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Supplementary Information for

Title: Warming winters in lakes: later ice onset promotes consumer overwintering and shapes springtime planktonic food webs

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I. Supplementary tables

II. Supplementary figures

Table S1. Mean and maximum daily light incidence at a 1-m depth averaged over three time periods during the 40 first days of the experiment. Maximum (98th percentile) and mean daily values are calculated based on data acquired during daylight hours. Averaged light incidence (avg; lux) and standard errors (se) are provided for three time periods: days 1–22 = treatment period; days 23–40 = carry-over effects of treatments; days 1–40 = total time period during which treatments had detectable (direct or carry-over) effects on light incidence. Deviations relative to controls (i.e., ice-on = day 1) are provided as a measure of effect size (bold); % increases calculated as: $((avg_{treatment} - avg_{control}) / avg_{control}) \times 100$.

	Mean daily light incidence						**Maximum** daily light incidence					
	days 1–22		*days 23–40*		*days 1–40*		*days 1–22*		*days 23–40*		*days 1–40*	
ice-on	avg (se)	%incr.	avg (se)	%incr.	avg (se)	%incr.	avg (se)	%incr.	avg (se)	%incr.	avg (se)	%incr.
Day 1	353.97 (12.04)	–	45.59 (3.69)	–	215.21 (5.06)	–	633.59 (19.45)	–	92.44 (7.03)	–	390.08 (8.09)	–
Day 8	653.94 (56.63)	**+84.7%**	69.37 (15.50)	**+52.1%**	390.88 (34.60)	**+81.6%**	1187.35 (108.27)	**+87.4%**	140.20 (29.13)	**+51.7%**	716.13 (67.10)	**+83.6%**
Day 15	1232.19 (52.53)	**+248.1%**	77.52 (16.42)	**+70.0%**	712.59 (36.25)	**+231.1%**	2300.24 (94.88)	**+263.0%**	153.39 (29.35)	**+66.0%**	1334.16 (65.37)	**+242.0%**
Day 22	1160.55 (58.42)	**+227.9%**	120.83 (23.63)	**+165.0%**	692.68 (25.30)	**+221.9%**	2189.88 (120.37)	**+245.6%**	244.45 (49.72)	**+164.4%**	1314.44 (51.16)	**+237.0%**

Summary of Table S1: As shown in Fig. S1a-d, light incidence decreased rapidly after ice cover onset in enclosures where ice was allowed to form on days 1 and 8 (blue and yellow), reaching light extinction after snowfall events (*ca*. day 10). In enclosures with later ice-on dates (days 15 and 22; orange and red), however, light attenuation took up to 40 days. Due to mild weather and, most importantly, no snowfall following ice cover onset on day 15 (orange), light availability remained high and declined more slowly. As a result, light incidence patterns were highly similar across enclosures with day 15 and 22 ice-on dates (orange and red) during the treatment period (days 1–22), receiving >200% more light at a 1-m depth relative to control conditions. Carry-over effects (days 23–40) of treatment were on average greater in enclosures where ice cover formed on day 22 (+165% relative to controls), compared to enclosures where ice cover formed on day 15 (~ +70% relative to controls). Overall, treatment effects on light incidence were not significantly different between enclosures with day 15 and 22 ice-on dates over the 40 first days of the experiment; i.e. both treatments received >200% more light relative to controls.

Table S2. Mean and maximum daily temperature at a 1-m depth averaged over the 40 first days of the experiment. Averages of maximum (98th percentile) and mean daily values are calculated based on data acquired during daylight hours. Averaged temperatures (avg; ºC) and standard errors (se) are provided for three time periods: days 1–22 = overall treatment period; days 23–40 = carry-over effects of treatments; days 1–40 = total time period during which treatments had detectable (direct or carry-over) effects on light incidence. Note that ice cover treatments had no detectable effect on water temperature; hence % deviations relative to controls were not calculated.

	Mean daily temperature			**Maximum** daily temperature		
	days 1–22	*days 23–40*	*days 1–40*	*days 1–22*	*days 23–40*	*days 1–40*
ice-on	avg (se)	avg (se)	avg (se)	avg (se)	avg (se)	avg (se)
Day 1	1.7 (0.04)	1.3 (0.07)	1.5 (0.05)	1.7 (0.04)	1.3 (0.07)	1.5 (0.06)
Day 8	1.8 (0.07)	1.4 (0.09)	1.6 (0.08)	1.8 (0.07)	1.4 (0.1)	1.6 (0.08)
Day 15	1.7 (0.04)	1.5 (0.03)	1.6 (0.03)	1.8 (0.03)	1.5 (0.03)	1.7 (0.03)
Day 22	1.7 (0.05)	1.5 (0.09)	1.6 (0.07)	1.8 (0.06)	1.5 (0.1)	1.7 (0.07)

Table S3. Parameter estimates of linear regression models for source-specific FA biomarkers, DHA, ARA, and EPA in zooplankton (associated with Fig. 3). Slopes are provided are an indication of effect size. SE = standard errors. DM = dry mass. *P*-values in bold and in bold italics are significant (<0.05) and marginally significant (≤0.1), respectively.

