

Seasonal Trophic Ecology and Diet Shift in the Common Sole *Solea solea* in the Central Adriatic Sea

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Supplementary Table S1. Values of %GSI, %HSI, stomach fullness (%), $\delta^{15}\text{N}$ (‰), $\delta^{13}\text{C}$ (‰), N%, C% and C/N measured seasonally in n=68 specimens of *Solea solea* captured in the Central Adriatic Sea between January and December 2019 for studying their diets.

Season	Size		%GSI	%HSI	Stomach fullness (%)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	N%	C%	C/N
Spring	M	mean	0.39	0.86	0.12	11.3	-18.4	19.41	45.87	2.39
		s.d.	0.24	0.23		0.8	0.5	2.77	2.09	0.25
	L	mean	0.71	1.23	0.17	11.2	-17.3	19.93	44.29	2.24
		s.d.	0.15	0.06		0.2	0.5	1.93	1.93	0.28
Summer	M	mean	0.38	0.85	0.14	11.0	-17.7	20.57	45.67	2.23
		s.d.	0.31	0.23		0.6	0.5	1.64	1.14	0.15
	L	mean	0.72	1.30	0.29	11.4	-17.4	20.49	45.21	2.22
		s.d.	0.37	0.44		0.6	0.5	1.62	2.45	0.16
Autumn	M	mean	0.54	0.93	0.19	11.2	-17.8	20.76	46.02	2.23
		s.d.	0.55	0.20		0.6	0.4	4.23	8.33	0.18
	L	mean	1.41	1.24	0.16	11.4	-17.4	23.82	52.52	2.23
		s.d.	1.06	0.32		0.3	0.2	6.76	13.11	0.25
Winter	M	mean	2.69	1.21	0.25	11.9	-17.7	21.28	46.68	2.20
		s.d.	3.57	0.50		0.5	0.5	1.74	1.84	0.15
	L	mean	5.29	1.52	0.25	12.0	-17.7	21.74	45.78	2.12
		s.d.	3.12	0.47		0.6	0.8	2.28	1.57	0.19

Supplementary Table S2. Results of univariate PERMANOVA main and pairwise tests carried out for the factors “Season” and “Size” on the %GSI, %HSI and stomach fullness values measured in n=477 specimens of *S. solea* captured between January and December 2019 in the Central Adriatic Sea for studying their diets. Pairwise comparisons are conducted on the interaction term “Season×Size” for pairs of level of factor “Season”. Only comparisons between pairs of consecutive seasons are showed. Level ‘M’ of factor ‘Size’= medium size; Level ‘L’ of factor ‘Size’=large size; df=degrees of freedom; MS= mean square; Pseudo-F= statistic F; t=statistic t for pairwise comparisons; Unique perms= number of permutations; p(MC)= probability level after Monte Carlo test; *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$; n.s. = not significant.

%GSI								
Main test for %GSI					Pair-wise comparison for factor "Sea×Size" within level 'M' of factor 'Size'			
Source	df	MS	Pseudo-F	P(MC)	Groups	t	Unique perms	p(MC)
Season	3	37.83	11.47	***	Spring vs summer	0.63	9825	n.s.
Size	1	28.59	8.67	**	Summer vs autumn	1.52	9943	n.s.
Sea×Size	3	6.86	2.08	n.s.	Autumn vs winter	1.77	9830	n.s.
Residuals	303	3.30			Winter vs spring	3.24	9831	**
Total	310				Pair-wise comparison for factor "Sea×Size" within level 'L' of factor 'Size'			
					Groups	t	Unique perms	p(MC)
					Spring vs summer	0.08	9876	n.s.
					Summer vs autumn	3.50	9834	***
					Autumn vs winter	0.00	9836	n.s.
					Winter vs spring	3.31	9840	**
%HSI								
Main test for %HSI					Pair-wise comparison for factor "Sea×Size" within level 'M' of factor 'Size'			
Source	df	MS	Pseudo-F	P(MC)	Groups	t	Unique perms	p(MC)
Season	3	0.88	3.29	*	Spring vs summer	3.95	9838	***
Size	1	4.19	16.60	***	Summer vs autumn	0.03	9832	n.s.
Sea×Size	3	1.38	1.81	n.s.	Autumn vs winter	3.94	9827	***
Residuals	304	76.80			Winter vs spring	1.66	9831	n.s.
Total	311	84.49			Pair-wise comparison for factor "Sea×Size" within level 'L' of factor 'Size'			
					Groups	t	Unique perms	p(MC)
					Spring vs summer	0.64	9844	n.s.

