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### Title

Elevated CO2 Enhances Otolith Growth in Young Fish (Supplementary Data)

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### Elevated CO<sub>2</sub> Enhances Otolith Growth in Young Fish

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**Abstract:** A large fraction of the carbon dioxide added to the atmosphere by human activity enters the sea, causing ocean acidification. We show that otoliths (aragonitic ear bones) of young fish grown under high CO<sub>2</sub> (low pH) conditions are larger than normal, contrary to expectation. We hypothesize that CO<sub>2</sub> moves freely through the epithelium around the otoliths in young fish, accelerating otolith growth while the local pH is controlled. This is the converse of the effect reported for structural biominerals.

Table column parameters are defined as follows:

- Experiment number – a sequential identification of experiments
- pCO<sub>2,atm</sub> (μatm) – partial pressure of carbon dioxide in infused gas
- Fish number – a unique number for each fish, in order of analysis
- Age (days) – age of the fish when sacrificed
- Number of otoliths – number of sagittal otoliths from a single fish represented by area and circularity; 1 = single otolith, 2 = mean for two otoliths
- Orientation – a code indicating the orientation of an otolith viewed in the scanning electron microscope; 1 = dorsal or ventral, 2 = lateral
- Area (μm<sup>2</sup>) – the total area of the otolith cross-section
- Circularity –  $4\pi \cdot \text{area} / \text{perimeter}^2$  (1 = circle, 0 = line)
- Dry weight (μg) – dry weight of fish

nd = no data

| Experiment number | pCO <sub>2,atm</sub> (µatm) | Fish number | Age (days) | Number of otoliths | Orientation | Area (µm <sup>2</sup> ) | Circularity | Dry weight (µg) |
|-------------------|-----------------------------|-------------|------------|--------------------|-------------|-------------------------|-------------|-----------------|
| 1                 | 380                         | 1           | 7          | 1                  | 1           | 1477                    | 0.829       | nd              |
| 1                 | 380                         | 2           | 7          | 2                  | 1           | 1412                    | 0.882       | nd              |
| 1                 | 380                         | 2           | 7          | 1                  | 2           | 1450                    | 0.847       | nd              |
| 1                 | 380                         | 3           | 7          | 1                  | 1           | 1507                    | 0.873       | nd              |
| 1                 | 380                         | 3           | 7          | 1                  | 2           | 1239                    | 0.868       | nd              |
| 1                 | 380                         | 4           | 7          | 2                  | 1           | 1205                    | 0.875       | nd              |
| 1                 | 380                         | 5           | 7          | 1                  | 1           | 1422                    | 0.889       | nd              |
| 1                 | 380                         | 6           | 7          | 1                  | 1           | 1154                    | 0.877       | nd              |
| 1                 | 380                         | 6           | 7          | 1                  | 2           | 1212                    | 0.877       | nd              |
| 1                 | 380                         | 7           | 7          | 1                  | 1           | 1353                    | 0.874       | nd              |
| 1                 | 380                         | 7           | 7          | 1                  | 2           | 1241                    | 0.875       | nd              |
| 1                 | 380                         | 8           | 7          | 2                  | 1           | 1280                    | 0.884       | nd              |
| 1                 | 380                         | 17          | 7          | 2                  | 1           | 1406                    | 0.886       | nd              |
| 1                 | 380                         | 18          | 7          | 1                  | 1           | 1169                    | 0.828       | nd              |
| 1                 | 380                         | 18          | 7          | 1                  | 2           | 1409                    | 0.722       | nd              |
| 1                 | 380                         | 19          | 7          | 2                  | 2           | 1354                    | 0.892       | nd              |
| 1                 | 380                         | 20          | 7          | 2                  | 1           | 1212                    | 0.889       | nd              |
| 1                 | 380                         | 21          | 7          | 2                  | 1           | 1319                    | 0.882       | nd              |
| 1                 | 380                         | 22          | 7          | 2                  | 1           | 1199                    | 0.869       | nd              |
| 1                 | 380                         | 23          | 7          | 2                  | 1           | 1511                    | 0.856       | nd              |
| 1                 | 2558                        | 9           | 7          | 2                  | 1           | 1652                    | 0.835       | nd              |
| 1                 | 2558                        | 10          | 7          | 1                  | 1           | 1495                    | 0.878       | nd              |
| 1                 | 2558                        | 10          | 7          | 1                  | 2           | 1620                    | 0.886       | nd              |
| 1                 | 2558                        | 11          | 7          | 1                  | 1           | 1449                    | 0.852       | nd              |
| 1                 | 2558                        | 11          | 7          | 1                  | 2           | 1481                    | 0.825       | nd              |
| 1                 | 2558                        | 12          | 7          | 2                  | 1           | 1605                    | 0.881       | nd              |
| 1                 | 2558                        | 13          | 7          | 2                  | 1           | 1657                    | 0.871       | nd              |
| 1                 | 2558                        | 14          | 7          | 1                  | 1           | 1487                    | 0.874       | nd              |
| 1                 | 2558                        | 15          | 7          | 2                  | 1           | 1494                    | 0.875       | nd              |
| 1                 | 2558                        | 16          | 7          | 2                  | 1           | 1675                    | 0.869       | nd              |
| 1                 | 2558                        | 24          | 7          | 2                  | 1           | 1376                    | 0.874       | nd              |
| 1                 | 2558                        | 25          | 7          | 2                  | 1           | 1407                    | 0.874       | nd              |
| 1                 | 2558                        | 26          | 7          | 1                  | 1           | 1505                    | 0.889       | nd              |
| 1                 | 2558                        | 26          | 7          | 1                  | 2           | 1569                    | 0.883       | nd              |
| 1                 | 2558                        | 27          | 7          | 2                  | 1           | 1439                    | 0.850       | nd              |
| 1                 | 2558                        | 28          | 7          | 1                  | 1           | 1603                    | 0.887       | nd              |
| 1                 | 2558                        | 28          | 7          | 1                  | 2           | 1526                    | 0.881       | nd              |
| 1                 | 2558                        | 29          | 7          | 2                  | 1           | 1586                    | 0.872       | nd              |
| 1                 | 2558                        | 30          | 7          | 2                  | 1           | 1674                    | 0.872       | nd              |
| 1                 | 2558                        | 31          | 7          | 2                  | 1           | 1467                    | 0.884       | nd              |
| 2                 | 380                         | 32          | 7          | 1                  | 2           | 1201                    | 0.801       | nd              |