ZOOPLANKTON	**Day 22**			**Day 45***		
	slope (SE)	*P*	R^2	slope (SE)	*P*	R^2
Source-specific FA biomarkers						
Total algal FA biomarkers (µg $mgDM^{-1}$)	0.992 (0.365)	**0.01**	0.345	0.61 (0.675)	0.385	0.069
% Algal FA biomarkers	0.327 (0.124)	**0.02**	0.332	0.454 (0.319)	0.182	0.156
Total bacterial FA biomarkers (µg $mgDM^{-1}$)	0.082 (0.039)	***0.059***	0.243	0.051 (0.045)	0.285	0.103
% Bacterial FA biomarkers	0.008 (0.023)	0.73	0.009	0.06 (0.042)	0.18	0.157
Total terrestrial FA biomarkers (µg $mgDM^{-1}$)	0.086 (0.031)	**0.015**	0.355	−0.025 (0.033)	0.467	0.049
% Terrestrial FA biomarkers	0.03 (0.009)	**0.007**	0.419	−0.001 (0.012)	0.929	0.001
Specific algal-derived FAs						
Total DHA (µg $mgDM^{-1}$)	0.205 (0.219)	0.366	0.059	0.427 (0.279)	0.154	0.175
% DHA	0.052 (0.06)	***0.1***	0.051	0.227 (0.084)	**0.02**	0.401
Total ARA (µg $mgDM^{-1}$)	0.056 (0.022)	**0.025**	0.311	−0.004 (0.007)	0.629	0.022
% ARA	0.02 (0.009)	**0.05**	0.247	−0.002 (0.004)	0.719	0.012
Total EPA (µg $mgDM^{-1}$)	0.23 (0.14)	0.124	0.161	0.022 (0.436)	0.961	0
% EPA	0.065 (0.064)	0.332	0.067	0.11 (0.215)	0.62	0.023

*****Note: missing zooplankton FA data for three high-treatment level sites on day 45.

Table S4. Parameter estimates of linear regression models for source-specific FA biomarkers, DHA, ARA, and EPA in seston (associated with Fig. S6). Slopes are provided are an indication of effect size. SE = standard errors. *P*-values in bold and in bold italics are significant (<0.05) and marginally significant (≤0.1), respectively.

SESTON	**Day 22**			**Day 45**		
	slope (SE)	*P*	R^2	slope (SE)	*P*	R^2
Source-specific FA biomarkers						
Total algal FA biomarkers (µg L^{-1})	0.363 (1.449)	0.806	0.004	1.2 (0.409)	**0.011**	0.381
% Algal FA biomarkers	0.402 (0.478)	0.414	0.048	0.9 (0.295)	**0.009**	0.399
Total bacterial FA biomarkers (µg L^{-1})	−0.007 (0.071)	0.926	0.001	0.005 (0.043)	0.908	0.001
% Bacterial FA biomarkers	0.014 (0.023)	0.567	0.024	−0.083 (0.074)	0.281	0.082
Total terrestrial FA biomarkers (µg L^{-1})	−0.007 (0.027)	0.805	0.005	0.003 (0.02)	0.881	0.002
% Terrestrial FA biomarkers	−0.004 (0.013)	0.782	0.006	−0.01 (0.02)	0.629	0.017
Specific algal-derived FAs						
Total DHA (µg L^{-1})	0.565 (0.571)	0.34	0.065	0.715 (0.264)	**0.017**	0.345
% DHA	0.16 (0.013)	0.223	0.104	0.58 (0.022)	**0.022**	0.322
Total ARA (µg L^{-1})	0.105 (0.042)	**0.026**	0.308	0	0	0
% ARA	0.092 (0.032)	**0.012**	0.374	0	0	0
Total EPA (µg L^{-1})	−0.455 (0.842)	0.597	0.02	0.157 (0.211)	0.47	0.038
% EPA	1.175 (0.637)	***0.086***	0.196	0.35 (0.187)	***0.082***	0.201

Table S5. Correlation coefficients (Pearson) between seston and zooplankton source-specific FA biomarkers, DHA, ARA, and EPA. *P*-values in bold are significant (<0.05). Selected panels are shown in Fig. S7.

SESTON–ZOOPLANKTON	**Day 22**		**Day 45**	
	r^2	*P*	r^2	*P*
Source-specific FA biomarkers				
% Algal FA biomarkers	0.226	0.399	0.248	0.413
% Bacterial FA biomarkers	0.16	0.554	−0.072	0.814
% Terrestrial FA biomarkers	−0.137	0.614	−0.263	0.385
Specific algal-derived FAs				
% DHA	0	0.524	0.31	0.303
% ARA	0.52	**0.039**	NA	NA
% EPA	0.266	0.32	0.016	0.959

Table S6. Comparison between lake and control raw data on days 22, 31, 45, 81, and during springtime (averaged across days 150, 166, and 183). This table includes main response parameters only. SEs are provided for controls; lake data only include one measurement per sampling day (i.e., no SE; except for spring averages). Ice-on dates: control = day 1; lake = a few days prior to the start of the experiment (see Methods). Chlorophyll concentrations and zooplankton biomass are expressed in µg/L; quantities of FAs are expressed in µg $mgDM^{-1}$.

