**Supporting Information for “Novel reverse radioisotope labelling experiment reveals carbon assimilation of marine calcifiers under ocean acidification conditions”**

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**Supporting Information**

**δ15N analysis of soft tissue and plankton samples**

Soft tissue (foot, mantle) and plankton samples (0.6–1.0 mg each) were dissected from the prepared specimens and tightly folded into 5–9 mm tin capsules for analysis of stable carbon and nitrogen isotopes (δ13C and δ15N) in an elemental analyzer (Flash 2000) coupled via a CONFLO III interface to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron Corporation). The δ15N values were calculated with respect to the atmospheric air standard. Alanine (δ13C VPDB –19.6‰, δ15Nair 11.0‰) was used as a working standard to calibrate the carbon and nitrogen data. A precision better than 0.1‰ (1 SD) was obtained for δ15N.

Clear trophic fractionation from the plankton feed to the bivalve specimens was observed in δ13C and δ15N (Fig. S1). Mean 13C enrichment of 3.9‰ and 4.4‰ in the foot and mantle tissues, respectively, was a consequence of trophic fractionation from the plankton diet to the bivalve consumer (Fig. S1). Mean fractionation of δ15N from the plankton feed to bivalve specimens was 6.4‰ and 6.7‰ for foot and mantle tissues respectively (Fig. S1).

***p*CO2 Treatment in the experiment**

Nishida et al. (2018) cultured specimens of *Scapharca broughtonii* in six different pCO2 treatments (332, 463, 653, 872, 1137, and 1337 μatm), and we used their specimens for our Δ14C analysis. Because an alkaline solution was used to adjust the *p*CO2 values in the 332 µatm tank, instead of aeration with fossil fuel–derived CO2 gas (Nishida et al., 2018), we excluded this treatment from our regression analysis calculations, although we also show the Δ14C and δ13C results in the 332-µatm tank here in the supporting information.

Table S1 Δ14C, δ14C and percent Modern Carbon of seawater DIC (Δ14CDIC, δ14CDIC and pMC) under six different *p*CO2 conditions. Mean ± 1 SD.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *p*CO2 (μatm) | Δ14CDIC (‰) | | δ14CDIC (‰) | | pMC (%) | |
| Mean | SD | Mean | SD | Mean | SD |
| 332 | 29.97 | 3.50 | 57.96 | 2.88 | 103.8 | 0.4 |
|  | 15.53 | 3.53 | 73.84 | 2.78 | 102.4 | 0.4 |
| 463 | 29.19 | 3.53 | 46.32 | 2.85 | 103.7 | 0.4 |
|  | 23.09 | 2.85 | 86.12 | 2.79 | 103.1 | 0.3 |
| 653 | 2.47 | 4.06 | 44.37 | 2.84 | 101.0 | 0.4 |
|  | 0.47 | 3.07 | 59.26 | 2.73 | 100.8 | 0.3 |
| 872 | -23.49 | 4.19 | 15.35 | 2.78 | 98.4 | 0.4 |
|  | -25.18 | 3.52 | 34.94 | 2.70 | 98.3 | 0.4 |
| 1138 | -34.06 | 3.54 | 26.57 | 2.67 | 97.4 | 0.4 |
|  | -32.80 | 3.58 | 23.94 | 2.69 | 97.5 | 0.4 |
| 1337 | -36.90 | 4.50 | -14.06 | 2.72 | 97.1 | 0.5 |
|  | -46.23 | 4.00 | 3.17 | 2.64 | 96.1 | 0.4 |

