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Supplementary Materials for

Plankton food web structure and productivity under ocean alkalinity enhancement

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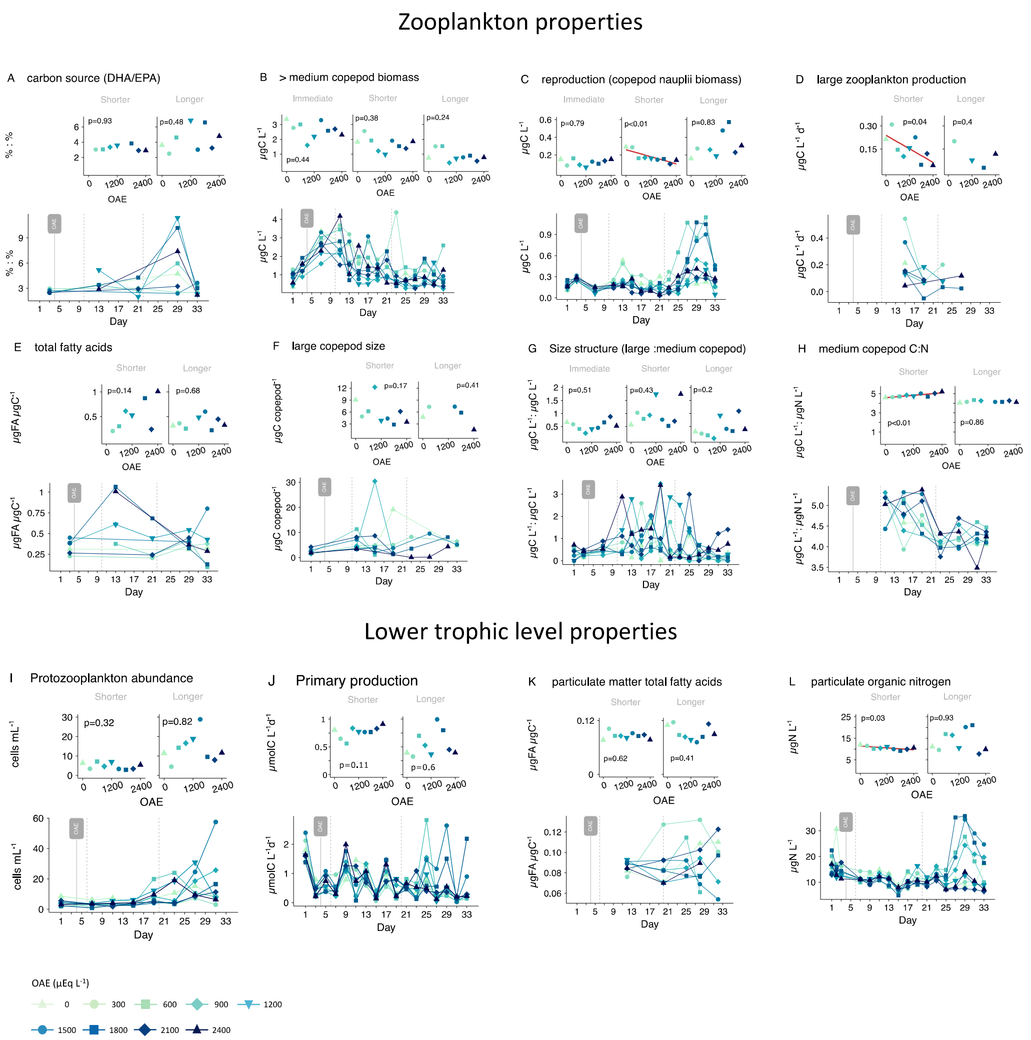

Fig. S1. Continuation of Figure 1.

Continuation of figure 1 reporting key metazoan zooplankton (A-H) and lower trophic level (I-L) responses to OAE. These responses are summarized in tables 1 and 2.

