Supplement 1

Text S1. Fatty acid analyses (detailed)

Two different methods were used: a fast method (direct methanolysis) for analyzing the FA profile in the total lipids (TL), and a more detailed method (lipid extraction and lipid class separation) for measuring the FA profile in the different lipid classes.

1. Direct methanolysis of shrimp muscle and heads followed by Gas chromatography analysis with flame ionization detection (GC-FID) gives the total amount of FAs and the FA profile in TL.
2. Lipid extraction (Folch method) of shrimp heads and muscle followed by lipid classes separation by high-performance liquid chromatography (HPLC) and direct methanolysis and GC-FID give the total amount of lipids and FAs and the FA profile in TL.

Direct methanolysis: A subsample of shrimp muscle (≈ 80 mg wet weight), shrimp head (≈ 50 mg dry weight) or salmon feed (≈ 50 mg dry weight) was weighed in a 16 ml glass tube for FA analysis. All samples (n = 20 for each location and time for the field samples and n = 50 from the laboratory experiment) were methylated and the respective fatty acid methyl esters (FAME) were analyzed on a HP-7890B gas chromatograph (Agilent, USA) with a flame ionization detector (GC-FID) according to a method described in (Meier et al. 2006) with the FA 19:0 added as an internal standard. 2.5 M dry HCl in methanol was used as a methylation reagent. The FAMEs were extracted using 2x2 ml of hexane. The combined extract was diluted or concentrated to obtain a suitable concentration (approximate 150 ng/µl) for gas chromatography. The samples (1 µl) were injected in pulsed splitless mode using a pressure of 25 psi in 2 min before open for purge flow to the split vent. Injector temperature was 280°C. Detector temperature was 300°C.

Lipid extraction (Folch method): Field samples of shrimp heads (n = 5 from each location and time) and one sample of shrimp muscle were freeze dried and homogenized to powder. From the laboratory experiment, head and muscle tissue from one specimen from Day 0 and one specimen from Day 17 were analyzed for lipid class composition.

A subsample of ≈ 500 mg was weighed into a 25 ml glass tube and extracted using a modified Folch method (Folch et al. 1957, Meier et al. 2006) with chloroform:methanol (2:1, v/v)). The TL percent (as weight of total sample) was determined gravimetrically. The extract was dissolved in chloroform to a concentration of 20 mg/ml and was kept at -80°C until further analysis.

The lipid classes triacylglycerol (TG), free fatty acids/cholesterol (FFA), glycerophosphoethanolamines (PE) and glycerophosphocholines (PC) were separated from the TL by HPLC on an Agilent 1100/1260 Infinity analytical/semi-preparative system (Agilent Technologies). Chromatography was performed on a cyanopropyl column (ACE 100Å HPLC Column, ACE 5µm CN 125x4.6mm (Advanced Chromatography Technologies). The gradient system was using a binary solvent system; (Hexane (A) and Chloroform:methanol (1:1; v/v) (B) and the gradient were programed to be 90% A and 10% B for 3 min, followed by a linear gradient from 10% B to 100% B for 7 min, 100% B for 8 min, followed by linear gradient from 100% B to 10% B for 1 min) was used at a flow rate of 1.0 ml/min and a column temperature of 30°C. A 1100 Binary pump was used connected to a fraction collector (1260 Preparative-scale) and a 1260 Infinity evaporative light scattering detector (Agilent Technologies) with the

evaporation tube at 40°C. The gas pressure was 3.5 bar/5 1 psi (nitrogen). The samples were dissolved in chloroform to a lipid concentration of 20 mg/ml and the injection volume was 20 µl. The TG fraction was collected from 1.5 min to 2.7 min, FFA/cholesterol was collected from 3–4.2 min, PE was collected from 7–8.5 min and PC was collected from 8.5–11 min.