	Day 22		**Day 31**		**Day 45**		**Day 81**		**Springtime**	
	Control	*Hertel*	*Control*	*Hertel*	*Control*	*Hertel*	*Control*	*Hertel*	*Control*	*Hertel*
Algae										
Total chlorophyll *a*	13.21 (1.38)	15.61	8.90 (1.31)	11.33	4.87 (0.68)	6.18	1.23 (0.29)	2.42	13.67 (1.48)	16.94 (2.48)
Green algae	0.54 (0.26)	0.04	0.00 (0.01)	0.05	0.00	0.00	0.00	0.00	7.69 (1.14)	8.13 (1.74)
Golden / Brown algae	4.99 (0.45)	4.68	2.74 (0.65)	4.19	1.88 (0.3)	2.59	0.60 (0.12)	1.10	5.88 (0.70)	7.01 (1.14)
Cryptophytes	6.03 (1.27)	8.68	6.16 (1.10)	6.07	1.86 (0.63)	2.58	0.29 (0.21)	1.22	0.00	0.05 (0.04)
PNF:HNF mass ratio	1.86 (0.40)	1.1	0.88 (0.08)	1.27	0.56 (0.07)	0.39	0.09 (0.01)	0.11	—	—
Zooplankton – biomass										
Total crustaceans	99.96 (8.85)	124.51	76.24 (11.27)	65.72	46.56 (8.34)	57.31	19.25 (5.14)	16.58	34.17 (5.51)	57.60 (14.76)
Cyclops	68.85 (8.9)	94.62	55.97 (9.75)	40.86	32.42 (5.48)	30.20	19.10 (3.21)	15.99	17.47 (3.11)	27.24 (8.05)
Bosmina	30.12 (7.3)	28.65	19.49 (3.73)	24.45	14.03 (4.15)	24.23	0.15 (0.11)	0.59	3.37 (1.17)	7.19 (2.29)
Total rotifers	13.1 (2.60)	9.8	13.58 (2.65)	18.00	26.01 (3.87)	35.73	10.67 (1.86)	8.13	10.32 (1.90)	16.49 (5.32)
Zooplankton – FAs										
Total algal FA biomarkers	13.60 (5.51)	12.61	—	—	34.16 (11.44)	32.76	—	—	—	—
% Algal FA biomarkers	5.59 (1.76)	15.63	—	—	12.68 (4.16)	13.24	—	—	—	—
% ARA	0.00	0.00	—	—	0.10 (0.08)	0.00	—	—	—	—
% DHA	1.99 (0.74)	5.68	—	—	2.78 (1.03)	—	—	—	—	—
% EPA	3.12 (0.99)	5.66	—	—	8.96 (3.41)	4.88	—	—	—	—

