	0	0.77	0.09	0.02	0	0	0	0	0	0	0	0.13	0	0.08	0	0
<i>Natronobacterium</i>	0	0	0	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0
Others ^a	1.30	14.60	15.88	0.29	35.10	2.88	30.31	2.06	3.56	4.96	3.17	1.46	6.05	2.86	3.18	30.23	1.92	2.01	2.22	3.87	26.80	12.04	5.06

a: “others” refers to the sequences that were unaffiliated (<94% similar) with any of the genera in the Halobacteria curated database used for phylogenetic classification.

Table S3. Assignment of genera to the 6 groups discussed in text. Description of groups is shown in the table footnote*.

	Number of datasets the genus is encountered in	% of datasets where the genus represented			
		>10%	5-10%	1-5%	<1%
Group 1: Consistently abundant genera					
<i>Halorientalis</i>	22	95.45	4.55	0	0
<i>Halorubrum</i>	23	26.09	43.48	26.09	4.35
<i>Halogramum</i>	22	18.18	36.36	22.73	22.73
Group 2: Moderately abundant genera					
<i>Halonotius</i>	21	14.29	4.76	38.10	42.86
<i>Haloferax</i>	19	21.05	5.26	52.63	21.05
<i>Halobiforma</i>	22	4.55	4.55	59.09	31.82
<i>Haloquadratum</i>	18	5.56	5.56	44.44	44.44
<i>Halolamina</i>	21	19.05	9.52	47.62	23.81
Group 3: Genera with occasional moderate abundance					
<i>Natronomonas</i>	22	0	13.64	72.73	13.64
<i>Halobellus</i>	23	0	13.04	60.87	26.09
<i>Halomicrobium</i>	22	0	45.45	50	4.55
<i>Haloplanus</i>	22	0	9.09	68.18	22.73
<i>Halorussus</i>	20	0	10	70	20
<i>Halorhabdus</i>	22	0	9.09	59.09	31.82
<i>Halostagnicola</i>	14	0	7.14	57.14	35.71
<i>Halorubellus</i>	17	0	5.88	47.06	47.06
Group 4: Consistently low-abundance genera					
<i>Halobacterium</i>	17	0	0	58.82	41.18
<i>Haloterrigena</i>	12	0	0	66.67	33.33
<i>Halovenus</i>	23	0	0	52.17	47.83
<i>Halopelagius</i>	19	0	0	52.63	47.37
<i>Halapricum</i>	19	0	0	68.42	31.58
Group 5: Genera with occasional low abundance					
<i>Natronorubrum</i>	19	0	0	42.11	57.89
<i>Salarchaeum</i>	9	0	0	44.44	55.56
<i>Halovivax</i>	15	0	0	40	60
<i>Haladaptatus</i>	14	0	0	35.71	64.29
<i>Halobaculum</i>	16	0	0	25	75
<i>Natronoarchaeum</i>	17	0	0	17.65	82.35
<i>Natronococcus</i>	15	0	0	13.33	86.67
<i>Salinarchaeum</i>	18	0	0	44.44	55.56
<i>Salinirubrum</i>	18	0	0	38.89	61.11
<i>Natrinema</i>	13	0	0	23.08	76.92
<i>Natronolimnobi</i>	10	0	0	30	70

<i>Halomarina</i>	11	0	0	18.18	81.82
<i>Halosimplex</i>	16	0	0	12.50	87.50
<i>Halomicroarcula</i>	19	0	0	31.58	68.42
Group 6: Consistently rare genera					
<i>Natrialba</i>	5	0	0	0	100
<i>Halalkalicoccus</i>	6	0	0	0	100
<i>Halococcus</i>	7	0	0	0	100
<i>Haloarchaeobius</i>	8	0	0	0	100
<i>Halopiger</i>	6	0	0	0	100
<i>Natronobacterium</i>	1	0	0	0	100
<i>Haloarchaeum</i>	1	0	0	0	100

Group 1: Genera that exceeded 10% in a few samples, exceeded 5% in the majority of samples

Group 2: Genera that exceeded 10% in just a few samples (1-4), exceeded 5% in a few samples (1-2), the majority between 1-5%

Group 3: Genera that never exceeded 10% in any sample, exceeded 5% in a few samples (1-2), the majority between 1-5%