Table S2 Δ14C, δ14C and percent Modern Carbon of foot tissue (Δ14Cfoot, δ14Cfoot and pMC) in experimental specimens reared under six different *p*CO2 conditions. Mean ± 1 SD.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| *p*CO2  (μatm) | Specimen  No. | Δ14Cfoot (‰) | | δ14Cfoot (‰) | | pMC (%) | |
| Mean | SD | Mean | SD | Mean | SD |
| 332 | 13 | 22.14 | 6.74 | 37.82 | 3.87 | 103.0 | 0.7 |
|  | 24 | 30.04 | 5.60 | 37.16 | 4.26 | 103.8 | 0.6 |
|  | 44 | 39.53 | 4.48 | 48.28 | 4.28 | 104.8 | 0.5 |
|  | 54 | 26.19 | 5.74 | 17.96 | 4.23 | 103.4 | 0.6 |
| 463 | 12 | 29.58 | 3.37 | 36.54 | 2.96 | 103.8 | 0.3 |
|  | 15 | 24.77 | 5.69 | 40.80 | 4.27 | 103.3 | 0.6 |
|  | 39 | 25.61 | 5.11 | 37.95 | 4.27 | 103.4 | 0.5 |
|  | 40 | 30.48 | 5.19 | 43.03 | 4.27 | 103.9 | 0.5 |
|  | 48 | 27.76 | 4.70 | 39.22 | 4.25 | 103.6 | 0.5 |
| 653 | 5 | 37.59 | 5.37 | 60.99 | 4.36 | 104.6 | 0.5 |
|  | 6 | 30.64 | 8.74 | 26.17 | 3.86 | 103.9 | 0.9 |
|  | 7 | 35.59 | 5.73 | 40.17 | 4.26 | 104.4 | 0.6 |
|  | 43 | 28.12 | 4.76 | 38.02 | 3.87 | 103.6 | 0.5 |
|  | 47 | 16.45 | 4.97 | 30.48 | 4.23 | 102.4 | 0.5 |
|  | 55 | 15.67 | 4.61 | 28.89 | 4.23 | 102.4 | 0.5 |
| 872 | 4 | 28.82 | 6.36 | 35.48 | 4.25 | 103.7 | 0.6 |
|  | 11 | 31.21 | 6.69 | 40.97 | 4.26 | 103.9 | 0.7 |
|  | 18 | 28.41 | 4.87 | 42.50 | 4.26 | 103.7 | 0.5 |
|  | 23 | 32.79 | 5.99 | 41.22 | 4.24 | 104.1 | 0.6 |
|  | 37 | 24.74 | 5.83 | 38.44 | 4.94 | 103.3 | 0.6 |
|  | 1 | 19.26 | 4.71 | 31.55 | 4.23 | 102.7 | 0.5 |
| 1138 | 14 | 11.63 | 4.76 | 28.51 | 4.23 | 102.0 | 0.5 |
|  | 25 | 41.32 | 5.23 | 45.30 | 4.28 | 105.0 | 0.5 |
|  | 29 | 28.81 | 5.17 | 36.93 | 4.95 | 103.7 | 0.5 |
|  | 34 | 34.28 | 5.00 | 40.14 | 4.26 | 104.2 | 0.5 |
|  | 46 | 36.06 | 4.31 | 47.13 | 4.28 | 104.4 | 0.4 |
|  | 16 | 24.26 | 7.22 | 46.84 | 3.90 | 103.2 | 0.7 |
| 1337 | 19 | 19.14 | 5.56 | 33.74 | 4.29 | 102.7 | 0.6 |
|  | 32 | 42.42 | 4.51 | 50.21 | 4.29 | 105.1 | 0.5 |
|  | 35 | 46.89 | 4.51 | 55.78 | 4.31 | 105.5 | 0.5 |
|  | 41 | 44.20 | 4.91 | 45.90 | 4.29 | 105.2 | 0.5 |
|  | 62 | 13.17 | 5.26 | -1.28 | 5.17 | 102.1 | 0.5 |

