Organic contaminants in zooplankton of Italian subalpine lakes: patterns of distribution and seasonal variations

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Supplementary materials

Table S1. Main morphology characteristics of deep subalpine lakes

Lake	Maggiore	Como	Iseo
Altitude - m a.s.l.	193	198	186
Area - km^2	213	146	61
Maximum depth - m	370	425	251
Mean depth - m	178	154	123
Volume - km^3	37	22	8
Main inflowing rivers	Ticino, Toce	Adda	Oglio
Outflowing river	Ticino	Adda	Oglio
Mean outflow discharge - $m^3 s^{-1}$	291.3	158	58.7
Theoretical renewal time - years	4.1	12.7	4.5/7.2
O_2 hypolimnetic - mg L^{-1}	8	8	0.2
Water temperature (min-max) - °C	7.1-17	7.6-20.3	10.7-18.3
Total phosphorus (min-max) - µg L^{-1}	2.5-23	8.25-22.8	8.9-25.7
Transparency (min-max) - Secchi disk m	4.5-10	4.9-8.1	3.5-6.2
Total suspended solids (min-max) - mg L^{-1}	0.5-3.5	2.5-3.3	2.5-2.8
Total nitrogen (min-max) - mg L^{-1}	0.9-1.3	1.07-1.12	0.7-0.9
Dissolved oxygen (min-max) - mg L^{-1}	8.5-10	9.9-11.9	8.5-11

Table S2. Monitoring Plan

Lake	Number of samples	Monitoring periods	Number of samples per size			Number of samples per season			
			≥200	≥450	≥850	Winter	Spring	Summer	Autumn
Maggiore	23	08/2015-11/2015 11/2016 - 08/2018		12	11	4	6	8	5
Como	38	06/2013 11/2016 - 11/2018	17	13	8	15	11	7	5
Iseo	12	11/2016 - 11/2018	9	3		3	4	2	3

Chemicals and solvents

All reagents were analytical reagent grade. LC–MS grade Chromasolv acetonitrile and concentrated formic acid were purchased from Sigma-Aldrich. Water (<18 MΩcm resistivity) was produced by a Millipore Direct-QUV water purification system (Millipore, Bedford, MA, USA). HybridSPE®Phospholipid Ultra cartridges (30 mg, 1 mL SPETubes) were obtained by Sigma Aldrich (St. Louis, Missouri, USA).

Table S3. List of PFAS compounds targeted in the present study, corresponding internal standards (ISs) and LC/MS/MS parameters for all target analytes and internal standards

Target analytes	Abbreviation	Precursor ion (*m/z*)	Product ions (*m/z*)	Collision energy	Corresponding ISs
Perfluorohexanoic acid	PFHxA	312.9	119.1	22	^{13}C2-PFHxA
			268.9	11	
Perfluoroheptanoic acid	PFHpA	362.9	169.0	18	^{13}C4-PFOA
			318.9	12	
Perfluorooctanoic acid	PFOA	412.9	169.0	19	^{13}C4-PFOA
			368.9	13	
Perfluorononanoic acid	PFNA	462.9	218.9	18	^{13}C5-PFNA
			418.9	13	
Perfluorodecanoic acid	PFDA	512.9	268.9	18	^{13}C2-PFDA
			468.9	13	
Perfluoroundecanoic acid	PFUnDA	562.9	268.8	20	^{13}C2-PFUnDA
			518.8	14	
Perfluorododecanoic acid	PFDoDA	612.9	318.8	20	^{13}C2-PFDoDA
			568.9	14	
Perfluorotridecanoic acid	PFTrDA	662.9	619.0	15	^{13}C2-PFDoDA
			369.0		
Perfluorotetradecanoic acid	PFTeDA	712.9	669.0	15	^{13}C2-PFDoDA
			419.0		
Perfluorobutane sulphonate	PFBS	298.9	80.2	44	^{13}C2-PFHxA
			99.1	32	
			79.9	40	
Perfluorohexane sulphonate	PFHxS	398.9	80.1	38	^{18}O2-PFHxS
			99.0	34	
			79.3	40	
Perfluorooctane sulphonate*	PFOS *	498.9	80.3	45	^{13}C4-PFOS
			99.1	45	
Perfluoro-n-[^{13}C2] hexanoic acid	$^{13}C_2$-PFHxA	314.9	269.9	11	n/a
Perfluoro-n-[^{13}C4] octanoic acid	$^{13}C_4$-PFOA	416.9	371.9	13	n/a
Perfluoro-n-[^{13}C5] nonanoic acid	$^{13}C_5$-PFNA	467.9	422.9	13	n/a
Perfluoro-n-[^{13}C2] decanoic acid	$^{13}C_2$-PFDA	514.9	469.9	13	n/a
Perfluoro-n-[^{13}C2] undecanoic acid	$^{13}C_2$-PFUnDA	564.9	519.8	14	n/a