Group 4: Genera that never exceeded 5% in any sample, the majority between 1-5%

Group 5: Genera that never exceeded 5% in any sample, the majority <1%

Group 6: Genera that were only present in a few samples, and were always <1%

*: The genera *Halosarcina*, *Halopenitus*, and *Haloarcula* did not satisfy the criteria for any of the above groups and so are not shown in the table. *Halosarcina* was encountered in 7 datasets, and its percentage abundance was 5-10% of the total Halobacteria community in 14.3% of the datasets, and <1% in 85.7% of the datasets. *Halopenitus* was encountered in 10 datasets, and its percentage abundance was 5-10% of the total Halobacteria community in 10% of the datasets, 1-5% in 30% of the datasets, and <1% in 60% of the datasets. *Haloarcula* was encountered in 19 datasets, and its percentage abundance was 5-10% of the total Halobacteria community in 5.3% of the datasets, 1-5% in 31.6% of the datasets, and <1% in 63.1% of the datasets.

Table S4. Classification of Halobacteriagenera into salinity-sensitive and salinity-indifferent. Pearson correlation coefficients are shown for the correlations between genera percentage abundance and sample salinity.

Genus	Pearson correlation coefficient	Genus	Pearson correlation coefficient
<i>Salinity-sensitive genera</i>		<i>Salinity-indifferent genera</i>	
1. Positive correlations			
<i>Halobellus</i>	0.70	<i>Halorientalis</i>	0.12
<i>Haloferax</i>	0.90	<i>Halorubrum</i>	0.16
<i>Halorhabdus</i>	0.62	<i>Halomicrobium</i>	-0.22
<i>Halapricum</i>	0.53	<i>Natronomonas</i>	-0.02
<i>Halovenus</i>	0.64	<i>Halolamina</i>	0.19
<i>Halomicroarcula</i>	0.65	<i>Haloplanus</i>	-0.06
2. Negative correlations		<i>Halonotius</i>	-0.11
<i>Halogram</i>	-0.82	<i>Haloquadratum</i>	-0.09
<i>Halorussus</i>	-0.86	<i>Halobacterium</i>	0.06
<i>Halobiforma</i>	-0.48	<i>Halopelagius</i>	-0.30
<i>Halostagnicola</i>	-0.79	<i>Haloarcula</i>	0.02
<i>Haloterrigena</i>	-0.84	<i>Halorubellus</i>	0.19
<i>Natronorubrum</i>	-0.97	<i>Salinarchaeum</i>	0.19
<i>Haladaptatus</i>	-0.73	<i>Salinirubrum</i>	-0.21
<i>Halovivax</i>	-0.88		

Supplementary figure legends

Figure S1. Map of Tunisia depicting sampling sites. Chott El-Djerid sampling site is shown in red circles (10 samples), Sebkheth Douz sampling site is shown in blue square (7 samples), Sebkheth El-Melah sampling site is shown in green triangle (2 samples), and Chott El-Fejej sampling site is shown in brown diamond (4 samples).

Figure S2. Effect of salinity on sample diversity in each sampling site. Rarefaction curves at both 97% (\square), and 94% (\diamond) were used to rank the samples diversity. Samples whose rarefaction curves lie on top are considered more diverse than samples whose rarefaction curves lie on bottom. Samples were given diversity rankings starting from the least diverse (rank 1) to the most diverse (rank 23). The same diversity rank was given to samples whose rarefaction curves intersected. Diversity rank of each sample (Y-axis) was plotted against the sample salinity (X-axis). Diversity rankings are shown for samples from Chott El-Djerid (A), Sebkheth Douz (B), and Chott El-Fejej (C).

