Supplementary information

Interactive effects of ocean acidification and temperature on oxygen uptake rates in *Calanus hyperboreus* nauplii

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Lipid content estimation and fatty acid composition

The lipid content and fatty acid compositions of the copepod nauplii obtained from the incubation assays were analysed at the Alfred Wegener Institute in Bremerhaven, Germany. To acquire sufficient sample material for the analysis, between 100 to 148 individual larvae were pooled per sample. Triplicate samples (except for Treatment 3, which had duplicates) were analysed for each treatment. Before lipid extraction, the samples were freeze-dried for 24 h and then mechanically homogenized using a Potter-Elvehjem homogenizer. Total lipids were extracted by using a modified protocol from Folch et al., (1957), with dichloromethane/methanol (2:1, v/v), followed by cleaning with 0.88% potassium chloride solution. The extracted lipids were then transformed into fatty acid methyl esters (FAMEs) and free fatty alcohols derived from wax esters by transesterification in methanol containing 3% concentrated sulfuric acid, at 80 °C for 4 h. The FAMEs and alcohols were separated via an Agilent 6890N Network gas chromatograph (Agilent Technologies, USA) with a DB-FFAP capillary column (30 m, 0.25 mm I.D., 0.25 µm film thickness), equipped with a flame-ionization detector using a temperature program (160 to 240 °C). The samples were injected at 160 °C with helium as the carrier gas. The FAMEs were identified using standard mixtures, and the total lipid content was quantified as the sum of FAs and fatty alcohols using an internal standard (23:0) that was added prior to lipid extraction. The fatty acids were expressed in the nomenclature A:B(n-X), where A represents the number of carbon atoms, B the amount of double bonds, and X is the position of the first double bond starting from the methyl end of the

carbon chain. The proportions of individual FAs were expressed as mass percentages of the total FA content.

Levels of stored lipids in the form of wax esters varied little across all samples, ranging from 70 to 79%. There was also a generally strong similarity in FA proportions between all samples from all four treatments. FA profiles were dominated by the *Calanus*-associated FAs 20:1 and 22: 1; sums of *Calanus*-associated FAs contributed on average to one third of the FA proportions. Furthermore, the diatom-associated FA 16:1(n-7) contributed largely (on average between 15 and 16%) to the FA composition of all samples. Relative proportions of the dinoflagellate-associated FA 18:4(*n*-3) varied, on average, between 6 and 8%. Proportions of the membrane-associated polyunsaturated FAs 20:5(n-3) and 22:6(n-3) were relatively low (≤ 5% in all samples).

The resulting data comprising the relative proportions of the most abundant fatty acids and fatty alcohols have already been published and are available at: 10.21334/npolar.2023.edc957ac.

Statistical analyses

```
> test.aov <- aov(rate.ind ~ pH*Temp, data = resp_rates)
> summary(test.aov)
            Df    Sum Sq   Mean Sq F value  Pr(>F)
pH           1 0.0000060 6.040e-06   0.799 0.37434
Temp         1 0.0000102 1.023e-05   1.352 0.24852
pH:Temp      1 0.0000664 6.643e-05   8.784 0.00406 **
Residuals   76 0.0005748 7.560e-06
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> model.tables(test.aov, "means")
Tables of means
Grand mean

0.003377977

 pH
pH
     7.5      8.1
0.003653 0.003103

 Temp
Temp
       0        3
0.003020 0.003736

 pH:Temp
     Temp
pH    0        3
  7.5 0.002384 0.004922
  8.1 0.003657 0.002550
```

```
> TukeyHSD(test.aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = rate.ind ~ pH * Temp, data = resp_rates)

$pH
                  diff         lwr         upr    p adj
8.1-7.5 -0.0005495282 -0.00177427 0.000675214 0.374335

$Temp
            diff           lwr         upr    p adj
3-0 0.0007150909 -0.0005096513 0.001939833 0.248517

$`pH:Temp`
                       diff           lwr           upr     p adj
8.1:0-7.5:0  0.0012729718 -0.0010114074  3.557351e-03 0.4642634
7.5:3-7.5:0  0.0025375908  0.0002532117  4.821970e-03 0.0234734
8.1:3-7.5:0  0.0001655627 -0.0021188164  2.449942e-03 0.9975323
7.5:3-8.1:0  0.0012646190 -0.0010197601  3.548998e-03 0.4700783
8.1:3-8.1:0 -0.0011074090 -0.0033917882  1.176970e-03 0.5825469
8.1:3-7.5:3 -0.0023720281 -0.0046564072 -8.764892e-05 0.0387922
```