Perfluoro-n-[^{13}C2] dodecanoic acid	$^{13}C_2$-PFDoDA	614.9	569.9	14	n/a
Perfluoro-n-hexane [^{18}O2] sulphonate	$^{18}O_2$-PFHxS	402.9	103.0	34	n/a
Perfluoro-n-octane [^{13}C4] sulphonate	$^{13}C_4$-PFOS	502.9	99.1	45	n/a

n/a not applicable; *sum of linear and branched isomers

Table S4. PFAS concentrations in zooplankton samples (ng g^{-1} ww)

	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	PFOS
Lake Maggiore (n=19)												
% positive samples	0	15.8	68.4	94.7	94.7	94.7	89.5	52.6	52.6	15.8	47.4	100.0
min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.3
median	<LOD	<LOD	0.1	0.2	0.4	0.2	0.1	<LOD	<LOD	<LOD	<LOD	3.9
max	<LOD	0.9	4.0	0.9	1.8	1.8	2.0	0.8	0.4	1.0	1.9	18.9
mean	<LOD	<LOD	0.6	0.3	0.6	0.4	0.3	0.1	0.0	<LOD	0.2	5.1
Lake Iseo (n=7)												
% positive samples	0	14.3	57.1	71.4	71.4	71.4	71.4	57.1	57.1	28.6	28.6	85.7
min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
median	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.07	0.03	<LOD	<LOD	<LOD	0.4
max	<LOD	0.1	9.4	0.1	0.3	0.3	0.3	0.2	0.2	4.3	0.3	1.8
mean	<LOD	<LOD	1.4	<LOD	0.1	0.08	0.09	0.06	0.05	0.7	<LOD	0.6
Lake Como (n=25)												
% positive samples	3.8	26.9	69.2	92.3	96.2	96.2	96.2	76.9	80.8	19.2	30.8	96.2
min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0	<LOD	<LOD	<LOD	<LOD	<LOD
median	<LOD	<LOD	<LOD	0.2	0.4	0.3	0.2	0.1	<LOD	<LOD	<LOD	2.5
max	<LOD	0.1	5.0	0.9	1.4	0.8	1.0	1.0	1.0	0.9	1.3	6.7
mean	<LOD	<LOD	0.4	0.3	0.5	0.3	0.3	0.2	0.1	<LOD	0.1	2.4
LOD	0.06	0.1	0.1	0.07	0.07	0.04	0.03	0.07	0.05	0.3	0.1	0.2

Table S5. DDT concentrations in zooplankton samples (ng g^{-1} dw). LODs are 0.1 ng g^{-1} dw

	op'DDE	**pp'DDE**	**op'DDD**	**pp'DDD**	**op'DDT**	**pp'DDT**
Lago Maggiore (n=22)						
% positive samples	100	100	100	100	100	100
min	1.1	4.5	2.5	4.1	0.4	1.6
median	4.0	21.4	11.6	9.7	2.0	6.3
max	20.2	38.3	36.0	37.2	11.2	21.3
mean	6.2	19.5	13.7	14.6	2.7	8.2
Lago Iseo (n=11)						
% positive samples	36.4	100	81.8	72.7	36.4	63.6
min	<LOD	0.5	<LOD	<LOD	<LOD	<LOD
median	<LOD	1.6	1.3	2.1	<LOD	0.2
max	20.0	2.7	15.8	7.6	2.3	3.3
mean	3.2	1.6	3.9	2.4	0.3	0.8
Lago Como (n=38)						
% positive samples	7.9	100	81.6	68.4	26.3	71.1
min	<LOD	0.3	<LOD	<LOD	<LOD	<LOD
median	<LOD	2.0	1.4	0.9	<LOD	0.9
max	7.4	14.0	23.9	14.3	1.5	12.4
mean	0.3	2.8	2.5	1.7	0.2	1.4

Table S6. PCB concentrations in zooplankton samples (ng g^{-1} dw). LODs are 0.1 ng g^{-1} dw