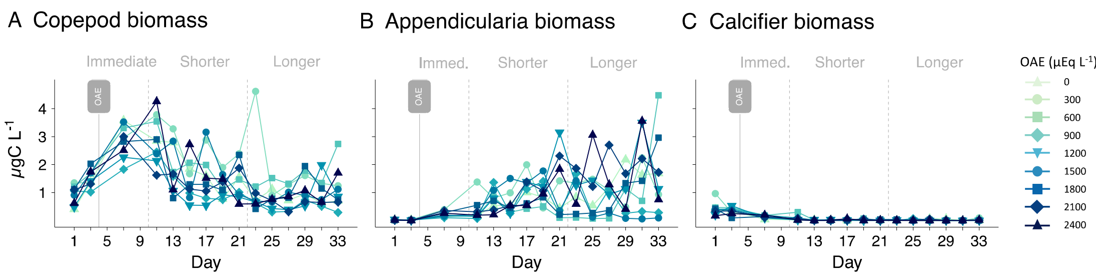

Fig. S2. Metazoan zooplankton biomass development.

Biomass development of key zooplankton groups, across size classes and development, under OAE. In the case of copepods, copepod nauplii are excluded.

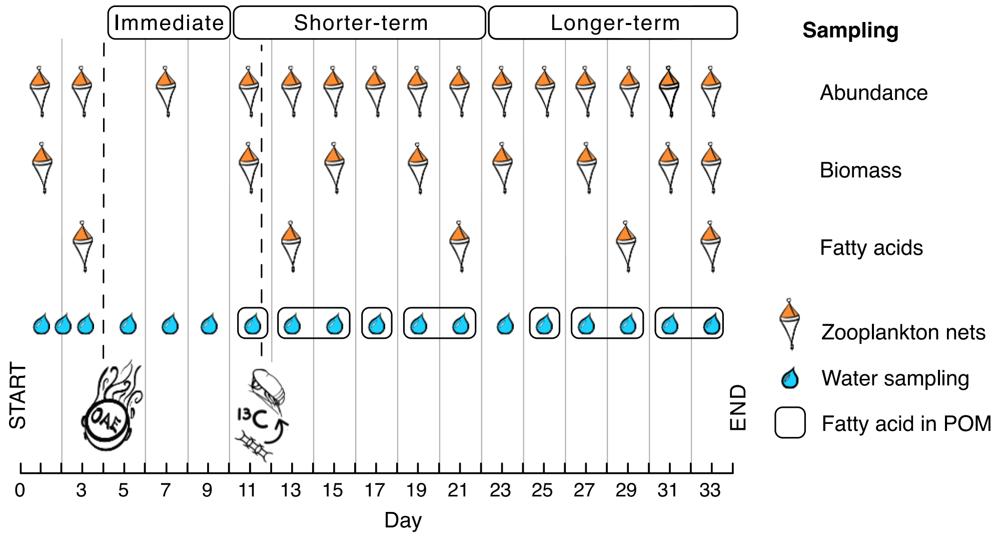

Fig. S3. Experimental schedule.

Experimental schedule including sampling of metazoan zooplankton and the water column, as well as OAE simulation and the enrichment in 13C, used to measure zooplankton production.

