

Using stable carbon and nitrogen isotopes to investigate the impact of desalination brine discharge on marine food webs

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Supplementary Information

page

2 Table S1. Stable isotope analysis full summary.

Full statistics results:

- 7 Table S2. PerMANOVA pairwise comparison among guild groups.
- 7 Table S3. Results of 3-way Permutation ANOVA for consumer TP.
- 7 Table S4. Permutation ANOVA pairwise comparison among consumer guild groups.
- 7 Table S5. Results of 3-way PerMANOVA main tests for phytoplankton sub-groups.
- 8 Table S6. Results of 3-way PerMANOVA main tests for phytoplankton sampling season.
- 8 Table S7. Results of 3-way PerMANOVA main tests for zooplankton sub-groups.
- 8 Table S8. Results of 3-way PerMANOVA main tests for zooplankton sampling season.

Table S1. Stable isotope analysis full summary. Comparison between Hadera and Ashqelon, and "impacted" brine (B) and "control" (C) among the examined functional groups of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, trophic position (TP), and C:N ratio (mean $\pm$ SD). Three-way pairwise PerMANOVA F and *p*-values are presented between sites (right columns) and between impact stations (a row after the mean values of stations) for each guild.

Functional group	Ashqelon							Hadera							PerMANOVA	
	B/C	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$ corr.	TP	C:N	n	B/C	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$ corr.	TP	C:N	n	F	<i>p</i>
Benthic primary producers (algae)																
	B	5.00	-11.90			8.55		B	5.30	-14.40			15.1			
	C	9.00	-13.40			5.41		C	4.00	-15.50			13.5			
								C	2.20	-7.60			14.6			
Avg. Algae B		5.00	-11.90			8.55	1		5.30	-14.40			15.1	1	-	-
Avg. Algae C		9.00	-13.40			5.41	1		3.1 $\pm$ 1.27	-11.5 $\pm$ 5.58			14.1 $\pm$ 0.75	2	1.08	0.66
B vs. C	-							F=0.46, <i>p</i> =0.66								
Avg. Algae		7.00 $\pm$ 2.82	-12.6 $\pm$ 1.06			6.98 $\pm$ 2.22	2		3.80 $\pm$ 1.5	-12.5 $\pm$ 4.27			14.4 $\pm$ 0.79	3	1.01	0.60
Benthic invertebrate																
Bryozoa	B	5.80	-5.30		2.49	21.71		C	2.50	-2.50		1.52	42.63			
Bryozoa	C	5.80	-3.60		2.51	31.60		C	2.20	-3.50		1.45	33.45			
<i>Phallusia nigra</i>	B	4.90	-19.90	-18.16	2.23	5.12										
<i>Phallusia nigra</i>	C	4.30	-19.50	-18.32	2.06	4.51		C	2.20	-19.80	-17.82	1.45	5.32			
<i>Littorina</i>	B	4.20	-6.20		2.04	8.40		B	4.10	-3.16		1.99	11.09			
Barnacles	B	9.10	-17.40	-16.78	3.46	4.03		C	6.10	-18.70	-24.38	2.48	4.03			
Oyster	B	7.30	-10.50	-6.08	2.95	7.85		C	6.71	-11.29	-7.64	2.77	7.03			
Worm	B	6.00	-5.30	7.99	2.56	16.82		C	6.70	-11.30	-18.59	2.59	3.43			
<i>Conomurex persicus</i>	C	6.40	-14.80	-14.80	2.67	3.36										
<i>Murex trunculus</i>	C	9.40	-15.50	-14.97	3.56	3.84										
<i>Oculina patagonica</i>	C	7.20	-7.10		2.92	18.01										
Shrimp	C	9.00	-15.90	-15.15	3.44	4.11										
Small shell								C	4.40	-19.60		2.10	3.37			
Hydrozoa								C	2.50	-19.70		1.53	3.73			
Sponge								C	3.50	-4.83		1.82	18.18			