Figure S3. Effect of salinity on sample diversity in sediment samples. Rarefaction curves at both 97% (\square), and 94% (\diamond) were used to rank the samples diversity. Diversity rankings are shown for all sediment samples from all sampling sites. While comparing diversity ranking to salinity was not possible for the salt crust samples as they all have the same salinity (~37%), those samples (n=5) showed the lowest level of diversity (average rarefaction rank=3.4 \pm 2 at the species level, and 3 \pm 1.4 at the genus level), which was probably a function of the saturated salinity. The diversity ranks for the salt crust samples were significantly lower than those for the sediment samples (Student T-test p-value=0.0001 at the species level, and 0.00008 at the genus level). Comparing diversity ranking to salinity was not possible for either the water or the biofilm samples due to the

small number of samples representing these two categories (n=1 for biofilm, and n=2 for water samples). However, the water samples in the dataset had higher rarefaction diversity rank (average rarefaction rank=7.8±5.3 at the species level, and 10.5±6.4 at the genus level) compared to the salt crust samples, albeit those values were only significantly higher at the genus level (Student T-test p-value=0.034).

Figure S4. Non-metric multidimensional scaling based on pairwise Bray-Curtis dissimilarity indices to highlight putative groupings by physical characteristics and geographical locations. Each symbol represents one sample. Samples don't seem to be grouped by their physical characteristics (A), or geographical location (B). Symbols in (A) (◇) sediment samples, (□) water samples, (○) salt crust samples, (△) biofilm. Symbols in (B) (◇) Chott El-Djerid, (□) Sebkhet Douz, (○) Sebkhet El-Melah, and (△) Chott El-Fejej samples.

Figure S5. Effect of sample salinity on the abundance of trehalose-producing genera in individual datasets. The sum of the percentage abundances of genera possessing OtsAB system based on either experimental/ genomic evidences (□), or predicted based on phylogenetic affiliations (◇) were plotted on the Y-axis versus salinity on the X-axis.