	PCB 18	**PCB 28+31**	**PCB 52**	**PCB 44**	**PCB 101**	**PCB 149**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 180**	**PCB 170**	**PCB 194**	**PCB 209**
Lago Maggiore (n=22)													
% positive samples	100	100	100	81.8	100	100	100	100	100	100	100	86.4	13.6
min	0.9	0.2	0.8	<LOD	2.8	0.6	0.3	1.5	1.3	0.7	0.3	<LOD	<LOD
median	4.3	1.4	3.3	1.7	7.9	3.8	2.9	9.0	7.6	6.1	2.3	0.7	<LOD
max	9.1	15.9	11.7	16.3	25.6	14.1	33.1	28.2	22.1	17.8	8.0	9.8	0.6
mean	4.4	3.2	4.3	3.8	9.1	4.2	5.0	11.0	9.5	6.5	2.8	1.6	<LOD
Lago Iseo (n=11)													
% positive samples	63.6	27.3	54.5	63.6	81.8	54.5	54.5	90.9	72.7	81.8	36.4	9.1	0
min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
median	0.7	<LOD	0.2	1.9	3.2	0.6	0.3	2.3	3.6	0.8	<LOD	<LOD	<LOD
max	12.4	6.9	46.3	6.3	17.1	29.5	7.5	37.2	18.9	25.1	11.6	2.0	<LOD
mean	2.5	0.9	6.0	2.3	5.0	3.7	1.3	7.3	5.1	5.0	1.4	0.2	<LOD
Lago Como (n=38)													
% positive samples	34.2	47.4	50.0	76.3	89.5	34.2	42.1	92.1	57.9	57.9	23.7	5.3	0
min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
median	<LOD	<LOD	<LOD	1.2	2.8	<LOD	<LOD	3.6	0.6	0.2	<LOD	<LOD	<LOD
max	20.1	4.6	8.8	7.2	20.7	41.6	10.8	24.2	14.8	14.8	7.2	0.1	<LOD
mean	1.8	1.0	0.6	1.8	4.3	2.2	0.6	4.3	2.3	1.6	0.3	<LOD	<LOD

Table S7. Taxa composition of zooplankton community in Lake Como and Maggiore

Lake	Size	Date	Season	Filter feeders (Cladocera) mg m^{-3}	Predators (Cladocera) mg m^{-3}	Cladocera mg m^{-3}	Copepods mg m^{-3}	Total biomass mg m^{-3}
Como	200	07/06/2013	Spring	96.6	0.1	96.7	19.9	116.6
Como	450	07/06/2013	Spring	34.5	0.1	34.6	4.3	38.9
Como	850	07/06/2013	Spring	14.3	0.3	14.6	0.0	14.6
Como	200	05/12/2016	Autumn	3.2	0.0	3.2	14.1	17.3
Como	450	05/12/2016	Autumn	0.3	0.0	0.3	1.1	1.4
Como	200	07/12/2016	Autumn	4.4	0.0	4.4	15.7	20.1
Como	450	07/12/2016	Autumn	1.6	0.0	1.6	4.9	6.5
Como	200	15/02/2017	Winter	1.0	0.0	1.0	20.3	21.3
Como	450	15/02/2017	Winter	1.1	0.0	1.1	3.3	4.4
Como	450	16/02/2017	Winter	0.4	0.0	0.4	1.5	2.0
Como	200	23/05/2017	Spring	38.0	3.8	41.8	33.6	75.4
Como	450	23/05/2017	Spring	30.6	2.9	33.5	4.0	37.4
Como	850	23/05/2017	Spring	22.0	3.1	25.1	0.0	25.1
Como	200	07/06/2017	Spring	13.0	1.3	14.3	0.5	14.8
Como	450	07/06/2017	Spring	15.0	0.9	15.9	0.1	16.1
Como	850	07/06/2017	Spring	10.6	5.1	15.6	0.0	15.6
Como	200	28/08/2017	Summer	40.3	2.1	42.4	24.1	66.5
Como	450	28/08/2017	Summer	13.0	1.5	14.5	0.3	14.8
Como	850	28/08/2017	Summer	0.2	0.8	1.0	0.0	1.0
Como	200	29/08/2017	Summer	12.3	1.0	13.2	17.1	30.4
Como	450	29/08/2017	Summer	3.6	1.3	4.9	0.1	5.0
Como	850	29/08/2017	Summer	0.1	1.4	1.4	0.0	1.4
Como	200	15/11/2017	Autumn	6.7	0.8	7.5	11.4	18.9
Como	200	16/11/2017	Autumn	3.3	0.3	3.6	10.0	13.6
Como	200	07/02/2018	Winter	0.4	0.0	0.4	8.0	8.5
Como	200	08/03/2018	Winter	0.5	0.0	0.5	5.6	6.0
Como	200	07/05/2018	Spring	45.7	0.6	46.3	58.0	104.3
Como	450	07/05/2018	Spring	37.4	0.2	37.6	0.2	37.8
Como	850	07/05/2018	Spring	20.1	0.6	20.7	0.0	20.7
Como	200	05/06/2018	Spring	21.8	2.0	23.8	9.4	33.2
Como	450	05/06/2018	Spring	22.3	1.8	24.0	0.0	24.0
Como	850	05/06/2018	Spring	33.7	4.1	37.8	0.0	37.8
Como	200	30/07/2018	Summer	64.4	1.4	65.7	20.9	86.7
Como	450	30/07/2018	Summer	19.5	1.7	21.2	0.0	21.2
Como	850	30/07/2018	Summer	1.7	3.5	5.3	0.0	5.3
Como	200	06/08/2018	Summer	61.3	0.6	61.9	12.1	74.0
Como	450	06/08/2018	Summer	17.6	1.1	18.6	0.0	18.6
Como	200	26/11/2018	Autumn	3.5	0.1	3.6	8.1	11.7
Maggiore	450	18/08/2015	Summer	12.1	1.2	13.3	0.5	13.8
Maggiore	850	18/08/2015	Summer	2.2	3.0	5.1	0.0	5.1
Maggiore	450	19/11/2015	Autumn	3.6	0.1	3.7	14.8	18.5