Sponge							C	4.16	-19.36		2.02	3.34			
Sponge							C	1.14	-18.92		1.13	4.22			
Avg. Benthic invertebrates B	6.21±1.76	-10.76±6.45	2.62±0.5	10.7±7.03	6			4.10	-3.16		1.99	11.09	1	2.44	0.12
Avg. Benthic invertebrates C	7.01±1.94	-12.73±6.04	2.86±0.6	10.9±11.6	6			3.54±1.8	-13.81±7.5		2.21±0.6	11.7±13.8	10	2.48	0.11
B vs. C	F=0.41, p=0.59						F=1.61, p=0.86								
Avg. Benthic invertebrates	6.60±1.81	-11.7±6.04	2.74±0.5	10.8±9.14	12			3.6±1.71	-12.85±7.8		1.90±0.5	11.7±13.2	11	3.41	0.07
Phytoplankton															
Microphytoplankton (Apr 2017)	B	4.93	-19.99		8.02		B	3.39	-22.04			10.95			
Microphytoplankton (Apr 2017)	B	5.03	-20.40		8.18		B	3.79	-19.74			9.86			
Avg. Micro- (Apr 2017) B	4.98±0.07	-20.2±0.28		8.10±0.1	2			3.6±0.28	-20.89±1.6			10.4±0.7	2		
Microphytoplankton (Apr 2017)	C	5.03	-21.75		7.74		C	4.26	-22.79			8.51			
Microphytoplankton (Apr 2017)	C	4.90	-21.28		7.46		C	4.36	-23.37			12.04			
Avg. Micro- (Apr 2017) C	4.96±0.09	-21.51±0.3		7.60±0.1	2			4.31±0.0	-23.08±0.4			10.3±2.5	2		
Microphytoplankton (Jan 2018)	B	3.35	-20.02		11.91		B	3.40	-14.50			12.36			
Microphytoplankton (Jan 2018)	B	3.17	-19.46		11.65		B	6.20	-15.50			10.08			
Avg. Micro- (Jan 2018) B	3.26±0.12	-19.74±0.4		11.8±0.2	2			4.80±1.9	-15±0.70			11.2±1.6	2		
Microphytoplankton (Jan 2018)	C	1.90	-18.93		12.26		C	3.10	-15.50			12.79			
Microphytoplankton (Jan 2018)	C	1.70	-21.37		12.53		C	4.80	-17.10			12.39			
Avg. Microphytoplankton (Jan 2018) C	1.8±0.14	-20.15±1.7		11.3±1.8	2			3.95±1.2	-16.3±1.13			12.6±0.2	2		
Avg. Microphytoplankton B	4.12±0.99	-19.96±0.4		9.9±2.12	4			4.2±1.24	-17.94±3.5			10.8±1.1	4	1.20	0.48
Avg. Microphytoplankton C	3.38±1.82	-20.83±1.3		10±2.77	4			4.13±0.7	-19.7±3.97			11.4±2.0	4	1.55	0.42
B vs. C	F=1.10, p=0.36						F=0.27, p=0.66								
Avg. Microphytoplankton	3.75±1.41	-20.4±0.99		10±2.28	8			4.16±1.0	-18.81±3.6			11.1±1.52	8	2.74	0.09
Nanophytoplankton (Apr 2017)	B	3.31	-22.33		5.07		B	3.39	-24.35			5.25			
Nanophytoplankton (Apr 2017)	C	3.64	-23.18		5.38		C	3.04	-24.31			5.01			
Nanophytoplankton (Jan 2018)	C	2.47	-23.90		12.18		B	2.51	-22.81			12.66			
Avg. Nanophytoplankton B	3.31	-22.33		5.07	1			2.95±0.6	-23.58±1.1			9.0±5.23	2	0.19	1.00
Avg. Nanophytoplankton C	3.05±0.82	-23.54±0.5		8.8±4.80	2			3.04	-24.31			5.01	1	0.15	1.00
B vs. C	F=0.48, p=0.66						F= 0.08 p=1								