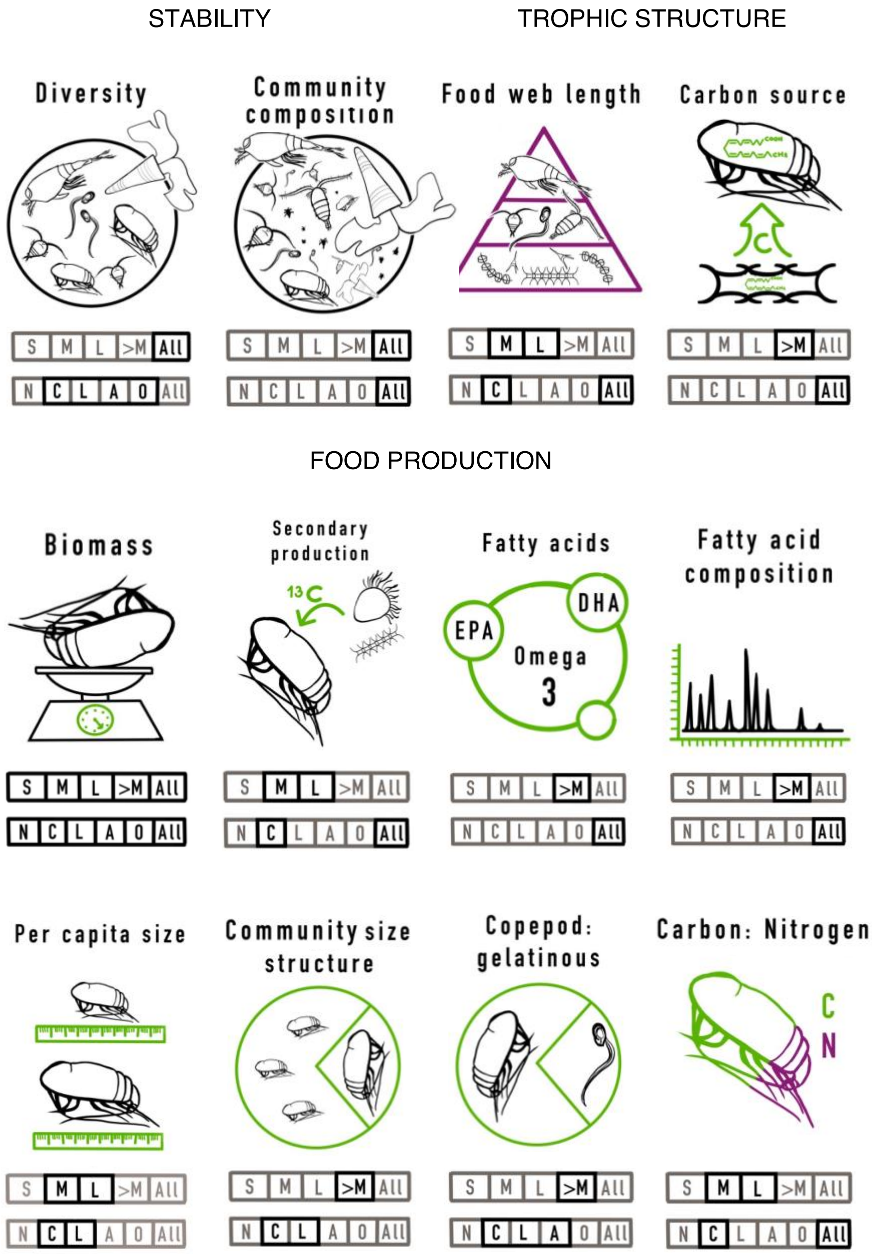

Fig. S4. Metazoan zooplankton samples overview.

Overview of zooplankton properties assessed, organized according to their food web implication. Green = carbon-based measurement, purple = nitrogen-based. Bar pairs underneath each symbol inform about the zooplankton size classes (upper bars) and taxonomic groups (lower bars) on which each property was assessed, indicated in black. When multiple size or taxonomic categories are enclosed together, these were measured together. Every marked taxon was assessed in every marked size class (factorially), except for production, which could only be measured in bulk zooplankton within the large size class, and except for copepod nauplii, which can only belong to the small size class. For the size bar: S = small, M = medium, L = large, >M = medium and large together, All = all. For the taxon bar: N = copepod nauplii, C = copepod and copepodite, excluding *Labidocera*, L = *Labidocera*, A = Appendicularia, O = other zooplankton, All = all.

Table S1. Summary of linear regressions for metazoan zooplankton responses to OAE.

Zooplankton responses to OAE through different phases of adjustment. Here we report the statistical outputs form the linear regressions fitted to phase averages. All zooplankton (size, taxonomy and development) samples in which each response was assessed are included.