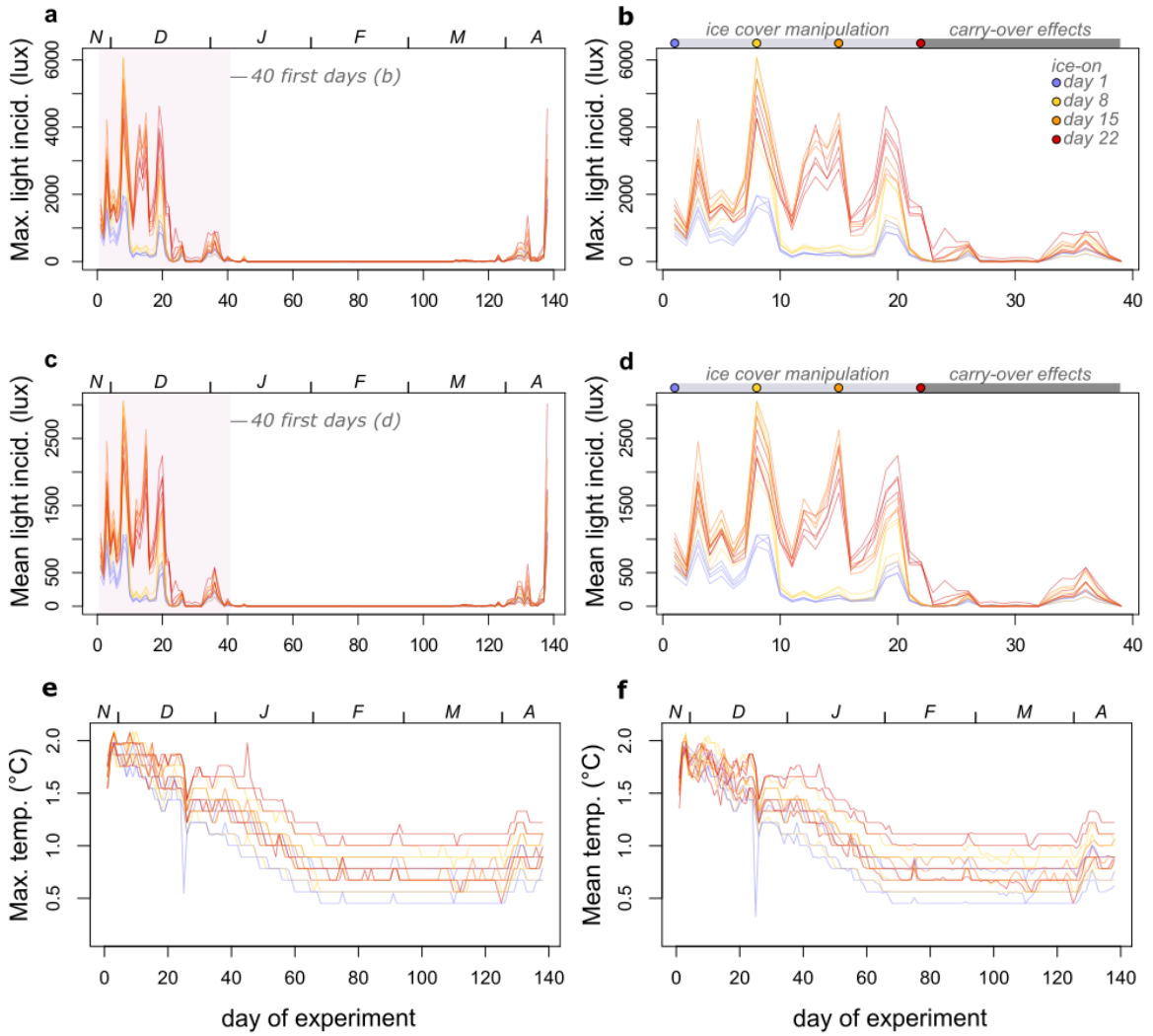

Figure S1. Time series of light incidence and temperature recorded at a depth of 1m. Light **(*A–D*)**: Temporal variation in **(*A, B*)** maximum daily light incidence (98th percentile) over the **(a)** 140 and **(b)** 40 first days of the experiment; panel b is a close-up of the shaded area in panel a. Temporal variation in **(*C, D*)** mean daily light incidence over the 140 **(*C*)** and 40 **(*D*)** first days of the experiment; panel d is a close-up of the shaded area in panel c. Differential fluctuations in light incidence between days 1 to 22 indicate short-term effects of ice manipulations (i.e., treatment period), while trends between days 23 and 40 indicate carry-over effects of ice manipulations. Temperature **(*E, F*)**: Temporal variation in **(*E*)** maximum and **(*F*)** mean temperature over the 140 first days. Each line represents an enclosure. Maximum and mean values were calculated based on data acquired during daylight hours; see Tables S1 and S2 for averages. Note that loggers reached their maximum memory capacity on day 140, only a few days before full ice-breakup. Colours refer to treatment levels; upper and lower horizontal axes indicate the time of the year and days of experiment, respectively. Detailed explanations regarding short-term and carry-over effects of treatments on light incidence are provided below Table S1 and in the Methods.

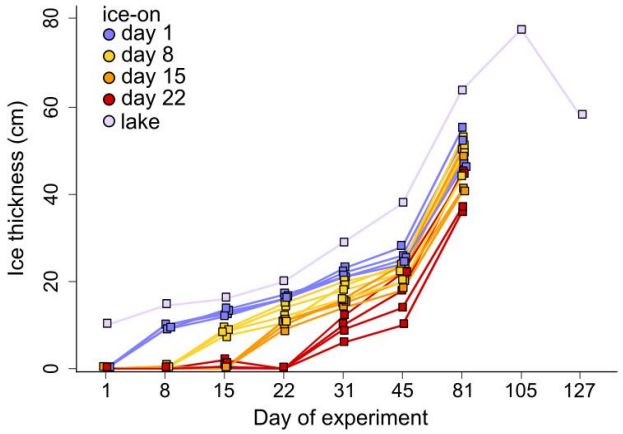

Figure S2. Effect of ice manipulation on ice thickness over the experiment. Colours refer to treatments. An offset on *x*-axis values was inserted to facilitate data visualisation.