Avg. Nanophytoplankton		3.14±0.60	-23.13±0.8		7.5±4.01	3		2.98±0.4	-23.8±0.87		7.6±4.35	3	-0.11	1.00
Avg. Phytoplankton B		3.96±0.93	-20.44±1.1		9.0±2.85	5		3.78±1.2	-19.82±4.0		10.2±2.67	6	0.36	0.65
Avg. Phytoplankton C		3.02±1.47	-17.57±1.73		9.6±3.10	6		3.9±0.79	-20.61±4.0		10.1±33.4	5	1.58	0.28
B vs. C	F=1.50, p=0.22							F=0.05, p=0.89						
Avg. Phytoplankton B		3.58±1.25	-21.1±1.56		9.3±2.86	11		3.84±1.0	-20.18±3.8		10.2±2.86	11	1.81	0.18
Zooplankton														
Planktonic crustacean (Jan 2017)	B	4.44	-16.43	-16.40	2.10	3.38		B	4.62	-16.83	-16.69	2.15	3.49	
	C	4.54	-19.92	-19.61	2.13	3.67								
	C	6.62	-19.22	-18.95	2.74	3.63								
Avg. Planktonic crustacean (Jan 2017) C		5.58±1.47	-19.57±0.5		2.43±0.4	3.65±0.0	2							
Planktonic crustacean (Apr 2017)	B	5.59	-15.10	-14.27	2.44	4.19								
	C	2.89	-17.19	-15.54	1.64	5.02								
Planktonic crustacean (Sep 2017)								B	7.23	-19.34	-19.36	2.92	3.33	
								C	6.35	-19.89	-20.08	2.66	3.16	
								C	6.48	-19.74	-19.74	2.70	3.36	
								C	6.97	-19.68	-19.37	2.84	3.66	
Avg. Planktonic crustacean (Sep 2017) C									6.6±0.32	-19.77±0.1		2.73±0.1	3.4±0.25	3
Planktonic crustacean (Jan 2018)	B	4.73	-19.99	-19.48	2.19	3.87		B	5.16	-20.56	-19.58	2.31	4.35	
	C	4.28	-21.31	-20.64	2.05	4.04		C	4.16	-22.10	-21.39	2.02	4.08	
								C	3.98	-22.70	-21.48	1.96	4.58	
Avg. Planktonic crustacean (Jan 2018) C									4.07±0.1	-22.4±0.42		2.0±0.03	4.3±0.35	2
Avg. Planktonic crustacean B		6.23±1.65	-20.15±1.0		3.16±0.4	3.03±0.7	3		5.60±1.3	-18.91±1.9		2.46±0.4	3.7±0.54	3
Avg. Planktonic crustacean C		4.58±1.53	-19.41±1.7		2.5±1.71	4.5±1.27	4		3.53±2.2	-21.13±0.2		2.43±0.4	3.76±0.6	5
B vs. C	F=0.99, p=0.47							F=0.73, p=0.47						
Avg. Planktonic crustacean		5.98±1.52	-17.73±1.4		2.8±0.45	2.9±0.79	7		4.9±2.63	-20.1±2.15		2.44±0.4	3.75±0.5	8
Planktonic invertebrates (Jan 2017)	B	6.61	-19.84		2.74	3.53		B	5.47	-20.86	-20.51	2.40	3.70	
	C	5.44	-18.90	-18.63	2.39	3.63		C	5.88	-20.87	-20.43	2.52	3.80	
Planktonic invertebrates (Apr 2017)	B	5.64	-21.25	-20.45	2.45	4.16		B	8.36	-16.27	-16.18	3.25	3.45	
	C	5.53	-21.58		2.42	3.89		C	3.23	-21.34	-21.12	1.74	3.57	