					Summer vs autumn	1.68	9840	n.s.
					Autumn vs winter	0.13	9926	n.s.
					Winter vs spring	0.78	9916	n.s.
Stomach fullness								
Main test for stomach fullness					Pair-wise comparison for factor "Sea×Size" within level 'M' of factor 'Size'			
Source	df	MS	Pseudo-F	P(MC)	Groups	t	Unique perms	p(MC)
Season	3	0.13	0.58	n.s.	Spring vs summer	1.30	9841	n.s.
Size	1	0.01	0.41	n.s.	Summer vs autumn	1.47	9827	n.s.
Sea×Size	3	0.22	0.96	n.s.	Autumn vs winter	0.15	9883	n.s.
Residuals	302	0.23			Winter vs spring	1.93	9894	n.s.
Total	309				Pair-wise comparison for factor "Sea×Size" within level 'L' of factor 'Size'			
					Groups	t	Unique perms	p(MC)
					Spring vs summer	0.21	9840	n.s.
					Summer vs autumn	3.05	9837	**
					Autumn vs winter	1.16	9950	n.s.
					Winter vs spring	0.14	9933	n.s.

Supplementary Table S3. Results of univariate PERMANOVA main and pairwise tests for the factors “Season” and “Size” carried out on the stomach fullness of female and male specimens of *Solea solea* captured between January and December 2019 in the Central Adriatic Sea for studying their diets. df=degrees of freedom; MS= mean square; Pseudo-F= statistic F; p(MC)= probability level after Monte Carlo test; n.s. = not significant.

Stomach fullness									
Main test for stomach fullness of FEMALES					Main test for stomach fullness of MALES				
Source	df	MS	Pseudo-F	P(MC)	Source	df	MS	Pseudo-F	P(MC)
Season	3	0.19	0.84	n.s.	Season	3	0.06	1.19	n.s.
Size	1	0.12	0.51	n.s.	Size	1	0.07	0.15	n.s.
Sea×Size	3	0.11	0.48	n.s.	Sea×Size	2	0.06	1.26	n.s.
Residuals	300	0.23			Residuals	159	0.05		
Total	307				Total	165			

Supplementary Table S4. List of the %W of the taxa found seasonally in the stomach contents of specimens of *Solea solea* captured between January and December 2019 in the Central Adriatic Sea for studying their diets.