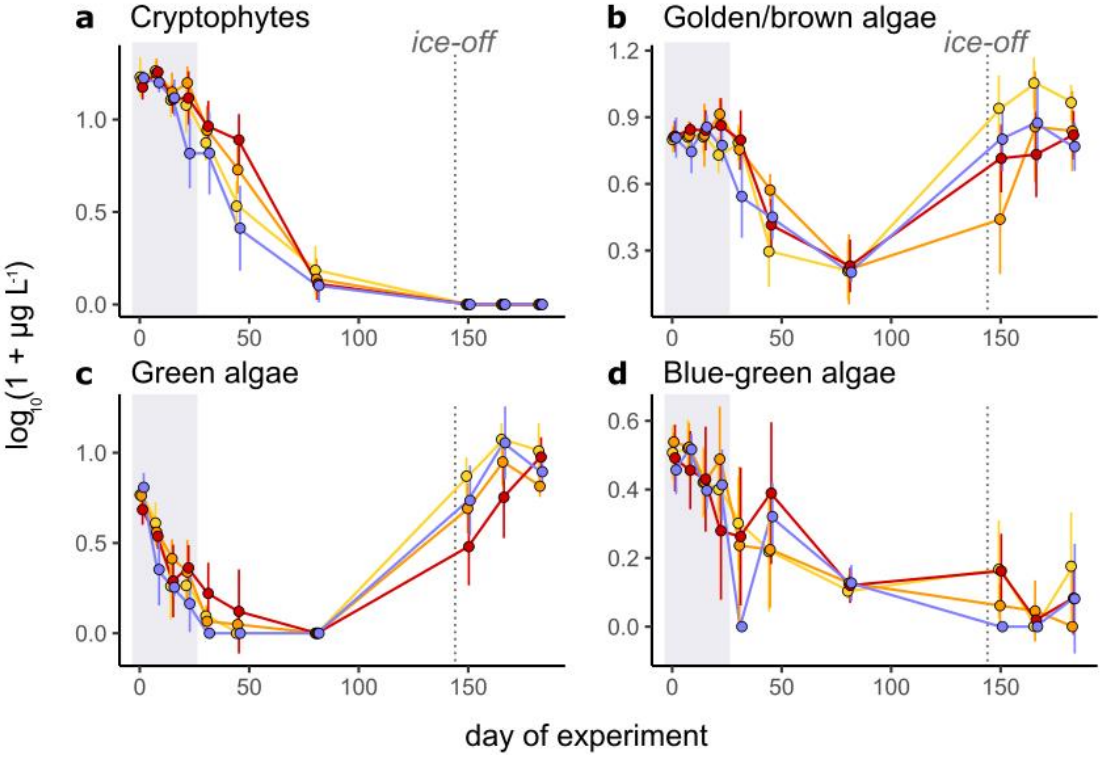

Figure S3. Temporal effects of ice manipulation on phytoplankton taxa. Time series of **(*A*)** cryptophytes, **(*B*)** golden/brown algae (e.g., diatoms, chrysophytes, dinoflagellates), **(*C*)** green algae, and **(*D*)** blue-green algae (cyanobacteria). Colours refer to treatment levels; legend as in Fig S1. Error bars represent 95% confidence intervals. Shaded gray areas illustrate the overall treatment period (i.e., days 1–22); carry-over effects on incident light were observable until day 40. Dashed lines indicate the timing of ice breakup (concomitant across enclosures).

Summary of Fig. S3: Positive effects of later ice-on on total chl-*a* concentration were driven by cryptophytes in early winter (until day 45; S3*A*); weak to no effects were detected in green and golden-brown algae during the same time period (S3*B*-*C*). In early spring, negative (likely indirect) effects of later ice-on on chl-*a* were exclusively driven by green algae (S3*C*); effects on golden-brown algae were marginally significant ($P < 0.01$; S3*B*). Blue-green algae showed no observable pattern over the experiment (S3*D*).