Table S3 Δ14C, δ14C and percent Modern Carbon of mantle tissue (Δ14Cmantle, δ14Cmantle and pMC) in experimental specimens reared under six different *p*CO2 conditions. Mean ± 1 SD.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| *p*CO2  (μatm) | Specimen  No. | Δ14Cmantle (‰) | | δ14Cmantle (‰) | | pMC (%) | |
| Mean | SD | Mean | SD | Mean | SD |
| 332 | 21 | 36.02 | 4.80 | 24.96 | 3.26 | 104.4 | 0.5 |
|  | 24 | 26.34 | 6.79 | 42.56 | 3.89 | 103.4 | 0.7 |
|  | 44 | 33.19 | 4.94 | 33.74 | 3.87 | 104.1 | 0.5 |
|  | 54 | 21.19 | 4.92 | 44.85 | 3.87 | 102.9 | 0.5 |
|  | 63 | 38.26 | 5.50 | 42.33 | 4.28 | 104.6 | 0.6 |
| 463 | 12 | 36.54 | 2.96 | 36.54 | 2.96 | 103.8 | 0.3 |
|  | 15 | 41.68 | 3.82 | 38.48 | 2.97 | 105.0 | 0.4 |
|  | 40 | 25.53 | 5.66 | 52.68 | 3.91 | 103.4 | 0.6 |
|  | 48 | 26.79 | 4.85 | 26.16 | 3.85 | 103.5 | 0.5 |
|  | 57 | 18.79 | 4.97 | 35.50 | 3.85 | 102.7 | 0.5 |
| 653 | 5 | 29.33 | 3.05 | 44.21 | 2.99 | 103.7 | 0.3 |
|  | 6 | 19.98 | 3.07 | 37.71 | 2.97 | 102.8 | 0.3 |
|  | 7 | 5.95 | 5.00 | 23.62 | 3.83 | 101.4 | 0.5 |
|  | 47 | 18.58 | 4.21 | 44.13 | 3.88 | 102.7 | 0.4 |
|  | 55 | 22.99 | 4.30 | 33.22 | 4.24 | 103.1 | 0.4 |
| 872 | 4 | 28.25 | 3.33 | 37.25 | 2.98 | 103.6 | 0.3 |
|  | 11 | 50.00 | 3.56 | 37.69 | 2.95 | 105.8 | 0.4 |
|  | 23 | 14.75 | 4.58 | -10.88 | 3.84 | 102.3 | 0.5 |
|  | 33 | 14.29 | 3.85 | 25.23 | 3.82 | 102.2 | 0.4 |
|  | 37 | 24.03 | 3.95 | 13.74 | 3.82 | 103.2 | 0.4 |
| 1138 | 1 | 25.34 | 4.52 | 40.16 | 3.87 | 103.3 | 0.5 |
|  | 14 | 26.91 | 7.55 | 27.24 | 4.27 | 103.5 | 0.8 |
|  | 25 | 37.73 | 5.38 | 44.15 | 4.30 | 104.6 | 0.5 |
|  | 29 | 24.36 | 6.43 | 33.65 | 4.28 | 103.2 | 0.6 |
|  | 34 | 24.15 | 4.97 | 33.92 | 4.28 | 103.2 | 0.5 |
|  | 46 | 17.11 | 5.28 | 25.32 | 4.26 | 102.5 | 0.5 |
| 1337 | 16 | 40.74 | 4.00 | 39.94 | 3.27 | 104.9 | 0.4 |
|  | 19 | 29.33 | 5.76 | 36.30 | 4.29 | 103.7 | 0.6 |
|  | 35 | 23.87 | 5.10 | 25.50 | 4.27 | 103.2 | 0.5 |
|  | 41 | 14.94 | 6.44 | 25.08 | 4.26 | 102.3 | 0.6 |
|  | 62 | 26.52 | 9.96 | 41.25 | 4.15 | 103.5 | 1.0 |

Table S4 Δ14C, δ14C and percent Modern Carbon of shell (Δ14Cshell, δ14Cshell and pMC) in experimental specimens reared under six different *p*CO2 conditions. Mean ± 1 SD.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| *p*CO2  (μatm) | Specimen  No. | Δ14Cshell (‰) | | δ14Cshell (‰) | | pMC (%) | |
| Mean | SD | Mean | SD | Mean | SD |
| 332 | 13 | -11.09 | 21.19 | 138.27 | 24.20 | 99.67 | 2.14 |
|  | 21 | 15.46 | 11.54 | 248.87 | 11.33 | 102.35 | 1.16 |
|  | 24 | 7.31 | 9.60 | 236.70 | 11.73 | 101.53 | 0.97 |
|  | 44 | 43.19 | 12.79 | 183.75 | 9.66 | 105.14 | 1.29 |
|  | 63 | 29.54 | 3.25 | 73.49 | 2.89 | 103.77 | 0.33 |
| 463 | 12 | 31.87 | 5.77 | 68.06 | 3.78 | 104.00 | 0.58 |
|  | 15 | 45.06 | 9.51 | 179.43 | 9.51 | 105.33 | 0.96 |
|  | 39 | 27.96 | 14.30 | 200.44 | 10.35 | 103.61 | 1.44 |
|  | 40 | 23.49 | 3.05 | 64.94 | 2.88 | 103.16 | 0.31 |
|  | 48 | 19.40 | 2.94 | 51.35 | 2.89 | 102.74 | 0.30 |
|  | 57 | 27.68 | 2.88 | 77.62 | 2.89 | 103.58 | 0.29 |
| 653 | 6 | -27.67 | 28.47 | 104.22 | 22.81 | 102.42 | 2.87 |
|  | 7 | -56.55 | 42.42 | 113.64 | 42.44 | 95.09 | 4.28 |
|  | 43 | -5.62 | 4.14 | 31.94 | 3.73 | 100.22 | 0.42 |
|  | 47 | 37.59 | 29.86 | 153.69 | 28.80 | 104.58 | 3.01 |
|  | 55 | 2.64 | 2.96 | 41.42 | 2.85 | 101.06 | 0.30 |
| 872 | 4 | 26.15 | 5.08 | 24.39 | 3.69 | 103.43 | 0.51 |
|  | 11 | 1.57 | 4.57 | 31.06 | 3.70 | 100.95 | 0.46 |
|  | 18 | -13.35 | 9.85 | 189.96 | 10.83 | 99.44 | 0.99 |
|  | 23 | -23.15 | 31.95 | 51.78 | 27.68 | 98.46 | 3.22 |
|  | 33 | -6.63 | 4.16 | 35.64 | 3.71 | 100.12 | 0.42 |
|  | 37 | -34.42 | 2.74 | 14.37 | 2.82 | 97.32 | 0.28 |
| 1138 | 1 | -0.81 | 11.12 | 87.79 | 11.08 | 100.71 | 1.12 |
|  | 14 | -18.32 | 23.10 | 23.18 | 22.26 | 98.56 | 2.33 |
|  | 25 | -80.28 | 21.08 | 78.07 | 23.15 | 92.70 | 2.12 |
|  | 29 | -49.77 | 23.74 | 46.29 | 24.15 | 95.77 | 2.39 |
|  | 34 | -27.93 | 2.89 | 6.36 | 2.77 | 97.97 | 0.29 |
|  | 46 | -40.51 | 3.25 | -6.30 | 2.79 | 96.71 | 0.33 |
| 1337 | 16 | 1.53 | 10.44 | 165.79 | 11.02 | 100.94 | 1.05 |
|  | 19 | -67.49 | 7.91 | 175.43 | 9.48 | 93.99 | 0.80 |
|  | 32 | -61.46 | 22.11 | 41.63 | 22.04 | 94.59 | 2.23 |
|  | 35 | -37.69 | 21.23 | 101.82 | 11.46 | 96.99 | 2.14 |
|  | 62 | -47.59 | 2.70 | -7.30 | 2.79 | 95.99 | 0.27 |