	Season					Season			
	Spring	Summer	Autumn	Winter		Spring	Summer	Autumn	Winter
Foraminifera					Sipuncula				
Elphidium	0.04	0.00	0.00	0.21	<i>Phascolosoma stephensoni</i>	10.43	0.00	2.14	0.00
Unid. Foraminifera	0.00	0.00	0.00	0.40	<i>Sipunculus nudus</i>	0.00	0.00	0.00	2.12
					Sipunculidae	0.00	0.00	1.32	1.54
Cnidaria					Arthropoda				
Hydrozoa	0.00	0.00	0.00	0.07	Crustacea				
					Decapoda				
Platyelminthes					<i>Upogebia tipica</i>	0.00	15.21	0.00	0.00
Cestoda	15.36	5.26	9.64	5.52	<i>Munida sp.</i>	0.00	0.00	0.00	0.46
					Crangonidae	0.00	0.00	0.00	0.14
Mollusca					Caridea	0.00	0.00	1.11	0.00
Scaphopoda					Unid. Decapoda	0.00	0.00	2.70	0.19
<i>Antalis inaequicostata</i>	0.00	0.00	0.00	1.57	Amphipoda				
Gastropoda					<i>Leucothoe lilljeborgi</i>	0.00	0.00	0.00	0.26
<i>Fusinus rostratus</i>	0.00	0.00	0.00	0.13	<i>Ampelisca sp.</i>	0.54	7.41	10.27	5.68
<i>Oxynoe olivacea</i>	0.67	0.00	0.00	0.00	Oedicerotidae	0.00	0.00	0.00	0.14
<i>Turritellinella tricarinata</i>	0.07	2.85	0.32	0.00	Gammaridea	0.00	0.00	0.21	0.15
<i>Alvania sp.</i>	0.00	0.00	1.99	0.00	Unid. Amphipoda	1.03	0.00	5.24	0.99
Unid. Gastropoda	0.00	0.16	0.00	0.00	Stomatopoda				
Bivalvia					<i>Squilla mantis</i>	0.75	0.00	1.03	0.00
<i>Peronidia albicans</i>	0.00	0.19	0.00	0.00	Tanaidacea				
<i>Petricola pholadiformis</i>	0.00	0.00	0.00	0.46	<i>Apseudopsis latreillii</i>	0.00	3.90	0.00	1.90
Corbulidae	0.67	0.00	0.00	0.00	<i>Apseudes spinosus</i>	0.00	0.00	0.48	0.41
Pectinidae	0.22	0.47	0.00	0.00	Unid. Tanaidacea	0.00	1.03	0.00	0.00
Veneridae	2.67	0.00	1.07	3.13	Unid. Crustacea	0.67	0.50	1.34	1.05
Unid. Mollusca	8.23	7.08	18.87	26.84					
					Echinodermata				
Anellida					<i>Paracentrotus lividus</i>	0.00	1.41	0.00	0.00
Polychaeta					<i>Holothuria sp.</i>	1.33	0.00	0.00	0.00
<i>Lepidasthenia sp.</i>	0.00	0.00	0.00	1.23	Unid. Echinodermata	2.49	2.92	1.51	1.84
Leanira	0.00	0.00	0.00	0.35					
Aphroditidae	0.61	0.00	0.00	0.00	Osteichthyes				
Nereididae	0.00	8.26	0.00	0.40	Fish fragments	0.45	0.00	2.37	1.39
Phyllodocidae	0.00	0.00	0.00	0.17	Fish scales	5.41	3.97	3.41	6.52
Glyceriformia	0.00	0.00	0.70	0.00	Larvae	11.64	5.76	0.23	5.43
<i>Sternaspis scutata</i>	0.00	0.00	3.89	0.00					
Terebellida	0.00	0.00	0.33	0.00	Other				
Maldanidae	0.00	0.00	0.00	0.20	Shells	6.28	10.36	1.50	12.68
Sabellidae	5.33	0.00	0.00	0.00	Vegetal rests	0.42	1.26	0.48	0.39
Spionidae	0.00	0.00	0.00	0.83	Eggs	0.86	0.00	0.32	2.28
Unid. Polychaeta	22.50	22.00	22.47	10.49	Sand	0.00	0.00	5.06	2.31
					Wood	0.00	0.00	0.00	0.18
					Unidentified material	1.33	0.00	0.00	0.00

Supplementary Table S5. Results of multivariate PERMANOVA main and pairwise tests carried out for the factors “Season” and “Size” on the results obtained with SCA (%W) carried out on specimens of *Solea solea* captured between January and December 2019 in the Central Adriatic Sea for studying their diets. Pairwise comparisons are conducted on the term “Season” and on the term “Size”. Only comparisons between pairs of consecutive seasons are showed. M= medium-size specimens; L= large-size specimens; df=degrees of freedom; MS= mean square; Pseudo-F= statistic F; t=statistic t for pairwise comparisons; Unique perms= number of permutations; p(MC)= probability level after Monte Carlo test; *=p ≤0.05; **=p ≤ 0.01; ***=p ≤ 0.001; n.s. = not significant.