FOOD WEB IMPLICATION	ZOOPLANKTON PROPERTY	SAMPLE	PHASE	STATISTICS		
				df	t	p
Stability	Diversity	all zooplankton	i	7	-0.35	0.74
			s	7	0.25	0.81
			l	7	-0.1	0.92
Trophic structure	Trophic level	large zooplankton	s	7	0.17	0.87
			l	3	0.5	0.65
		medium zooplankton	s	7	1.28	0.24
			l	7	0.5	0.63
		medium copepod	s	7	0.66	0.53
			l	6	0.79	0.46
	Carbon source	PUFA:SFA	s	5	-1.62	0.17
			l	6	-0.1	0.92
		DHA:EPA	s	5	-0.1	0.93
			l	6	0.75	0.48
		C18:1n-7	s	5	-1.38	0.23
			l	6	-1	0.35
Food production: quantity	Biomass	all zooplankton	i	7	-0.75	0.48
			s	7	-0.75	0.48
			l	7	0.06	0.95
		all appendicularia	i	7	0.67	0.52
			s	7	0.25	0.81
			l	7	0.69	0.51
		all calcifiers	i	7	0.95	0.37
			s	7	-0.19	0.86
			l	7	-2.06	0.08
		all other zooplankton	i	7	-1.62	0.15
			s	7	-2.17	0.07
			l	7	0.33	0.75
		copepodites	i	7	-1.06	0.33
			s	7	-0.59	0.57
			l	7	-0.91	0.4
		adult copepod	i	7	1.32	0.23
			s	7	-1.76	0.12
			l	7	-2.01	0.08
		Labidocera	s	7	-0.12	0.91
			l	7	1.7	0.13
		>medium zooplankton	i	7	-1.03	0.34
			s	7	-0.58	0.58
			l	7	-0.13	0.9
		>medium copepods	i	7	-0.82	0.44
			s	7	-0.95	0.38
			l	7	-1.29	0.24
	Reproduction	copepod nauplii biomass	i	7	0.28	0.79
			s	7	-4.08	<0.01
			l	7	0.22	0.83
	Production	large zooplankton	s	7	-2.54	0.04
			l	2	-1.07	0.4
		medium zooplankton	s	7	-2.26	0.06
			l	6	-1.06	0.33
		medium copepod	s	6	-2.28	0.06
			l	6	-1.78	0.13
Food production: quality	Fatty acids	total FAs	s	5	1.74	0.14
			l	6	0.44	0.68
		DHA:EPA	s	5	-0.1	0.93
			l	6	0.75	0.48
		PUFAs	s	5	0.75	0.49
			l	6	0.17	0.87
	Size	large copepod	s	7	-1.52	0.17
			l	3	-0.95	0.41
		large *Labidocera*	s	3	1.44	0.25
			l	2	-0.45	0.7
		medium copepod	s	7	0.4	0.7
			l	7	0.44	0.67
		medium *Temora*	s	7	-0.13	0.9
			l	7	-1.72	0.13
	Size structure	Large: medium copepod	i	7	0.69	0.51
			s	7	0.83	0.43
			l	7	1.44	0.2
	Copepod: gelatinous	>medium zooplankton	i	7	-0.62	0.56
			s	7	-0.53	0.61
			l	7	-0.29	0.78
	Carbon: nitrogen	large zooplankton	s	7	-0.38	0.72
			l	3	-0.2	0.86
		medium zooplankton	s	7	1.89	0.1
			l	7	3.3	0.01
		medium copepod	s	7	3.84	<0.01
			l	6	0.18	0.86

Table S2. Summary of linear regressions of metazoan zooplankton biomass and fatty acid composition to OAE.

Zooplankton biomass (A) and fatty acid (B) composition responses to OAE. We fitted linear regressions to the calculated ecological (based on Bray-Curtis dissimilarity) and environmental distances (based on Euclidean distance) to quantitatively assess the effect of OAE on these multivariate data sets. We further tested for a correlation between the environmental and ecological distances using Mantel tests. In both cases we established a $p>0.05$ as non-significant . Response phases: i = immediate, s = shorter-term, l = longer-term.

Zooplankton response	Phase	OAE effect (linear regressions)			Mantel test	
		df	t	p	r	p
Community composition	i	34	-0.1	0.93	0.01	0.47
	s	34	-0.45	0.66	0.03	0.42
	l	34	-1.09	0.28	-0.15	0.8
Fatty acid composition	s	19	0.91	0.38	0.13	0.21
	l	26	-1.49	0.15	-0.24	0.62

Table S3. Equations used in the study.