Table S5 Δ14C, δ14C and percent Modern Carbon (Δ14Cplankton, δ14Cplankton and pMC) of plankton samples (*Tetraselmis tetrathele*, *Pavlova lutheri*) cultured in modern seawater. Mean ± 1 SD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Plankton species | Δ14Cplankton (‰) | | δ14Cplankton (‰) | | pMC (%) | | Date of sampling | Details of sampling\* |
| Mean | SD | Mean | SD | Mean | SD |
| *T. tetrathele* | 4.95 | 2.36 | 8.92 | 2.28 | 101.3 | 0.2 | 11 Dec. 2015 | 1 |
|  | 8.58 | 2.57 | -0.42 | 2.27 | 101.7 | 0.3 | 11 Dec. 2015 | 1 |
|  | 2.72 | 2.45 | 10.39 | 2.29 | 101.1 | 0.2 | 11 Dec. 2015 | 1 |
|  | 9.68 | 2.33 | 12.86 | 2.28 | 101.8 | 0.2 | 11 Dec. 2015 | 1 |
|  | 0.69 | 3.50 | 22.31 | 2.95 | 100.9 | 0.4 | 11 Dec. 2015 | 1 |
|  | 6.52 | 3.64 | 22.69 | 2.96 | 101.4 | 0.4 | 11 Dec. 2015 | 1 |
|  | 8.73 | 2.96 | 23.69 | 2.96 | 101.7 | 0.3 | 11 Dec. 2015 | 1 |
|  | 8.04 | 3.88 | 12.49 | 2.79 | 101.6 | 0.4 | 24 Jan. 2015 | 2 |
|  | 7.78 | 3.09 | 2.83 | 2.81 | 101.6 | 0.3 | 24 Jan. 2015 | 2 |
|  |  |  |  |  |  |  |  |  |
| *P. lutheri* | 8.37 | 2.88 | 4.94 | 2.27 | 101.6 | 0.3 | 11, Dec. 1015 | 1 |
|  | 12.84 | 3.03 | 8.57 | 2.27 | 102.1 | 0.3 | 11, Dec. 1015 | 1 |
|  | 12.90 | 2.99 | 11.99 | 2.28 | 102.1 | 0.3 | 11, Dec. 1015 | 1 |
|  | 12.15 | 2.74 | -2.26 | 2.26 | 102.0 | 0.3 | 11, Dec. 1015 | 1 |
|  | 6.32 | 2.92 | -7.29 | 2.26 | 101.4 | 0.3 | 11, Dec. 1015 | 1 |
|  | 9.10 | 3.26 | 32.06 | 2.84 | 101.7 | 0.3 | 24 Jan. 2015 | 2 |
|  | 9.65 | 3.82 | 9.01 | 2.79 | 101.8 | 0.4 | 24 Jan. 2015 | 2 |
|  | 14.06 | 4.09 | 15.77 | 2.80 | 102.2 | 0.4 | 24 Jan. 2015 | 2 |