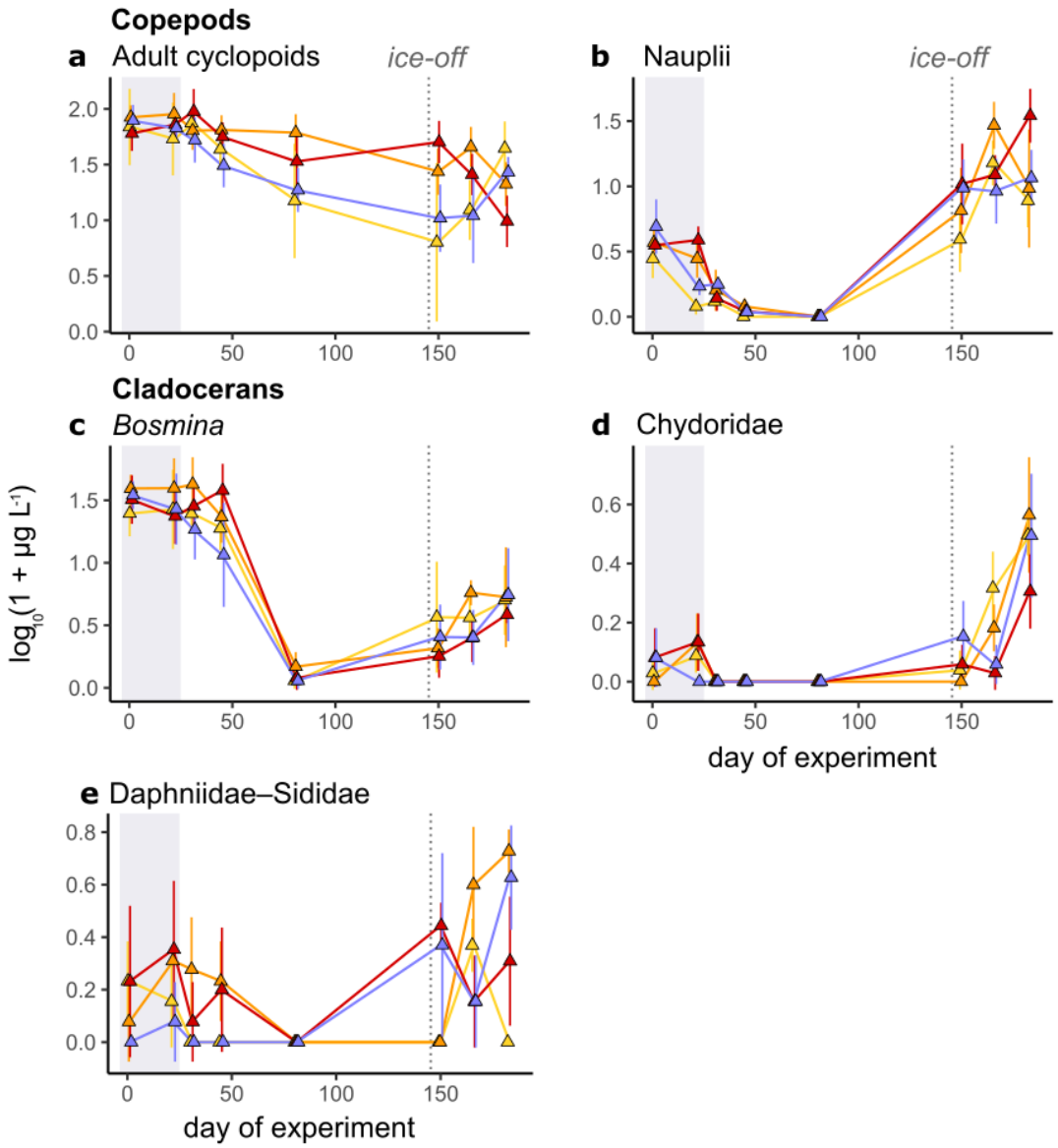

Figure S4. Temporal effects of ice manipulation on crustacean copepod and cladoceran taxa: **(*A*)** adult cyclopoids, **(*B*)** nauplii (immature copepods), **(*C*)** *Bosmina*, **(*D*)** Chydoridae, and **(*E*)** Daphniidae–Sididae. Colours refer to treatment levels; legend as in Fig S1. Error bars represent 95% confidence intervals. Shaded gray areas illustrate the overall treatment period (i.e., days 1–22); carry-over effects on light availability were observable until day 40. Dashed lines indicate the timing of ice breakup (concomitant across enclosures). A slight offset on *x*-axis values was introduced to facilitate data visualisation.

Summary of Fig. S4: Positive effects of ice cover manipulation on crustacean densities were driven by adult cyclopoid copepods (predominant species: *Cyclops scutifer*; S4*A*); in early spring, effects were attributable to both mature and immature (nauplii) cyclopoid copepods (S4*A*-*B*). Note that no calanoids have been reported in our source lake over the last decade; hence immature (nauplii) copepods are assumed to be members of cyclopoids. Cladocerans of the genus *Bosmina* were generally abundant in early winter (and positively responded to treatment on day 45) but did not survive through the season (S4*C*); other cladoceran taxa did not show any observable patterns over the experiment (S4*D*-*E*).