Here we report on the series of equations and transformations applied to calculate selected food web parameters presented in this study. Further details on how we applied these formulas can be found in the materials and methods sections. ZP = metazoan zooplankton, POM = particulate organic matter, prosome = anterior region of the body of invertebrates (in copepods, roughly corresponds to the bulk of the biomass).

PROPERTY	MEASUREMENT (units)	EQUATION	VARIABLES	SOURCE
1. COPEPOD PER CAPITA SIZE	Biovolume (mm^3)	$= \frac{4}{3}\Pi\frac{PL}{2}\frac{PW}{2}\frac{PH}{2}$	PL = prosome length (mm) PW = prosome width (mm) PH = prosome height (same as PW)	14
	Wet mass (μg)	$= (1.024 * biovolume)1000$	1.024 = seawater gravity (gcm^{-3}) at 22°C; assumed similar to copepods´	
	Dry mass (μg)	$= 0.162 * wetmass$	0.162 = constant	
	C mass (μg)	$= 0.48 * drymass$	0.48 = constant	
2. DIVERSITY	Shannon index (H')	$= -1\sum(p_i(\ln p_i))$	p_i = taxon-specific biomass relative to total biomass $\ln p_i$ = natural log of p_i	
3. FOOD WEB LENGTH	Zooplankton trophic level ($\delta^{15}N$)	$= ZP\delta^{15}N_t - POM\delta^{15}N_{t\to t-1}$	$ZP\delta^{15}N_t$ = zooplankton $\delta^{15}N$ on sampling day t $POM\delta^{15}N_{t\to t-1}$ = mean POM $\delta^{15}N$ across sampling days t and the previous one	
4. SECONDARY PRODUCTION	AT%^{13}C	$= \frac{X}{X+1}100$	$X = (\frac{\delta^{13}C}{1000} + 1)0.0111802$	73
	Production ($\mu gCL^{-1}d^{-1}$)	$= \frac{ZPAT\%^{13}C_t - ZPAT\%^{13}C_{t0}}{POMAT\%^{13}C_{t0\to t} - ZPAT\%^{13}C_{t0}}\frac{ZPbiomass_{t\to t\pm 1}}{t-t_0}$	$ZPAT\%^{13}C_t$ = ZP enrichment on day t $ZPAT\%^{13}C_{t0}$ = ZP baseline enrichment $POMAT\%^{13}C_{t0\to t}$ = mean POM enrichment of measurements between t_0 and t $ZPbiomass_{t\to t\pm 1}$ = mean ZP biomass (μgCL^{-1}) at t and the sampling days directly before and after $t - t_0$ = days	
5. COMMUNITY COMPOSITION	Normalized zooplankton biomass	$= \frac{ZPbiomass - ZPbiomas_{min}}{ZPbiomass_{max} - ZPbiomas_{min}}$	$ZPbiomass$ = biomass of each ZP group $ZPbiomas_{min}$ = minimum biomass for each group, per treatment and across time $ZPbiomas_{max}$ = maximum biomass for each group, per treatment and across time	87

Table S4. Metazoan zooplankton biomass conversion factors.

Compilation of all zooplankton biomass conversion factors (µgC per ind) applied to calculate zooplankton biomass (µgC per L) from their abundances (ind per L). Factors were averaged from ZP carbon biomass measured either directly in an elemental analyzer, or indirectly via image-based carbon calculations, depending on sample availability. This table presents factors for ZP groups that both contributed to more than 0.1% of total biomass and that were found on more than two time points, per treatment. Abundances were resolved to a more detailed taxonomic and developmental level than biomass measurements, which is why in many case the same factor is applied to multiple count groups. In most cases, no temporal trends were observed in per capita biomass, so factors are calculated on average values for the whole experiment.
[1]Small copepod(ite) factors are calculated by subtracting the average (across treatments and time) copepod nauplii biomass from the average small bulk ZP biomass from the Gran Canaria 2019 mesocosm campaign. Both campaigns took place in the same location and season, and followed the same sampling strategy. [2]Small gelatinous ZP factors used came from the average small Appendicularia biomass from the Gran Canaria 2019 mesocosm campaign. [3]Large copepod(ite) factors were calculated both from copepod and selected bulk zooplankton samples which only contained copepods. [4]Large *Labidocera* factors were calculated from individuals sampled both in the water column and in the sediment traps, as we did not observe consistent biomass differences according to origin. Grey color = factor applied was averaged across all treatments.