\*Details of sampling: (1) centrifuged, and dried in a freeze-dry machine, (2) collected by a MF-Millipore membrane filter

Table S6 δ13C and δ15N of foot and mantle tissues of experimental specimens reared under six different *p*CO2 conditions.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *p*CO2 (μatm) | Specimen No. | δ13Cfoot (‰) | δ15Nfoot (‰) | δ13Cmantle (‰) | δ15Nmantle (‰) |
| 332 | 13 | –20.0 | 7.2 | –19.3 | 7.3 |
|  | 21 | –20.5 | 7.0 | –19.8 | 7.2 |
|  | 24 | –20.1 | 6.2 | –19.5 | 7.3 |
|  | 44 | –20.6 | 6.3 | –19.6 | 6.6 |
|  | 54 | –19.6 | 8.0 | –19.7 | 7.7 |
|  | 63 | –20.2 | 6.4 | –19.6 | 6.6 |
| 463 | 12 | –20.1 | 7.5 | –18.8 | 8.2 |
|  | 15 | –20.3 | 7.8 | –18.9 | 7.9 |
|  | 39 | –19.4 | 8.2 | –19.3 | 8.5 |
|  | 40 | –21.1 | 7.1 | –19.6 | 7.4 |
|  | 48 | –20.1 | 7.0 | –19.7 | 7.4 |
|  | 57 | –19.2 | 7.5 | –19.8 | 8.0 |
| 653 | 5 | –19.0 | 9.3 | –19.0 | 8.8 |
|  | 6 | –20.6 | 6.1 | –19.4 | 7.9 |
|  | 7 | –20.1 | 7.8 | –19.3 | 8.4 |
|  | 43 | –19.9 | 7.7 | –19.8 | 7.3 |
|  | 47 | –19.3 | 8.2 | –19.8 | 7.9 |
|  | 55 | –20.3 | 7.4 | –19.1 | 8.3 |
| 872 | 4 | –19.9 | 7.2 | –19.5 | 7.4 |
|  | 11 | –19.4 | 8.5 | –19.4 | 8.9 |
|  | 18 | –19.3 | 7.3 | –19.2 | 7.3 |
|  | 23 | –20.9 | 5.5 | –19.7 | 6.1 |
|  | 33 | –19.9 | 6.7 | –19.7 | 7.1 |
|  | 37 | –20.4 | 6.7 | –19.3 | 7.9 |
| 1137 | 1 | –20.7 | 6.3 | –20.5 | 6.5 |
|  | 14 | –18.9 | 8.5 | –19.4 | 8.1 |
|  | 25 | –19.5 | 8.2 | –19.7 | 7.5 |
|  | 29 | –19.3 | 8.8 | –19.4 | 8.1 |
|  | 34 | –20.9 | 6.0 | –20.4 | 7.0 |
|  | 46 | –20.5 | 7.3 | –19.6 | 8.0 |
| 1337 | 16 | –19.2 | 8.8 | –19.2 | 8.8 |
|  | 19 | –20.7 | 7.5 | –19.4 | 8.4 |
|  | 26 | –20.2 | 10.6 |  |  |
|  | 32 | –20.3 | 7.8 | –19.1 | 8.6 |
|  | 35 | –20.1 | 6.9 | –19.3 | 10.7 |
|  | 41 | –20.5 | 6.8 | –19.6 | 7.4 |
|  | 62 |  |  | –19.9 | 7.5 |

Table S7 δ13C of plankton samples cultured in natural seawater and collected by a MF-Millipore membrane filter.

|  |  |  |  |
| --- | --- | --- | --- |
| Plankton species | δ13Cplankton (‰) | δ14Nplankton (‰) | Date of sampling |
| *T. tetrathele* | –24.0 | 0.8 | 19 Dec. 2013 |
|  | –21.9 | 1.7 | 24 Oct. 2013 |
| *P. lutheri* | –23.8 | 1.3 | 19 Dec. 2013 |
|  |  |  |  |
| Equal mixture of two species | –23.5 | 1.1 | 19 Dec. 2013 |
|  | –22.1 | 0.9 | 24 Oct. 2013 |

**Figure S1** δ13C vs δ15N in plankton and foot and mantle tissues.