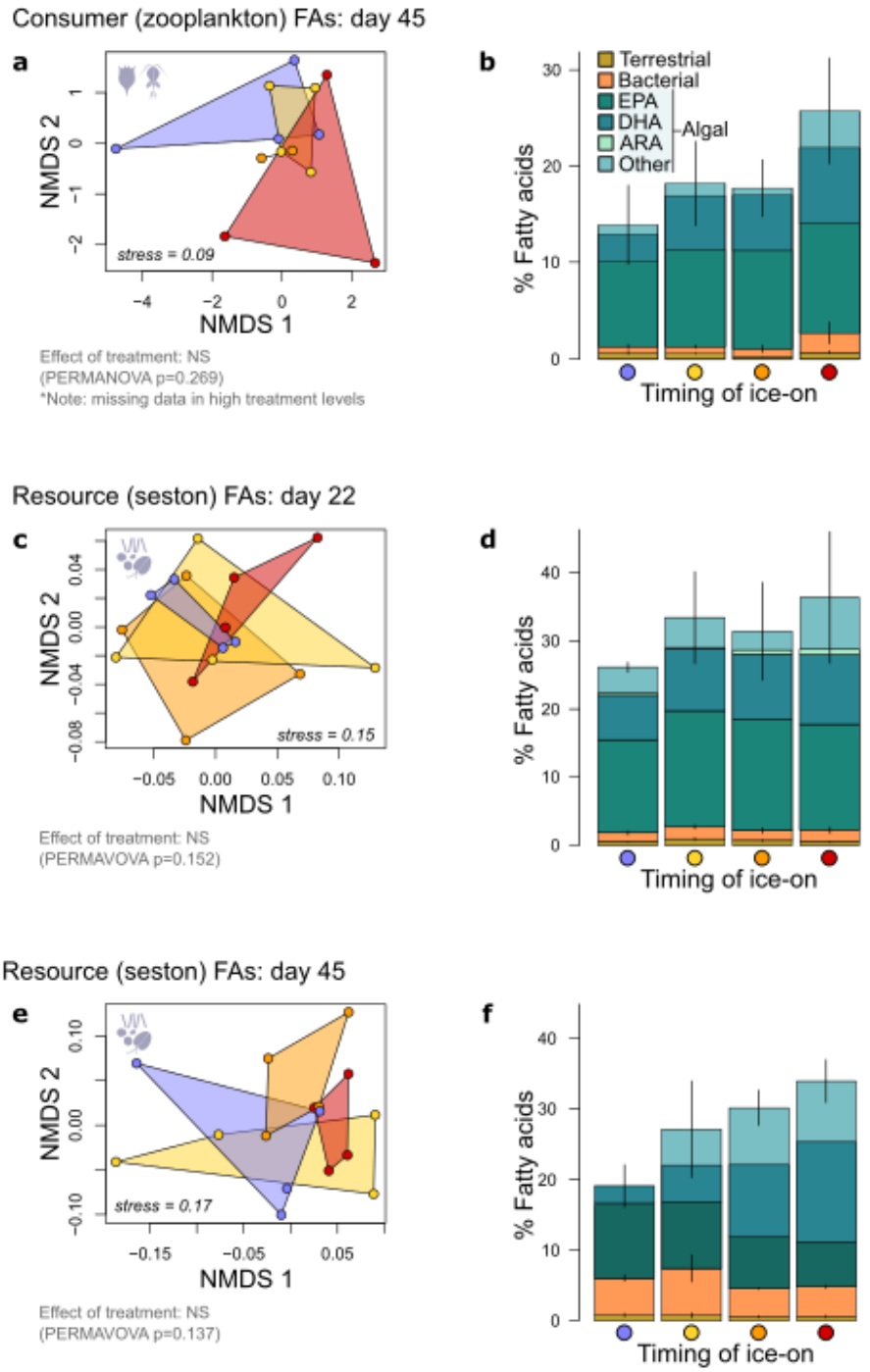

Figure S5. Effect of ice-cover manipulation on zooplankton and seston fatty acids. **(*A*, *C*, *E*)** NMDS representation of **(*A*)** zooplankton (day 45) and **(*C*, *E*)** seston (days 22 and 45) fatty acid (FA) composition, using all individual FAs. Zooplankton FA units: µg FA mg^{-1} dry mass; seston: µg FA l^{-1}. Convex hulls illustrate differences in FA signatures among treatments. Colours refer to treatment levels; legend as in Fig. S1. **(*B*, *D*, *F*)** Relative proportions (%) of FA biomarkers derived from algal (green), bacterial (orange), and terrestrial (brown) sources cumulated in **(*B*)** zooplankton (day 45) and **(*D*, *F*)** seston (days 22 and 45). Error bars represent standard errors on total FA biomarkers; proportions of EPA, DHA, and ARA are represented within algal FA biomarkers. Notes: missing data in high treatment levels for zooplankton on day 45 (a-b); zooplankton %ARA were not visually discernable on day 45 (<0.1%). Model coefficients are provided in Tables S3 and S4.