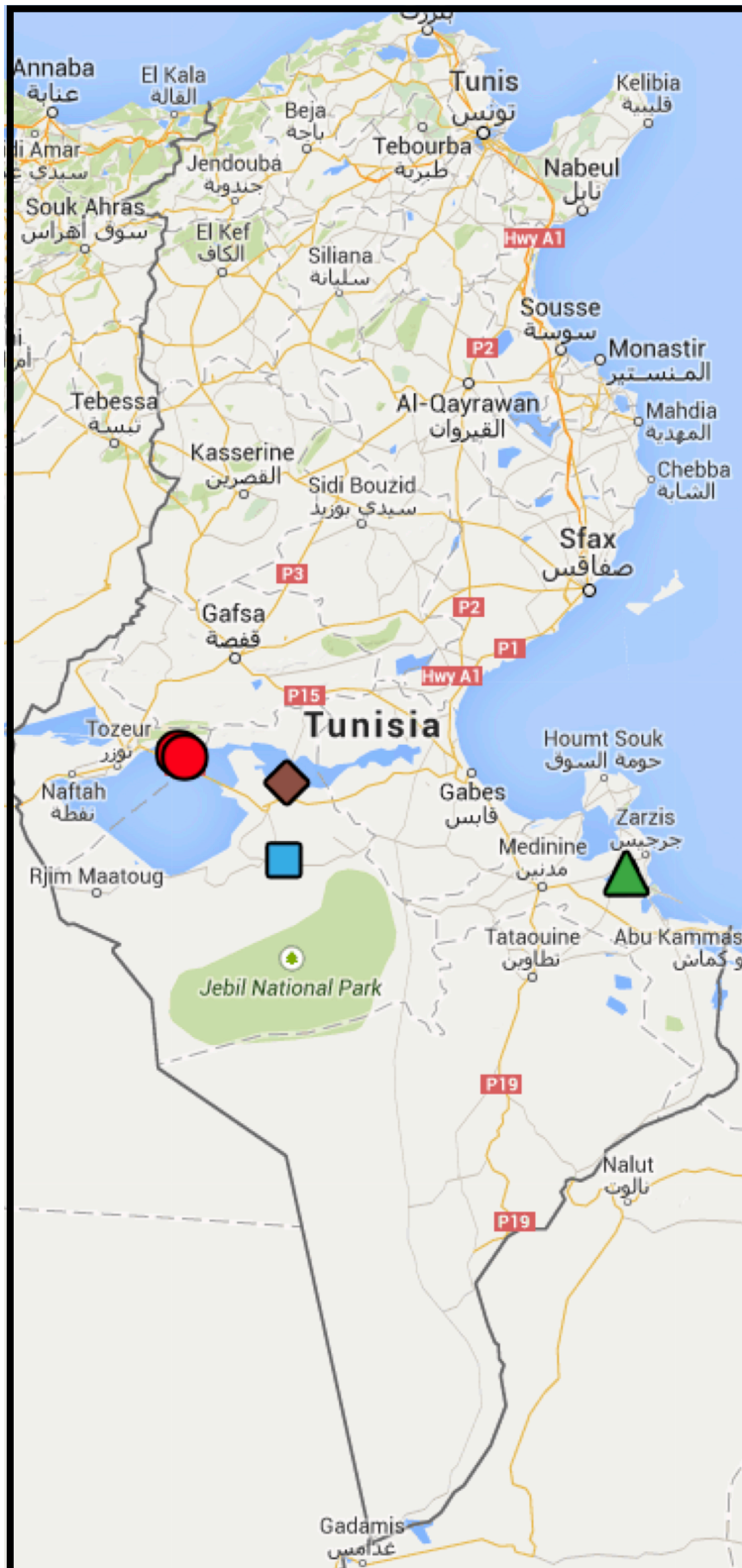


Figure S1.