Maggiore	850	19/11/2015	Autumn	0.4	0.7	1.2	0.0	1.2
Maggiore	450	16/11/2016	Autumn	3.3	0.5	3.9	0.8	4.7
Maggiore	850	16/11/2016	Autumn	ND	ND	ND	ND	3.9
Maggiore	450	18/01/2017	Winter	2.0	0.0	2.0	1.7	3.8
Maggiore	850	18/01/2017	Winter	0.4	0.3	0.7	0.0	0.7
Maggiore	450	17/05/2017	Spring	5.8	1.9	7.7	5.5	13.2
Maggiore	850	17/05/2017	Spring	18.5	4.5	23.0	0.0	23.0
Maggiore	450	08/08/2017	Summer	24.6	2.3	26.9	3.6	30.5
Maggiore	850	08/08/2017	Summer	1.1	1.7	2.8	0.0	2.8
Maggiore	450	20/11/2017	Autumn	11.1	1.5	12.7	6.3	19.0
Maggiore	850	20/11/2017	Autumn	0.4	0.7	1.2	0.0	1.2
Maggiore	450	24/01/2018	Winter	2.7	0.4	3.1	1.6	4.7
Maggiore	850	24/01/2018	Winter	0.5	0.1	0.6	0.0	0.6
Maggiore	450	09/05/2018	Spring	8.4	0.1	8.5	30.8	39.3
Maggiore	850	09/05/2018	Spring	2.1	0.1	2.2	0.0	2.2
Maggiore	450	07/08/2018	Summer	12.1	1.2	13.3	0.5	13.8
Maggiore	850	07/08/2018	Summer	1.4	1.6	3.0	0.0	3.0
Maggiore	450	20/11/2018	Autumn	3.6	0.1	3.7	14.8	18.5