High-performance thin layer chromatography (HPTLC) was used for visualization of the lipid classes of the TL and the fraction from the HPLC. The mobile phase system for the method is described in (Olsen and Henderson 1989) and the HPTLC plates were first developed to a distance of 4.5 cm from the origin using a polar phase (methyl acetate: isopropanol: chloroform: methanol: 0.25 % KCl (25:25:25:10:9) as the solvent system. The plate was then dried in a vacuum desiccator until the solvent had evaporated, and the plate was thereafter developed in neutral solvent phase (hexane: diethylether: acetic acid (80:20:2)) to 9 cm. The separated lipid classes were detected by spraying the plate with 3% cupric acetate in 8% phosphoric acid, followed by heating at 160ºC for 20 min.

Total lipid and all four lipid classes were methylated, and the FAME was analyzed on GC-FID.

GC-FID analysis: The FAME extracts were analyzed using a CP-Wax 52 CB column (25 m × 0.25 mm fused silica capillary, coated with polyethylene-glycol of 0.25 µm film thickness, Varian-Chrompack, Middelburg, Netherlands). The oven temperature was programmed to hold at 90°C for 2 min, then from 90°C to 150°C at 30°C/min and then to 225°C at 2.5°C/min and hold there for 35 min, for a total analysis time of 75 min. Helium (99.9999%) was used as mobile phase at 1 ml/min for 45 min, followed by a flow increase to 3 ml/min which was held for 30 min. Injector and detector temperatures were 270°C and 300°C, respectively.

The method can distinguish between 97 different FAs that are identified based on comparison of retention time with standard mixtures of FAME (GLC-463 from Nu-Chek Prep. Elysian, MN, USA), retention index card, and mass spectrum library (GC-MS) (http://www.chrombox.org). However, only the 50 FAs that contribute with more than 0.1% of the total FAs are included in the data analysis. The data are presented either as FA profiles (% of total FAs) or normalised to tissue weight (mg/100 mg wet weight) (Tables S1–S5).

References

Folch J, Lees M, Stanley GS (1957) A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry 226:497–509

Meier S, Mjøs SA, Joensen H, Grahl-Nielsen O (2006) Validation of a one-step extraction/methylation method for determination of fatty acids and cholesterol in marine tissues. Journal of Chromatography A 1104:291–298

Olsen RE, Henderson RJ (1989) The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. Journal of Experimental Marine Biology and Ecology 129:189–197

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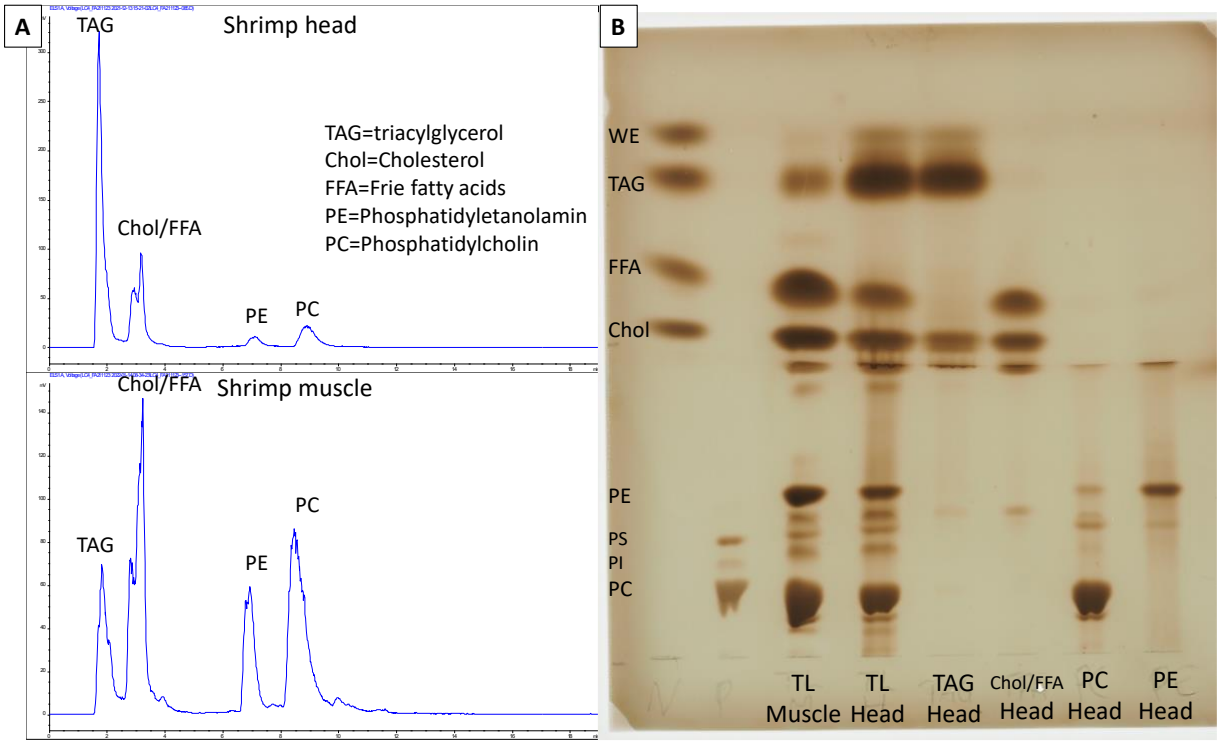