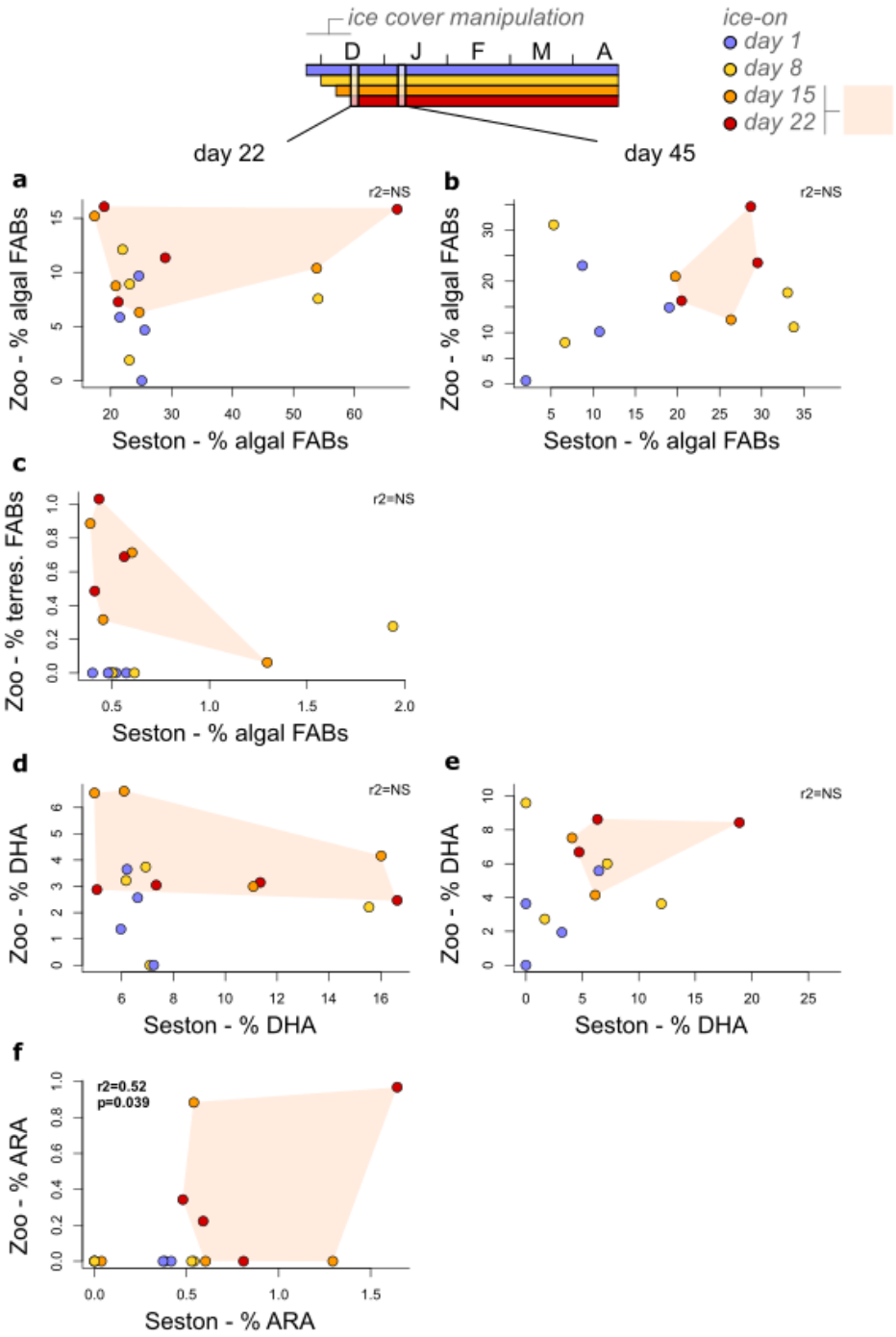

Figure S6. Selected relationships between seston and zooplankton FAs on days 22 and 45: **(*A-B*)** % algal FA biomarkers, **(*C*)** % terrestrial FA biomarkers, **(*D-E*)** % DHA, and **(*F*)** % ARA. Convex hulls delineate "orange" and "red" ice-cover treatments (i.e., ice-on = days 15 and 22). Only relationships including FAs that significantly increased in zooplankton are shown in this figure; see Table S5 for model coefficients of all tested relationships.

Summary of Fig. S6: Although mostly non-significant ($P > 0.05$), these relationships indicate that winter-active zooplankton may preferentially retain and cumulate specific types of FAs in early winter (red and orange data points often falling in upper quadrants; delineated with convex hulls), regardless of seston FA composition.

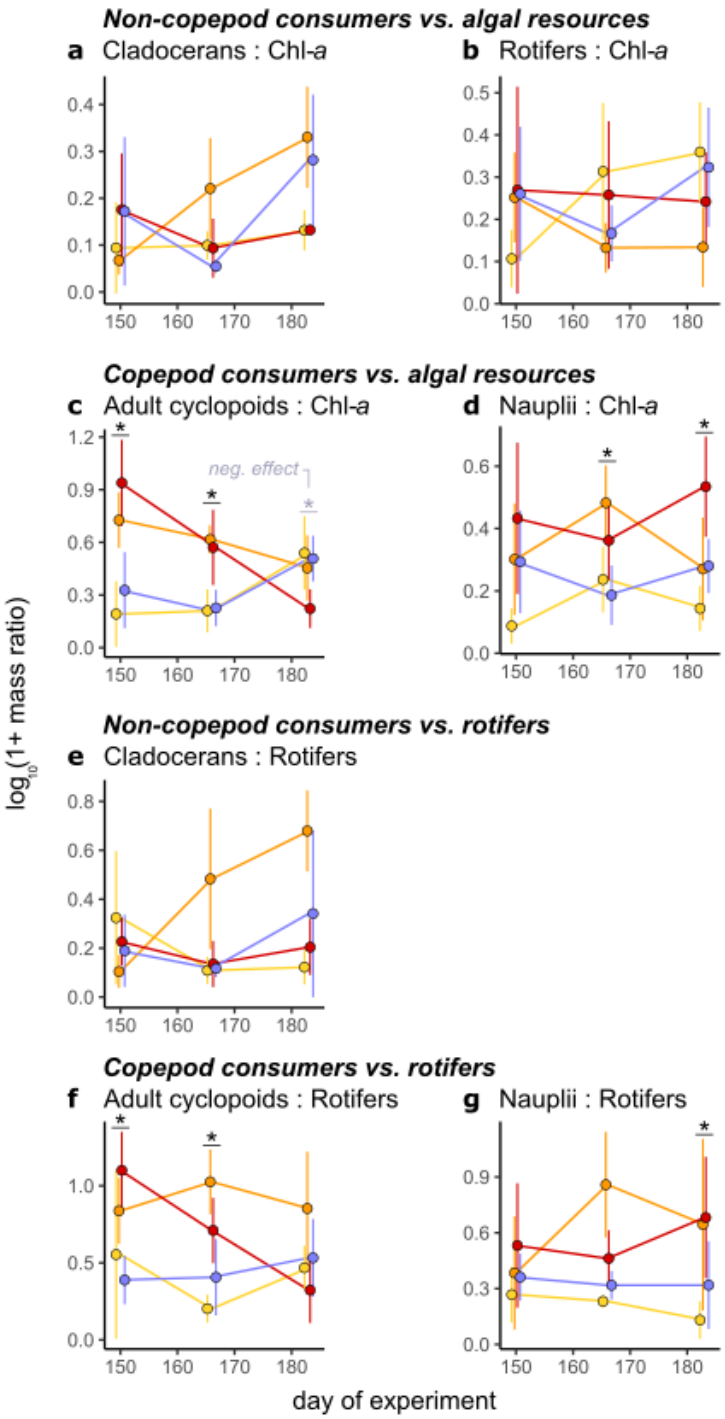

Figure S7. Copepod versus non-copepod consumer–resource and consumer–consumer mass ratios in spring. Asterisks denote significant ($P < 0.05$) overall effects of ice cover manipulation on springtime mass ratios (measured as parameter estimates of LMMs).

Summary of Fig. S7: In enclosures with later ice-on, where enhanced cyclopoid copepod under-ice survival was observed, we found relatively more (adult or immature) cyclopoid copepod biomass than chl-*a* (S8*C-D*) and rotifer (S8*F-G*) biomass in spring. Spring trends in mass ratios differed between mature and immature (nauplii) copepods over time, likely owing to transitions in their population dynamics.