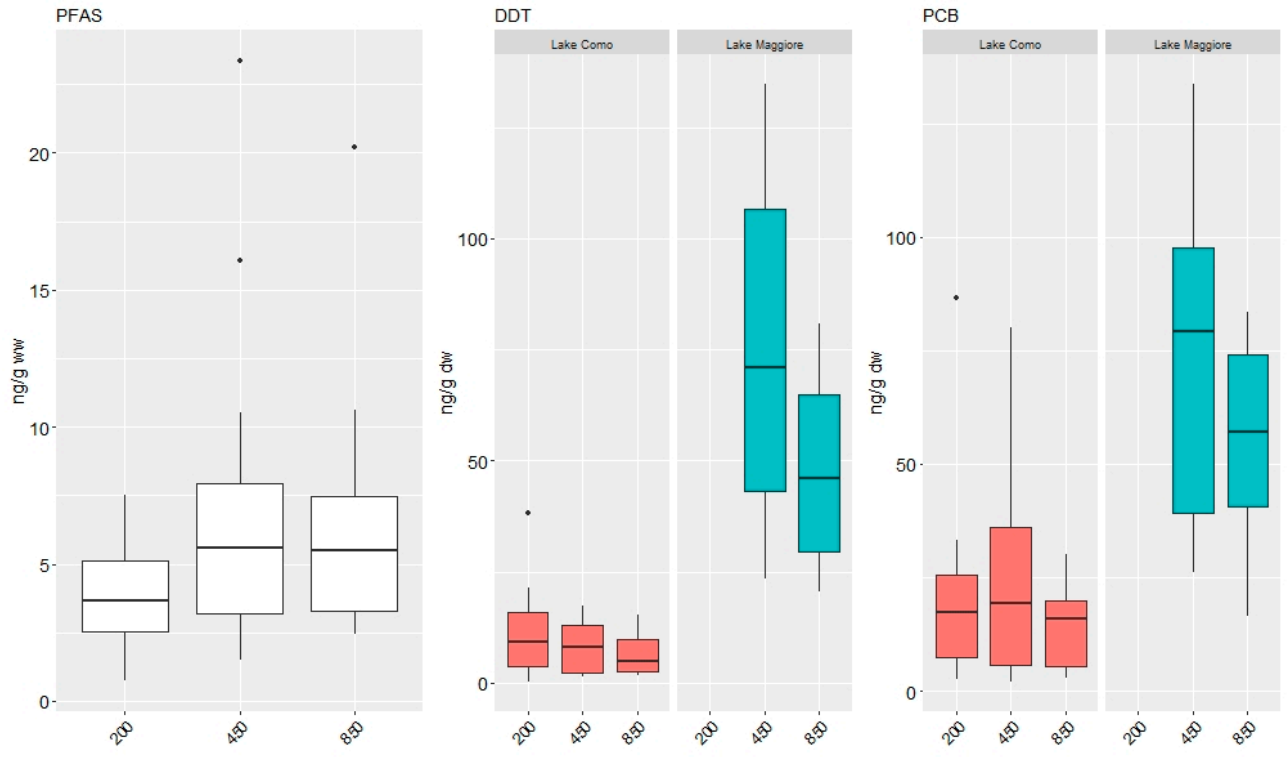

Figura S1. Contaminant trend in different zooplankton size

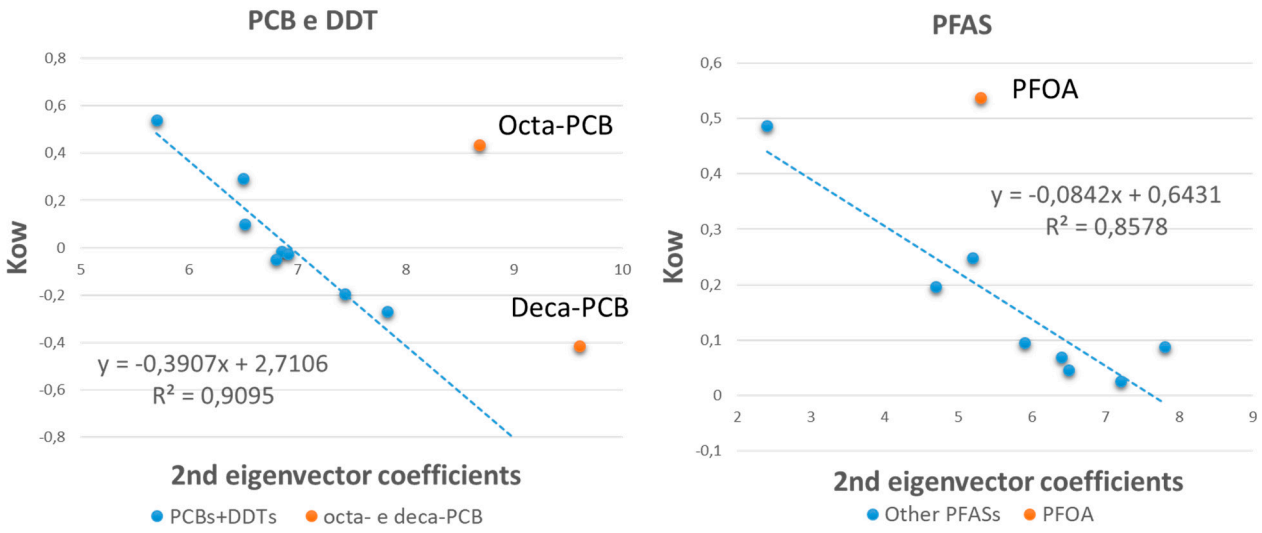

Figura S2. Correlation between coefficients of 2nd eigenvector in PCA and Kow