Fig. S1. Chromatogram of [A] HPLC separation of triacylglycerol (TG), free fatty acids (FFA), glycerophosphoethanolamines (PE) and glycerophosphocholines (PC) from shrimp heads (top) and shrimp muscle (below). [B] Thin layer chromatography (TLC) analysis of the lipid classes in the total lipid (TL) of muscle and head, and from the four selected peaks from the HPLC separation of head lipids (TG, FFA/Chol, PE, PC).

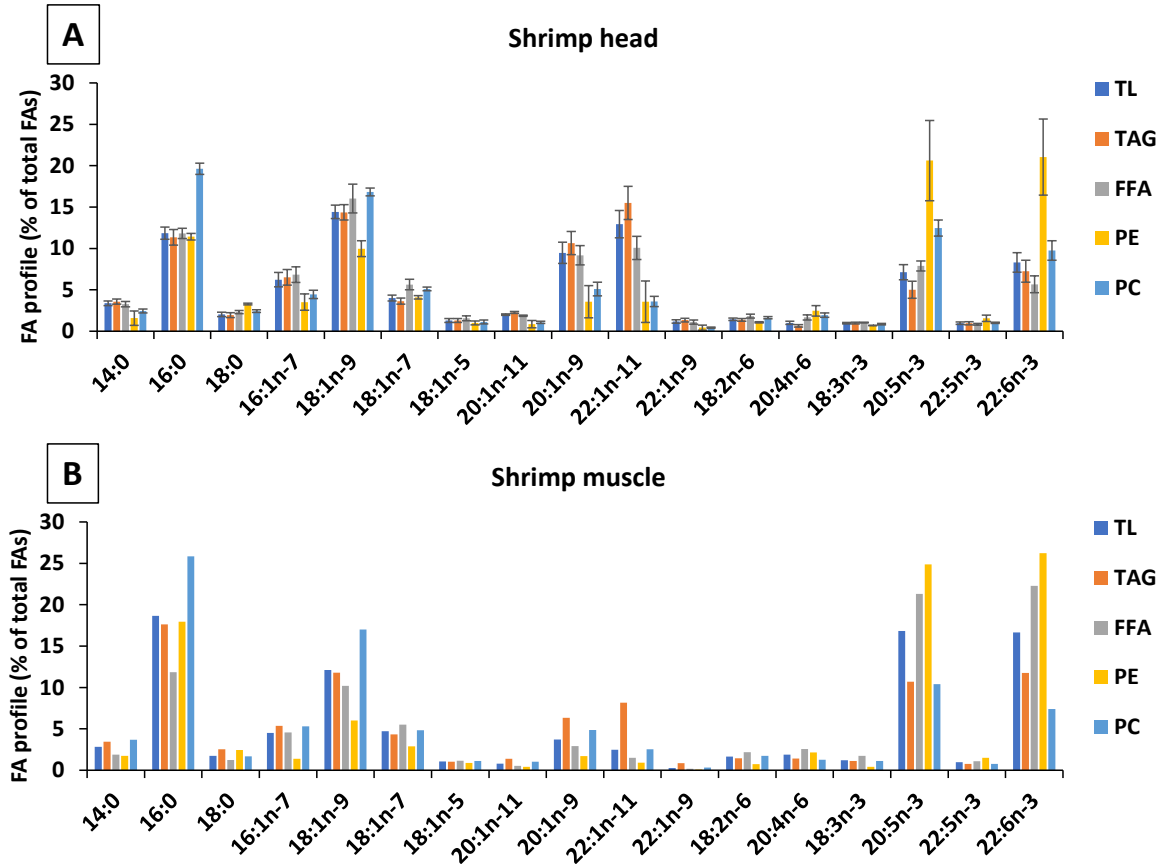

Fig. S2. Fatty acid (FA) composition of different lipid classes in field sampled shrimp heads (n = 5) [A] and muscle (n = 1) [B]. Data are given for total lipid (TL), triacylglycerol (TG), free fatty acids (FFA), glycerophosphoethanolamines (PE) and glycerophosphocholines (PC). The data are shown as mean (± SD) for shrimp heads. Only 17 FAs (out of 50 FAs) that contribute with more than 1% of the total FAs are shown in the figure.