Diet composition (%W)								
Main test for diet composition of <i>S. solea</i> (%W)					Pair-wise comparison for factor "Sea×Size" within level 'M' of factor 'Size'			
Source	df	MS	Pseudo-F	P(MC)	Groups	t	Unique perms	p(MC)
Season	3	4545.3	5.70	***	Spring vs summer	1.91	9949	**
Size	1	5848.5	7.34	***	Summer vs autumn	2.01	9942	**
Sea×Size	3	1299	1.63	n.s.	Autumn vs winter	3.00	9951	***
Residuals	355	797.18			Winter vs spring	2.23	9941	***
Total	362				Pair-wise comparison for factor "Sea×Size" within level 'L' of factor 'Size'			
					Groups	t	Unique perms	p(MC)
					Spring vs summer	0.59	9933	n.s.
					Summer vs autumn	1.11	9930	n.s.
					Autumn vs winter	2.09	9930	***
					Winter vs spring	1.56	9923	*
					Pair-wise comparison for factor "Sea×Size" within levels of factor 'Size'			
					Groups	t	Unique perms	p(MC)
					M vs L in spring	2.56	9945	***
					M vs L in summer	2.25	9944	n.s.
					M vs L in autumn	1.14	9955	n.s.
					M vs L in winter	0.85	9945	***

Supplementary Table S6. Output of SIMPER analysis conducted on the diet composition (%W) of *Solea solea* within each season both considering overall results and differences in the diets of medium- (M) and large- (L) size specimens. SIMPER analysis was conducted on the Bray-Curtis resemblance matrix of transformed biomass data. Only comparisons between consecutive pairs of seasons are showed. Cut-off for low contribution at 60%. Avg. Ab=average abundance; Avg. Sim=average similarity; Contrib %= percentage of variance explained by the explanatory variables; Cum %= cumulative percentage of variance explained by the explanatory variables.

OVERALL RESULTS OF DIET COMPOSITION									
Spring					Spring vs summer				
Average similarity: 21.8 %					Average dissimilarity = 82.2 %				
Taxon	Av.Abund	Av.Sim	Contrib %	Cum %	Taxon	Spring Av.Abund	Summer Av.Abund	Contrib %	Cum %
Fish larvae	0.21	10.08	46.35	46.35	Polychaeta	0.19	0.23	19.82	19.82
Polychaeta	0.19	5.36	24.64	71	Fish larvae	0.21	0.12	17.72	37.53
Summer					Shells	0.1	0.15	13.07	50.61
Average similarity: 16.5 %					Fish scales	0.12	0.08	12.73	63.33
Taxon	Av.Abund	Av.Sim	Contrib %	Cum %	Summer vs autumn				
Polychaeta	0.23	6.22	37.64	37.64	Average dissimilarity = 85.6 %				
Fish larvae	0.12	3.11	18.84	56.48	Summer	Autumn			
Shells	0.15	2.75	16.62	73.09	Taxon	Av.Abund	Av.Abund	Contrib %	Cum %
Autumn					Polychaeta	0.11	0.26	18.3	18.3
Average similarity: 18.3 %					Fish scales	0.18	0.08	13.11	31.41
Taxon	Av.Abund	Av.Sim	Contrib %	Cum %	<i>Ampelisca</i> sp.	0.09	0.13	10.94	42.35
Polychaeta	0.26	12.54	68.56	68.56	Fish larvae	0.15	0.01	9.81	52.16
Winter					Mollusca	0.08	0.07	7.04	59.2
Average similarity: 16.1 %					Amphipoda	0.04	0.04	4.93	64.13
Taxon	Av.Abund	Av.Sim	Contrib %	Cum %	Autumn vs winter				
					Average dissimilarity = 88.9 %				
					Autumn	Winter			