Planktonic invertebrates (Sep 2017)	C	5.51	-21.53	-20.83	2.41	4.06											
		5.52±0.01	-21.5±0.03		2.4±0.01	3.97±0.1	2										
	B	8.30	-19.37	-18.17	3.24	4.57											
	C	8.24	-18.66		3.22	3.42											
Planktonic invertebrates (Sep 2017) C	C	9.12	-17.98	-17.98	3.48	3.36											
		8.68±0.62	-18.32±0.5		3.34±0.2	3.4±0.04	2										
		6.85±1.34	-20.1±0.97		2.8±0.39	4.09±0.5	3	6.9±2.04	-18.56±3.2		2.83±0.6	3.57±0.2	2	0.25	0.80		
		6.76±1.77	-19.73±1.7		2.78±0.5	3.67±0.3	5	4.55±1.9	-21.1±0.33		2.13±0.5	3.7±0.15	2	6.05	0.08		
B vs. C		F=0.05, p=0.94						F=3.32, p=0.30									
Avg. Planktonic invertebrates		6.79±1.52	-19.88±1.40		2.8±0.44	3.83±0.4	8	5.73±2.1	-19.83±2.4		2.48±0.6	3.6±0.15	4	1.72	0.22		
Chaetognatha (Jan 2017)	C	7.00	-19.44	-19.39	2.85	3.40											
Chaetognatha (Apr 2017)								B	8.07	-19.00	-19.19	3.17	3.16				
								C	7.85	-19.53	-19.45	3.10	3.43				
	C	6.15	-19.77	-20.13	2.60	2.99											
	C	7.05	-21.11		2.87	2.84											
Avg. Chaetognatha (Apr 2017) C		6.60±0.63	-20.4±0.94		2.7±0.18	2.92±0.1	2										
Chaetognatha (Sep 2017)								B	7.20	-18.97	-18.93	2.91	3.40				
								C	7.85	-20.16	-19.89	3.10	3.63				
								C	9.11	-18.45	-18.42	3.47	3.38				
								C	6.79	-19.29	-19.22	2.79	3.43				
Avg. Chaetognatha (Sep 2017) C									7.91±1.16	-19.3±0.85		3.12±0.34	3.48±0.12	3			
Chaetognatha (Jan 2018)	B	8.30	-19.75		3.23	3.19		B	7.81	-19.33	-19.52	3.09	3.16				
	C	7.87	-19.80	-19.58	3.11	3.58		C	6.60	-20.18	-20.13	2.74	3.40				
								C	7.62	-19.61	-19.59	3.03	3.37				
									7.11±0.72	-19.89±0.4		2.89±0.21	3.39±0.02	2			
Avg. Chaetognatha (Jan 2018) C									7.69±0.44	-19.1±0.19		3.06±0.13	3.24±0.13	3	2.51	0.50	
Avg. Chaetognatha B		8.30	-19.75		3.23	3.19	1		7.63±0.9	-19.53±0.64		3.04±0.26	3.44±0.09	6	1.44	0.27	
Avg. Chaetognatha C		7.01±0.70	-20.03±0.7		2.86±0.20	3.20±0.34	4										
B vs. C		F=1.54, p=0.40						F=0.27, p=0.65									
Avg. Chaetognatha		7.27±0.83	-19.97±0.65		2.93±0.24	3.20±0.29	5		7.65±0.74	-19.39±0.56		3.05±0.21	3.37±0.14	9	1.67	0.22	
Jellyfish (Jan 2018) (<i>Rhopilema nomadica</i>)	C	6.96	-21.99	-19.76	2.84	5.61											