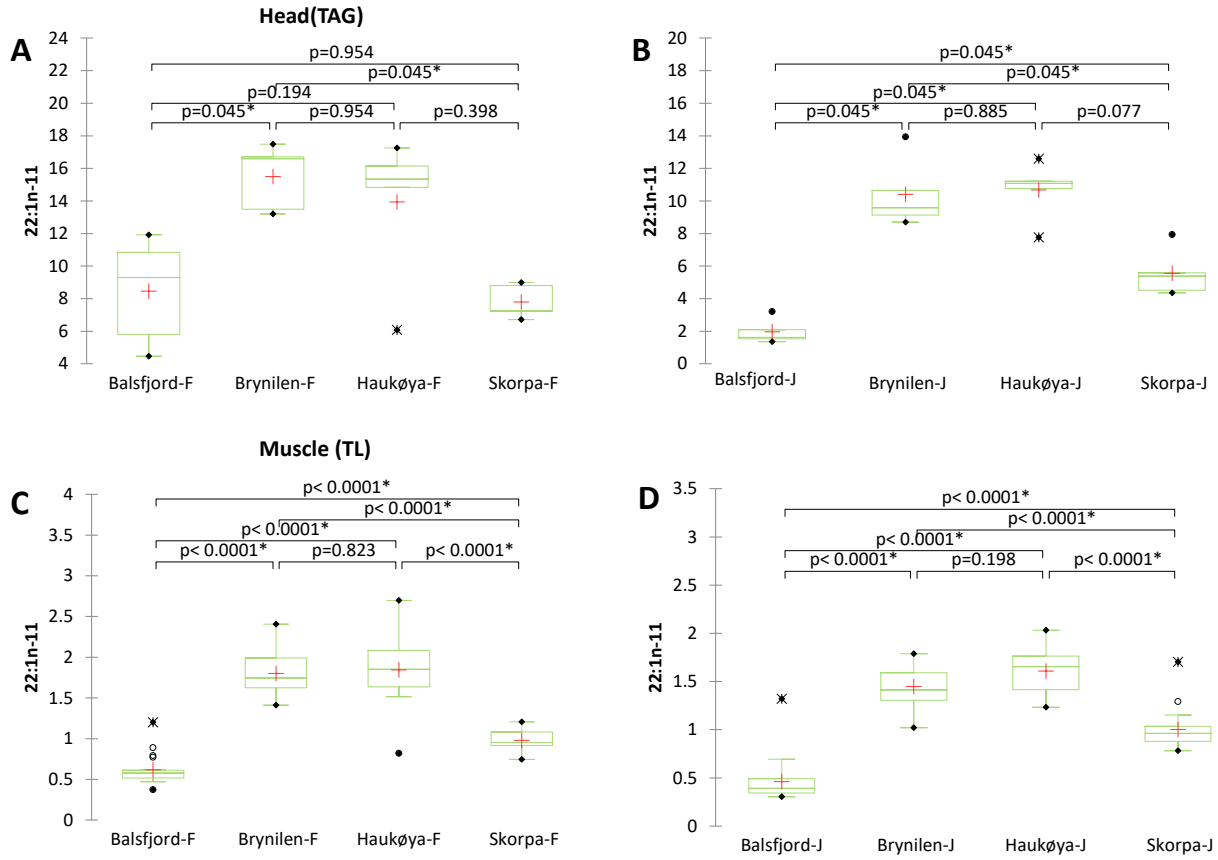

Fig. S3. Relative proportion of the *Calanus* fatty acid trophic marker (FATM) 22:1n-11 (% of total FAs) in (A,B) shrimp heads triacylglycerol (TG) (n = 5) and (C,D) muscle total lipids (TL) (n = 20) collected at 4 fjord sites in northern Norway—Kvænangen (Skorpa, Brynilen, Haukøya) and Balsfjord (Svartnes)—in (A,C) February (F) or (B,D) June (J) 2021. Box plots show the median (50th percentile) and interquartile range (IQR) (25th and 75th percentiles) as horizontal green lines (box), with whiskers indicating range of the data (1.5 x IQR). Dots and asterisks show outliers. Mean is shown with a red cross. Horizontal black lines show the pairwise comparisons of groups, with