***Supplementary material***

**Sex-specific differences in essential lipid requirements of**

***Daphnia magna***

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**Supplementary Tables**

**Table S1:** Parameters of the non-linear regression model (modified Von Bertalanffy growth curves, see methods for details) fitted to the sterol-limited growth responses of *D. magna*. *g*0 is the estimated growth rate (d-1) without sterol or EPA supply, b the Von Bertalanffy growth coefficient in mg C (*µ*g sterol)-1 or mg C (*µ*g EPA)-1 and *g*∞ the asymptotic maximum growth rate (d-1). Values in parentheses represent 95 %-CI.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | g0  d-1 | |  | b  mg C (*µ*g sterol)-1 | |  | g∞  d-1 | |  | R² |
| males | 0.046 | (0.033-0.058) |  | 6.20 | (5.13-7.55) |  | 0.24 | (0.23-0.25) |  | 0.95 |
| males + EPA | 0.069 | (0.047-0.092) |  | 6.43 | (5.10-8.20) |  | 0.35 | (0.33-0.36) |  | 0.93 |
| females | 0.044 | (0.032-0.057) |  | 9.53 | (7.93-11.6) |  | 0.26 | (0.25-0.27) |  | 0.96 |
| females + EPA | 0.058 | (0.040-0.076) |  | 8.21 | (7.22-9.38) |  | 0.43 | (0.42-0.44) |  | 0.97 |
|  | d-1 | |  | mg C (*µ*g EPA)-1 | |  | d-1 | |  |  |
| males | 0.32 | (0.31-0.33) |  | 1.04 | (0.67-1.55) |  | 0.37 | (0.36-0.37) |  | 0.81 |
| females | 0.37 | (0.36-0.38) |  | 0.89 | (0.54-1.41) |  | 0.43 | (0.42-0.44) |  | 0.74 |

**Table S2:** Results of the two-factorial ANOVA used to assess differences in clutch sizes, egg dry masses, and maternal dry mass investments in reproduction in the first three reproduction cycles on the different food treatments (Fig. 5). Clutch size and maternal investment data were log-transformed to comply with ANOVA assumptions.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | factor |  | df |  | *F* |  | *P* |
| clutch size | clutch |  | 2/45 |  | 122.9 |  | <0.001 |
|  | food |  | 2/45 |  | 75.0 |  | <0.001 |
|  | clutch×food |  | 4/45 |  | 5.6 |  | <0.001 |
| egg dry mass | clutch |  | 2/45 |  | 385.2 |  | <0.001 |
|  | food |  | 2/45 |  | 150.7 |  | <0.001 |
|  | clutch×food |  | 4/45 |  | 5.3 |  | 0.001 |
| maternal investment | clutch |  | 2/45 |  | 366.7 |  | <0.001 |
|  | food |  | 2/45 |  | 169.3 |  | <0.001 |
|  | clutch×food |  | 4/45 |  | 3.5 |  | 0.013 |

**Table S3:** Results of the two-factorial ANOVA used to assess differences in dry mass and lipid content (µg individual-1) between sexes (males, females with and without eggs) and among food treatments (Fig. 6). EPA was detected in the animals only after supplementation. Thus, differences in EPA content between sexes were assessed using a one-factorial ANOVA. Dry mass data are based on n = 6, lipid data on n = 3 replicates. Dry mass data were log-transformed to comply with ANOVA assumptions.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | factor |  | df |  | *F* |  | *P* |
| dry mass | sex |  | 2/45 |  | 1363.5 |  | <0.001 |
|  | food |  | 2/45 |  | 169.1 |  | <0.001 |
|  | sex×food |  | 4/45 |  | 5.2 |  | 0.002 |
| total fatty acids | sex |  | 2/18 |  | 1190.2 |  | <0.001 |
|  | food |  | 2/18 |  | 267.5 |  | <0.001 |
|  | sex×food |  | 4/18 |  | 90.6 |  | <0.001 |
| LIN | sex |  | 2/18 |  | 1318.1 |  | <0.001 |
|  | food |  | 2/18 |  | 455.4 |  | <0.001 |
|  | sex×food |  | 4/18 |  | 168.6 |  | <0.001 |
| ALA | sex |  | 2/18 |  | 896.3 |  | <0.001 |
|  | food |  | 2/18 |  | 153.3 |  | <0.001 |
|  | sex×food |  | 4/18 |  | 72.4 |  | <0.001 |
| EPA | sex |  | 2/6 |  | 539.0 |  | <0.001 |
|  | food |  | – |  | – |  | – |
|  | sex×food |  | – |  | – |  | – |
| cholesterol | sex |  | 2/18 |  | 147.4 |  | <0.001 |
|  | food |  | 2/18 |  | 14.8 |  | <0.001 |
|  | sex×food |  | 4/18 |  | 3.2 |  | 0.037 |