Fish larva	C	6.43	-19.48	2.69	3.09								
Avg. Zooplankton B		6.23±1.57	-18.81±2.2	2.6±0.46	3.84±0.5	7	6.74±1.44	-18.89±1.61	2.78±0.42	3.51±0.38	8	0.55	0.58
Avg. Zooplankton C		6.24±1.61	-19.85±1.4	2.6±0.47	3.75±0.7	15	6.37±1.70	-20.27±1.18	2.67±0.50	3.61±0.37	13	0.28	0.73
B vs. C	F=0.57, p=0.58						F=2.21, p=0.15						
Avg. Zooplankton		6.23±1.56	-19.52±1.7	2.63±0.46	3.78±0.65	22	6.51±1.58	-19.74±1.48	2.71±0.46	3.57±0.37	21	0.82	0.90
Cephalopoda*													
<i>Sepia officinalis</i>	C	8.09	-18.31	-18.48	3.17	3.18							
Fish													
Demersal (fish fed) (<i>Epinephelus</i> sp.)	C	10.67	-16.82	-16.97	3.93	3.19							
Demersal (invertebrate fed) (<i>Oblada melanura</i>)	C	10.81	-18.34	-18.58	3.97	3.11							
Demersal (invertebrate fed) (<i>Sparus aurata</i>)	C	12.57	-16.61	-16.83	4.49	3.13							
Avr. Demersal (invertebrate fed)		11.69±1.24	-17.47±1.22	4.23±0.36	3.12±0.01	2							
Herbivore (<i>Siganus</i> sp.)	C	4.20	-9.13	-8.86	2.03	3.63	C	10.70	-16.02	-16.97	3.94	3.22	
Pelagic fish (<i>Scomberomorus commerson</i>)	C	9.90	-17.21	-17.45	3.71	3.11							
Pelagic fish (<i>Caranx crysos</i>)	C	11.82	-19.13	-16.90	4.27	5.61							
Avr. Pelagic fish		10.86±1.35	-18.17±1.35	3.99±0.40	4.36±1.76	2							
Rocky fish (<i>Diplodus</i> sp.)	C	12.16	-16.47	-16.71	4.37	3.11							
Avg. all Fish (all C)		10.03±2.75	-16.50±3.12	3.74±0.80	3.51±0.86	8	10.70	-16.02	3.94	3.22	1	0.05	0.88

*added to Fish group for calculations

Table S2. PerMANOVA pairwise comparison among guild groups.

pairs		pseudo F	R ²	p-value	p-adjusted	sig
Algae	Benthic invertebrate	0.25	<0.01	0.761	1	
Algae	Phytoplankton	15.85	0.38	0.001	0.01	*
Algae	Zooplankton	24.69	0.34	0.001	0.01	*
Algae	Fish	7.09	0.37	0.022	0.22	
Benthic invertebrate	Phytoplankton	15.41	0.26	0.001	0.01	*
Benthic invertebrate	Zooplankton	23.47	0.26	0.001	0.01	*
Benthic invertebrate	Fish	6.25	0.17	0.011	0.11	
Phytoplankton	Zooplankton	25.29	0.28	0.001	0.01	*
Phytoplankton	Fish	32.15	0.52	0.001	0.01	*
Zooplankton	Fish	16.29	0.23	0.001	0.01	*

Significance codes: '*' 0.05, '.' 1.

Table S3. Results of 3-way Permutation ANOVA for consumer TP. the factors: Guild, Site (Hadera or Ashqelon) and Station ("impacted" or "control"), after 10,000 permutations.

Factor	df	SS	MS	Iter	p-value	sig
Guild	2	4.81	2.41	5000	<0.01	**
Station	1	<0.01	<0.01	51	1	
Guild × Station	1	0.01	0.01	51	0.92	
Site	1	<0.01	<0.01	51	1	
Guild × Site	2	2.53	1.26	3717	0.04	*
Station × Site	1	0.14	0.14	51	0.72	
Guild × Station × Site	1	0.01	0.01	51	1	
Residuals	67	21.66	0.32			

Significance codes: '***' 0.01, '**' 0.05, '.' 1.

Table S4. Permutation ANOVA pairwise comparison among consumer guild groups.

pairs		pseudo F	p-value	p-adjusted	sig
Fish	Benthic invertebrate	3.78	<0.01	2.27×10 ⁻⁴	***
Fish	Zooplankton	4.50	<0.01	2.00×10 ⁻⁵	***
Benthic invertebrate	Zooplankton	-1.82	0.06	6.83×10 ⁻²	.

Significance codes: '***' 0.001, '.' 0.1.

Table S5. Results of 3-way PerMANOVA main tests for phytoplankton sub-groups (type), the factors: Phyto type, Site (Hadera or Ashqelon) and Station ("impacted" or "control"), after 10,000 permutations.