corresponding p-values, where significant differences are marked with an asterisk ($p<0.05$) (Kruskal-Wallis test).

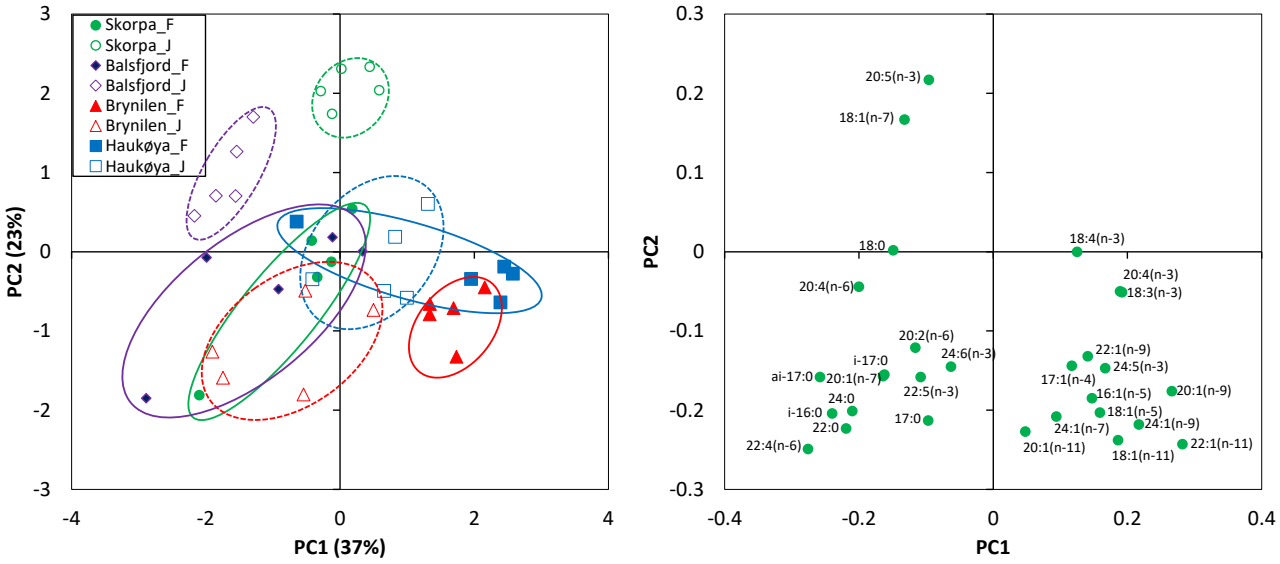

Fig. S4. Principal component analysis (PCA) of 50 FAs from storage fat, triacylglycerol (TG), extracted from heads of northern shrimp caught in 2 fjords in northern Norway—Kvænangen (Skorpa, Brynilen, Haukøya) and Balsfjord (Svartnes)—in February (F) and June (J) 2021. n = 5 for each site and sampling date. The first 2 axes of the score plot (left panel) explain 60% of the total variance (axis 1 = 37%, axis 2 = 23%). In the loading plot (right panel), only the FAs that contribute the most to the grouping are included in the figure (loading values > average loading).