**Table S4:** Results of the two-factorial ANOVA used to assess differences in the concentrations (µg (mg dry mass)-1) of linoleic acid (LIN; A), α-linolenic acid (ALA; B), eicosapentaenoic acid (EPA; C), and cholesterol (D) in male and female somata as well as eggs produced in the third reproduction cycle (Fig. S4). EPA was detected in animals and eggs only after supplementation. Thus, differences in EPA concentrations were assessed using a one-factorial ANOVA. Data are based on n = 3 replicates. Asterisks (\*) indicate *P* values that were not significant after Holm-Bonferroni correction.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | factor |  | df |  | *F* |  | *P* |
| LIN | sex |  | 2/18 |  | 161.4 |  | <0.001 |
|  | food |  | 2/18 |  | 89.7 |  | <0.001 |
|  | sex×food |  | 4/18 |  | 5.0 |  | 0.007 |
| ALA | sex |  | 2/18 |  | 122.9 |  | <0.001 |
|  | food |  | 2/18 |  | 20.2 |  | <0.001 |
|  | sex×food |  | 4/18 |  | 3.3 |  | 0.034\* |
| EPA | sex |  | 2/6 |  | 6.0 |  | 0.037\* |
|  | food |  | – |  | – |  | – |
|  | sex×food |  | – |  | – |  | – |
| cholesterol | sex |  | 2/18 |  | 1.6 |  | 0.23 |
|  | food |  | 2/18 |  | 7.0 |  | 0.006 |
|  | sex×food |  | 4/18 |  | 1.4 |  | 0.29 |

**Supplementary Figures**

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**Fig. S1:** Schematic depiction of the applied experimental set-ups (Experiment 1-3).



**Fig. S2:** Size-specific juvenile somatic growth rates of male and female *D. magna* reared on *S. obliquus* and *S. obliquus* supplemented with cholesterol or EPA. Growth rates were calculated from the increase in body length up to day four of the experiment. Data represent means of n = 6 ± SD. Bars labeled with the same letters are not significantly different (Tukey’s HSD, *P* < 0.05, following ANOVA, sex×food interaction effect, *P* = 0.07).



**Fig. S3:** Body length (mm) of female and male *D. magna* reared on *S. obliquus* and *S. obliquus* supplemented with cholesterol or EPA. Females and males were subsampled at birth, at day four of the experiment, and when females deposited their first-, second-, and third-clutch eggs in their brood chambers. Females and males did not differ in body size at birth (ANOVA, *F*1,18 = 0.79, *P* = 0.39) but females were almost twice as large as males at the end of the experiment in all food treatments. Data represent means of n = 6 ± SD (error bars mostly smaller than symbol sizes).**Fig.S3_neu.TIF**

**Fig. S4:** Concentration (µg (mg dry mass)-1) of linoleic acid (LIN; A), α-linolenic acid (ALA; B), eicosapentaenoic acid (EPA; C), and cholesterol (D) in male and female somata as well as eggs produced in the third reproduction cycle. Data represent means of n = 3 ± SD. Data points labeled with the same letters are not significantly different (Tukey’s HSD, *P* < 0.05, following ANOVA; Table S4).