Fish scales	0.18	6.84	42.58	42.58
Fish larvae	0.15	4.37	27.17	69.75
Medium size				
Average similarity: 19.2 %				
Taxon	Av.Abund	Av.Sim	Contrib %	Cum %
Fish larvae	0.18	7.07	36.85	36.85
Polychaeta	0.18	4.69	24.43	61.27
Large size				
Average similarity: 11.5 %				
Taxon	Av.Abund	Av.Sim	Contrib %	Cum %
Polychaeta	0.2	5.93	51.73	51.73
Fish scales	0.1	2.44	21.25	72.98

Taxon	Av.Abund	Av.Abund	Contrib %	Cum %
Polychaeta	0.11	0.26	18.3	18.3
Fish scales	0.18	0.08	13.11	31.41
<i>Ampelisca</i> sp.	0.09	0.13	10.94	42.35
Fish larvae	0.15	0.01	9.81	52.16
Mollusca	0.08	0.07	7.04	59.2
Amphipoda	0.04	0.04	4.93	64.13

Winter vs spring				
Average dissimilarity = 83.4 %				
Taxon	Winter Av.Abund	Spring Av.Abund	Contrib %	Cum %
Fish larvae	0.15	0.21	17.15	17.15
Polychaeta	0.11	0.19	15.45	32.59
Fish scales	0.18	0.12	15.13	47.72
Shells	0.05	0.1	8.37	56.09
<i>Ampelisca</i> sp.	0.09	0.02	6.64	62.74

DIET COMPOSITION OF MEDIUM- AND LARGE-SIZE SPECIMENS				
M vs L in spring				
Average dissimilarity = 89.3 %				
Taxon	M Av.Abund	L Av.Abund	Contrib %	Cum %
Polychaeta	0,16	0,31	24,32	24,32
Fish larvae	0,25	0,02	17,47	41,78
Fish scales	0,12	0,14	14,83	56,61
Shells	0,09	0,13	12,01	68,62

M vs L in summer				
Average dissimilarity = 85.7 %				
Taxon	M Av.Abund	L Av.Abund	Contrib %	Cum %
Polychaeta	0,21	0,26	20,25	20,25
Shells	0,15	0,14	14,14	34,39
Fish larvae	0,16	0,03	12,59	46,98
<i>Ampelisca</i> sp.	0,15	0,07	9,82	56,80
Fish scales	0,10	0,04	9,28	66,08

M vs L in autumn				
Average dissimilarity = 81.1 %				
Taxon	M Av.Abund	L Av.Abund	Contrib %	Cum %
Polychaeta	0,26	0,27	23,49	23,49
<i>Ampelisca</i> sp.	0,15	0,11	14,29	37,78
Fish scales	0,10	0,04	9,98	47,77
Mollusca	0,10	0,03	7,59	55,35
Fish fragments	0,04	0,08	7,11	62,46

M vs L in winter				
Average dissimilarity = 87.9 %				
Taxon	M Av.Abund	L Av.Abund	Contrib %	Cum %
Fish scales	0,20	0,14	14,54	14,54
Fish larvae	0,21	0,02	14,46	29,00
Polychaeta	0,14	0,06	9,44	38,43
Fish eggs	0,03	0,12	9,09	47,52
<i>Ampelisca</i> sp.	0,12	0,04	8,29	55,81
Mollusca	0,07	0,10	7,18	63,00

Supplementary Table S7. Values of seasonal diet diversity observed in n=477 specimens of *Solea solea* captured in the Central Adriatic Sea between January and December 2019 for studying their diets. s.d. = standard deviation.