SIZE	COUNT GROUP	BIOMASS GROUP	METHOD	OAE (μEqL^{-1})	FACTOR ($\mu gCind^{-1}$) *immediate*	*shorter*	*longer*
all	copepod nauplii	copepod nauplii	elemental analyzer	0	0.029		0.024
				300			0.027
				600			0.03
				900			0.025
				1200			0.034
				1500			0.032
				1800			0.032
				2100			0.029
				2400			0.029
small	Calanoid *Oithona* *Oncaea* *Temora*	copepod(ite)	elemental analyzer[1]	all	0.089		
small	*Oikopleura* *Obelia*	Appendicularia	elemental analyzer[2]	all	0.089		
small	Foraminifera Gastropoda Pteropoda Radiolaria	bulk zooplankton	elemental analyzer	all	0.066		
medium	Calanoid *Corycaeus* *Microsetella* *Oithona* *Oncaea* *Paracalanus* *Temora* Harpacticoida	copepod(ite)	elemental analyzer	0	1.054		
				300	1.193		
				600	1.234		
				900	0.981		
				1200	1.072		
				1500	1.086		
				1800	1.145		
				2100	0.901		
				2400	0.763		
medium	*Oikopleura* *Obelia*	Appendicularia	images	0	0.117		
				300	0.114		
				600	0.13		
				900	0.133		
				1200	0.123		
				1500	0.247		
				1800	0.138		
				2100	0.181		
				2400	0.144		

table continued in the next page

SIZE	COUNT GROUP	BIOMASS GROUP	METHOD	OAE (μEqL^{-1})	FACTOR ($\mu gCind^{-1}$) immediate	shorter	longer
medium	Foraminifera Gastropoda Pteropoda Radiolaria Pluteus	bulk zooplankton	elemental analyzer	0		0.564	
				300		0.598	
				600		0.766	
				900		0.694	
				1200		0.712	
				1500		0.826	
				1800		0.672	
				2100		0.686	
				2400		0.58	
large	Calanoid *Centropages* *Oithona* *Oncaea* *Paracalanus* *Temora*	copepod(ite)	elemental analyzer[3]	all		5.058	
large	*Oikopleura*	Appendicularia	images	0		0.137	
				300		0.369	
				600		0.226	
				900		0.339	
				1200		0.201	
				1500		0.284	
				1800		0.1	
				2100		0.361	
				2400		0.126	
large	Foraminifera Gastropoda Pteropoda Radiolaria	bulk zooplankton	elemental analyzer	0		3.687	
				300		6.023	
				600		3.687	
				900		3.687	
				1200		3.891	
				1500		2.017	
				1800		3.922	
				2100		3.687	
				2400		3.954	
large	*Labidocera*	*Labidocera*	elemental analyzer[4]	all		34.209	

Table S5. Metazoan zooplankton fatty acids.

Abundant (contributing more than 0.1% to total for each sample) fatty acids (%) measured in zooplankton. Phase average values are reported (S = shorter-term, L = longer-term). Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were calculated from the individual fatty acids listed in this table. blank = no data, - = below 0.1% threshold.