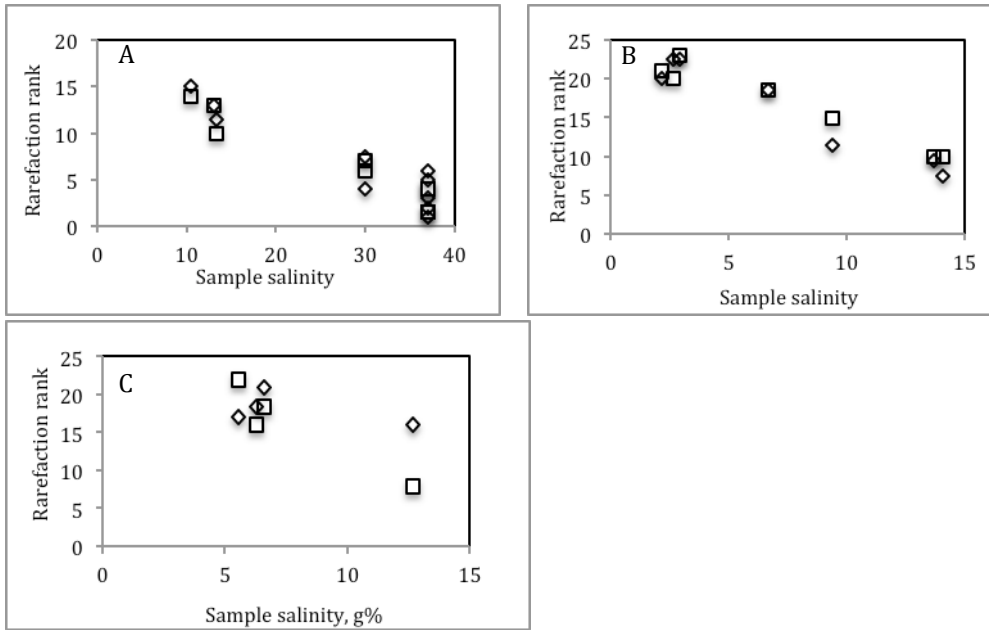


Figure S2

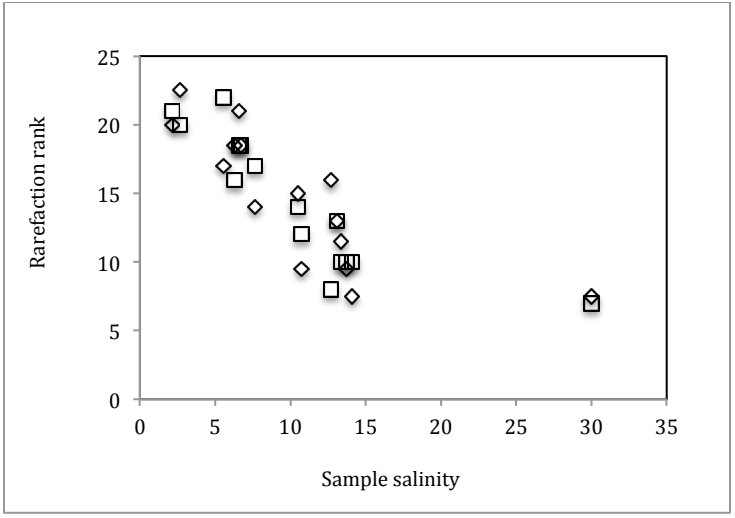


Figure S3

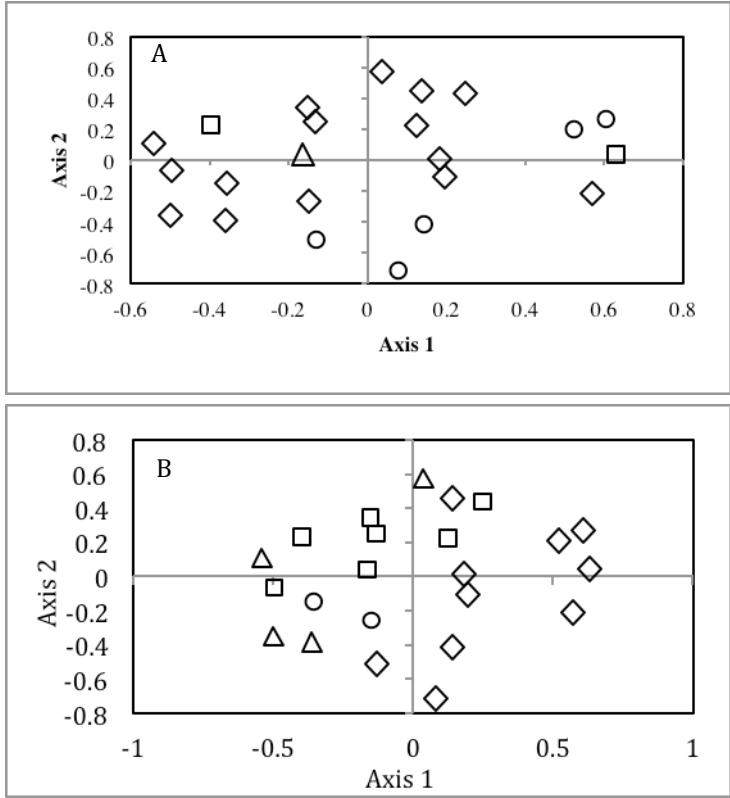


Figure S4.

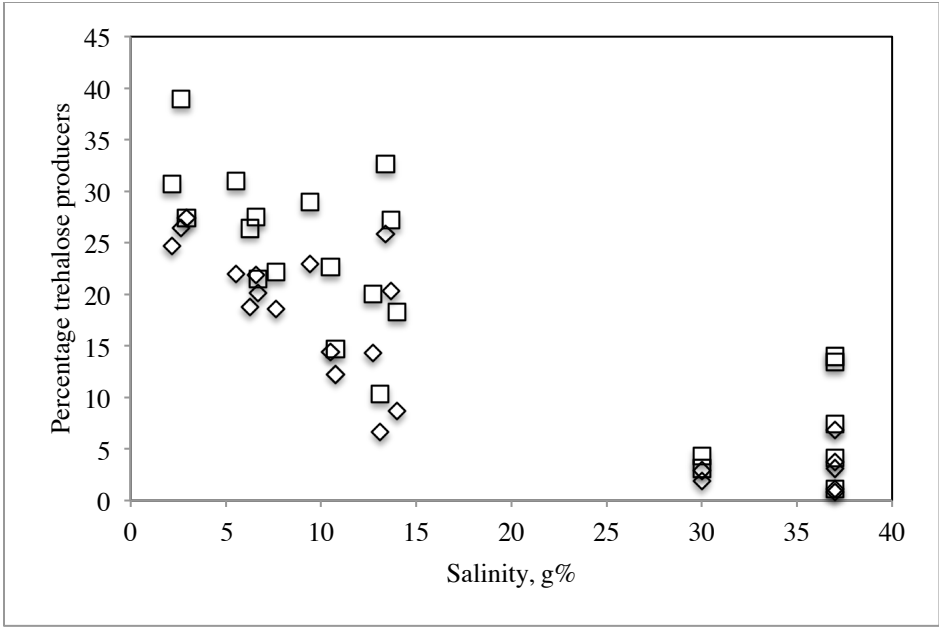


Figure S5.