Season	Diet diversity (H')	
	mean	s.d.
Spring	0.45	0.48
Summer	0.45	0.55
Autumn	0.38	0.42
Winter	0.59	0.51

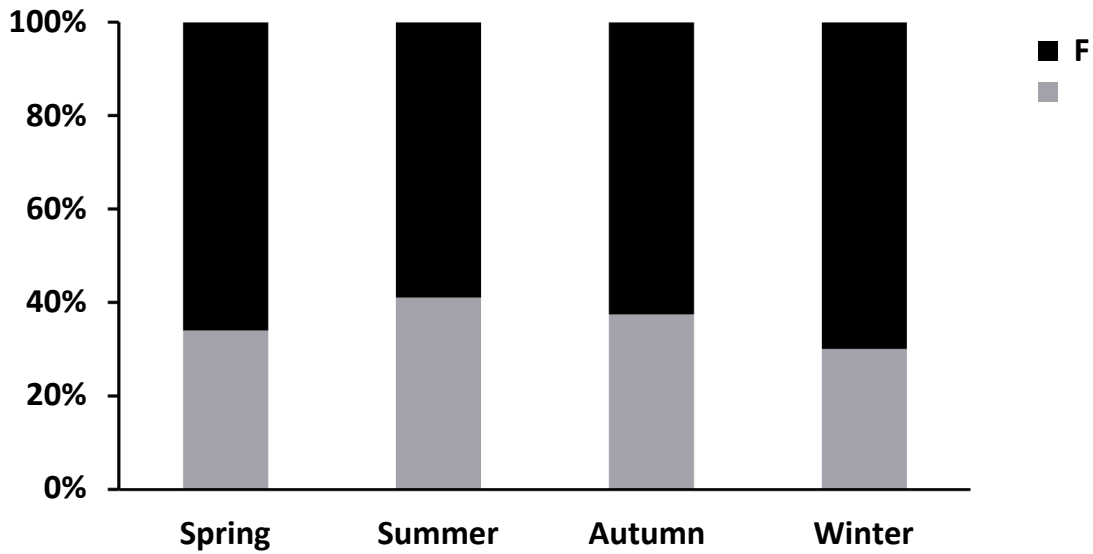
Supplementary Table S8. Results of univariate PERMANOVA main and pairwise tests carried out for the factors “Season” and ‘Size’ on the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N values of n=68 specimens of *Solea solea* captured in the Central Adriatic Sea between January and December 2019 for studying their diets. Pairwise comparisons are conducted on the term “Season” for pairs of level of factor “Season”. Only comparisons between pairs of consecutive seasons are showed. M= medium-size specimens; L= large-size specimens; df=degrees of freedom; MS= mean square; Pseudo-F= statistic F; t=statistic t for pairwise comparisons; Unique perms= number of permutations; p(MC)= probability level after Monte Carlo test; *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$; n.s. = not significant.

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$								
Main test for $\delta^{13}\text{C}$					Pair-wise comparison within pairs of levels of factor 'Season'			
Source	df	MS	Pseudo-F	P(MC)	Groups	t	Unique perms	P(MC)
Season	3	1.68	3.1	*	Spring vs summer	0.93	9947	n.s.
Size	1	2.68	4.9	*	Summer vs autumn	0.27	9950	n.s.
Sea×Size	3	0.53	1.0	n.s.	Autumn vs winter	2.64	9948	**
Residuals	60	0.55			Winter vs spring	2.02	9954	*
Total	67							
					Pair-wise comparison within pairs of levels of factor 'Size'			
					Groups	t	Unique perms	P(MC)
					M vs. L	2.21	9954	*
$\delta^{15}\text{N}$								
Main test for $\delta^{15}\text{N}$					Pair-wise comparison within pairs of levels of factor 'Season'			
Source	df	MS	Pseudo-F	P(MC)	Groups	t	Unique perms	P(MC)
Season	3	1.47	4.84	*	Spring vs summer	0.0	9849	n.s.
Size	1	0.17	0.56	n.s.	Summer vs autumn	0.2	9850	n.s.
Sea×Size	3	0.14	0.47	n.s.	Autumn vs winter	3.5	9853	**
Residuals	60	0.30			Winter vs spring	2.7	9840	*
Total	67							
$\delta^{13}\text{C}$								
Main test for $\delta^{13}\text{C}$								
Source	df	MS	Pseudo-F	P(MC)				
Season	3.0	0.20	0.8	n.s.				