Factor	df	SS	MS	pseudo F	R ²	p-value	sig
Phyto type	1	<0.01	<0.01	5.29	0.24	0.01	*
Site	1	<0.01	<0.01	0.60	0.03	0.55	
Station	1	<0.01	<0.01	0.58	0.02	0.58	
Phyto type × Site	1	<0.01	<0.01	0.76	0.03	0.48	
Phyto type × Station	1	<0.01	<0.01	0.06	<0.01	0.95	
Site × Station	1	<0.01	<0.01	0.29	0.01	0.76	
Phyto type × Site × Station	1	<0.01	<0.01	0.05	<0.01	0.96	
Residuals	14	<0.01	<0.01	0.65		0.55	
Total	21	<0.01	1	5.29			

Significance codes: '*' 0.05, '.' 1.

Table S6. Results of 3-way PerMANOVA main tests for phytoplankton sampling season, the factors: Season, Site (Hadera or Ashqelon) and Station ("impacted" or "control"), after 10,000 permutations.

Factor	df	SS	MS	pseudo F	R ²	p-value	sig
Season	1	<0.01	<0.01	8.48	0.25	0.0005	***
Site	1	<0.01	<0.01	0.93	0.03	0.40	
Station	1	<0.01	<0.01	0.88	0.02	0.42	
Season Site	1	<0.01	<0.01	6.78	0.20	0.003	**
Season × Station	1	<0.01	<0.01	0.83	0.02	0.44	
Site × Station	1	<0.01	<0.01	0.61	0.02	0.55	
Season × Site × Station	1	<0.01	<0.01	0.59	0.02	0.56	
Residuals	14	<0.01	<0.01	0.42			
Total	21	<0.01	1				

Significance codes: '***' 0.001, '**' 0.01, ' ' 1.

Table S7. Results of 3-way PerMANOVA main tests for zooplankton sub-groups (type), the factors: Zoo type, Site (Hadera or Ashqelon) and Station ("impacted" or "control"), after 10,000 permutations.

Factor	df	SS	MS	pseudo F	R ²	p-value	sig
Zoo type	4	0.01	<0.01	3.89	0.24	0.02	*
Site	1	<0.01	<0.01	0.42	<0.01	0.61	
Station	1	<0.01	<0.01	2.92	0.05	0.08	.
Zoo type × Site	2	<0.01	<0.01	3.17	0.09	0.03	*
Zoo type × Station	2	<0.01	<0.01	0.48	0.01	0.69	
Site × Station	1	<0.01	<0.01	1.13	0.02	0.30	
Zoo type × Site × Station	2	<0.01	<0.01	2.56	0.08	0.06	.
Residuals	31	<0.01	<0.01	0.49			
Total	44	<0.01	1				

Significance codes: '.', 0.10, '*' 0.05, ' ' 1.

Post-hoc zooplankton sub-group:

Planktonic crustacean vs. Chaetognaths: SS - 17.09, pseudo F - 0.38, p-value - 0.001, p-adjusted - 0.009, sig *

Table S8. Results of 3-way PerMANOVA main tests for zooplankton sampling season, the factors: Season, Site (Hadera or Ashqelon) and Station ("impacted" or "control"), after 10,000 permutations.

Factor	df	SS	MS	pseudo F	R ²	p-value	sig
Season	1	<0.01	<0.01	1.18	0.03	0.29	
Site	1	<0.01	<0.01	<0.01	<0.01	0.99	
Station	1	<0.01	<0.01	1.19	0.03	0.28	
Season × Site	1	<0.01	<0.01	2.40	0.05	0.11	
Season × Station	1	<0.01	<0.01	0.46	0.01	0.59	
Site × Station	1	<0.01	<0.01	0.22	<0.01	0.76	
Season × Site × Station	1	<0.01	<0.01	0.09	<0.01	0.88	
Residuals	37	<0.01	<0.01	0.87			
Total	44	<0.01	1				

Significance codes: ' ' 1.