FATTY ACID FORMULA	MEAN FA (%)																	
	0		300		600		900		1200		1500		1800		2100		2400	
	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L
C12:0		0.4	0.6	0.7	0.6	0.3	0.2		1.6	-		0.4	0.7	0.5	0.4	0.4	-	0.5
C14:0		2.3	3	2.7	1.4	1.8	1.1		1.6	2.3		3.4	1	2.6	1.5	1.7	1.1	2.1
C14:1, 2OH-C10:0		-	0.1	0.1	-	-	-		-	-		0.2	-	-	-	-	-	0.2
C15:0		-	0.9	-	0.4	-	-		0.3	-		-	0.3	-	-	0.4	0.3	-
C16:0		50.5	43.3	49.1	51.6	51.7	51.8		52.9	50.2		64.3	51.9	47.6	52.1	51.3	51.2	46.8
C16:1		0.7	1.7	1.5	0.4	0.3	0.3		0.3	0.9		1.5	0.3	0.5	0.4	0.7	0.3	0.8
C16:3n-4		0.2	1.1	-	0.4	-	0.4		0.6	-		0.2	0.3	-	0.8	-	0.5	-
C17:0		0.3	1.2	0.4	0.4	0.3	0.4		0.3	0.2		0.3	0.3	0.5	0.4	-	0.3	0.3
C17:1; 3-OH-C12		-	-	0.2	-	-	-		-	-		0.4	-	-	-	-	-	-
C18:0; C18:1n9t		31.7	27.4	30.8	35.2	33.4	35.5		32.8	30.4		31.2	36.6	30.2	35.4	33.3	36.4	39
C18:1n7c		0.8	1.5	1.2	0.3	2.4	0.2		0.3	0.8		0.6	0.3	0.9	0.3	0.6	0.2	0.5
C18:1n9c		4.7	4.4	5.3	4.9	4.7	4.8		4.7	4.6		4.8	4.6	4.3	4.9	4.5	4.5	3.3
C18:2n6c		0.9	0.8	0.9	0.5	0.6	0.5		0.5	1.1		1.4	0.5	0.9	0.5	-	0.5	0.5
C18:3n3		0.5	-	0.2	-	0.5	-		-	0.8		0.3	-	0.4	-	-	-	0.2
C18:3n6		0.3	-	0.3	0.2	0.2	-		-	0.3		0.2	-	0.3	-	0.2	0.1	0.3
C20:0		0.4	0.3	0.4	0.4	0.3	0.4		0.3	0.3		0.4	0.3	0.4	0.4	0.4	0.4	0.4
C20:1n9c		0.2	-	-	-	0.3	-		-	0.2		0.2	0.3	0.3	-	-	-	0.5
C20:2n6c		-	-	-	-	-	-		-	0.2		-	-	-	-	-	-	-
C20:3n6		-	0.1	-	-	-	-		-	0.3		0.7	-	-	-	-	-	-
C20:4n6c		0.2	0.2	0.2	0.1	0.1	-		-	0.3		0.3	0.1	0.3	-	0.1	0.1	0.2
C20:5n3c		1.3	3	2	0.8	0.9	0.7		0.9	1		2.4	0.7	2	0.7	1.5	0.9	0.6
C22:0		1.7	-	-	-	1.7	-		-	2.8		-	-	1.6	-	-	-	2.3
C22:1n9c		0.3	0.4	0.4	0.4	0.2	0.4		0.3	0.2		0.2	0.3	0.5	0.2	-	0.5	0.2
C22:6n3		4.1	9.2	4.9	2.6	3.7	2.4		3.4	5		6.5	2.3	7.8	2	4.9	2.5	3.3
C23:0		-	-	-	-	-	-		-	-		-	-	0.2	-	-	-	-
C24:0		0.3	0.3	0.4	-	0.3	-		-	0.2		0.4	0.1	0.4	-	-	-	-
C24:1; C22:5n3		0.2	0.4	-	0.1	0.1	0.2		0.1	0.2		0.2	-	0.3	0.1	-	0.1	0.2
SFAs		**86.5**	**77**	**84**	**89.8**	**88.9**	**89.9**		**89.7**	**85**		**84.6**	**90.7**	**82.5**	**90.2**	**87.4**	**89.7**	**90**
MUFAs		**6.3**	**7.9**	**8.2**	**5.6**	**5.3**	**5.8**		**5.3**	**6.5**		**4.5**	**5.4**	**6.3**	**5.7**	**5.8**	**5.5**	**5**
PUFAs		**7.2**	**14.5**	**7.6**	**4.5**	**5.8**	**4.1**		**5**	**8.4**		**10.4**	**3.9**	**11.1**	**4**	**6.7**	**4.6**	**4.8**