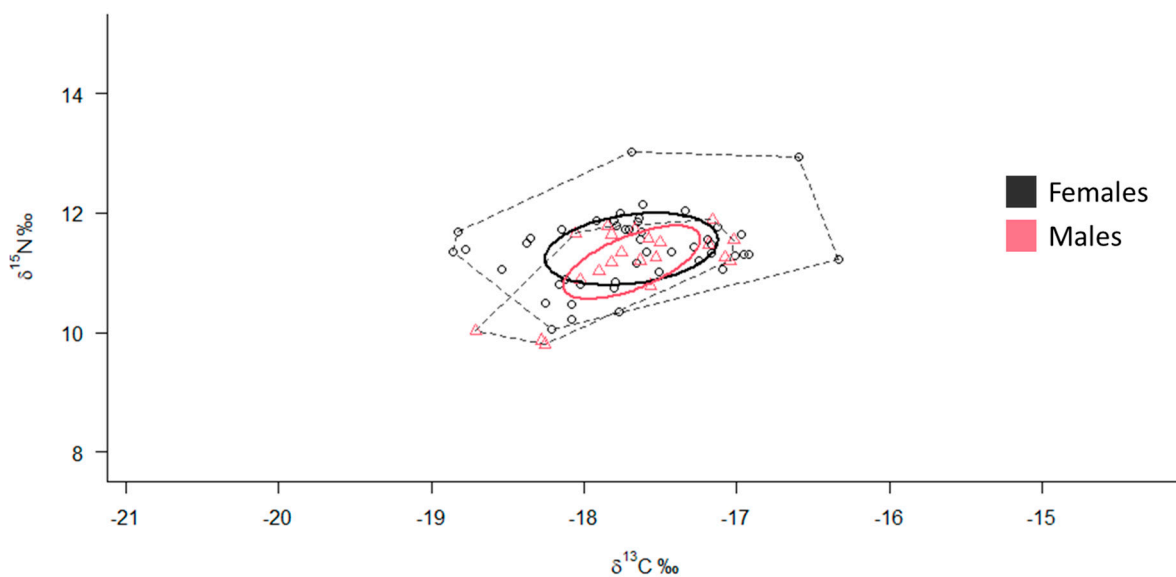
Size	1.0	2.51	1.0	**
Sea×Size	3.0	0.39	1.6	n.s.
Residuals	60.0	0.24		
Total	67.0			
C:N				
Main test for C:N				
Source	df	MS	Pseudo-F	P(MC)
Season	3	0.04	1.1	n.s.
Size	1	0.09	2.7	n.s.
Sea×Size	3	0.01	0.3	n.s.
Residuals	60	0.03		
Total	67			

Supplementary Table S9. Seasonal proportional contribution of each food source to the diet of n=477 specimens of *Solea solea* captured in the Cetral Adriatic Sea between January and December 2019 for studying their diets. SD= standard deviation.

Season	Proportional contribute									
	Pelagic fish		Nephtyidae		<i>Ampelisca</i> sp.		Fish larvae		Cucumariidae	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Spring	0.23	0.13	0.40	0.11	0.11	0.07	0.09	0.06	0.18	0.07
Summer	0.22	0.08	0.38	0.09	0.17	0.08	0.08	0.05	0.15	0.06
Autumn	0.33	0.07	0.28	0.08	0.24	0.07	0.05	0.04	0.10	0.05
Winter	0.31	0.12	0.48	0.09	0.07	0.04	0.05	0.03	0.10	0.04



Supplementary Figure S1. Seasonal percentage of female and male specimens of *Solea solea* (n=477) captured in the Central Adriatic Sea between January and December 2019.



Supplementary Figure S2. $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ scatterplot with standard ellipses corrected for small sample size population (SEAC) overlaid for male (n=20) and female (n=48) specimens of *Solea solea* captured in the Central Adriatic Sea between January and December 2019 (p interval=0.4).