|   |      |    |   |   |   |      |       |    |
|---|------|----|---|---|---|------|-------|----|
| 2 | 380  | 33 | 7 | 2 | 1 | 1251 | 0.885 | nd |
| 2 | 380  | 34 | 7 | 2 | 1 | 1417 | 0.867 | nd |
| 2 | 380  | 35 | 7 | 2 | 1 | 1317 | 0.879 | nd |
| 2 | 380  | 36 | 7 | 2 | 1 | 1357 | 0.879 | nd |
| 2 | 380  | 37 | 7 | 1 | 1 | 1276 | 0.873 | nd |
| 2 | 380  | 38 | 7 | 2 | 1 | 1309 | 0.878 | nd |
| 2 | 380  | 39 | 7 | 2 | 1 | 1325 | 0.884 | nd |
| 2 | 380  | 40 | 7 | 2 | 1 | 1402 | 0.884 | nd |
| 2 | 380  | 41 | 7 | 1 | 1 | 1331 | 0.884 | nd |
| 2 | 380  | 42 | 7 | 2 | 1 | 1441 | 0.846 | nd |
| 2 | 380  | 43 | 7 | 2 | 1 | 1163 | 0.871 | nd |
| 2 | 380  | 44 | 7 | 2 | 1 | 1363 | 0.885 | nd |
| 2 | 380  | 45 | 7 | 2 | 1 | 1314 | 0.859 | nd |
| 2 | 380  | 46 | 7 | 2 | 1 | 1324 | 0.801 | nd |
| 2 | 380  | 47 | 7 | 2 | 1 | 1528 | 0.881 | nd |
| 2 | 2558 | 48 | 7 | 2 | 1 | 1497 | 0.877 | nd |
| 2 | 2558 | 49 | 7 | 2 | 1 | 1612 | 0.852 | nd |
| 2 | 2558 | 50 | 7 | 1 | 1 | 1522 | 0.845 | nd |
| 2 | 2558 | 50 | 7 | 1 | 2 | 1352 | 0.803 | nd |
| 2 | 2558 | 51 | 7 | 1 | 1 | 1561 | 0.836 | nd |
| 2 | 2558 | 52 | 7 | 1 | 1 | 1487 | 0.866 | nd |
| 2 | 2558 | 53 | 7 | 2 | 1 | 1405 | 0.866 | nd |
| 2 | 2558 | 54 | 7 | 2 | 1 | 1524 | 0.860 | nd |
| 2 | 2558 | 55 | 7 | 1 | 1 | 1662 | 0.890 | nd |
| 2 | 2558 | 55 | 7 | 1 | 2 | 1447 | 0.870 | nd |
| 2 | 2558 | 56 | 7 | 2 | 1 | 1611 | 0.879 | nd |
| 2 | 2558 | 57 | 7 | 1 | 1 | 1645 | 0.883 | nd |
| 2 | 2558 | 57 | 7 | 1 | 2 | 1474 | 0.877 | nd |
| 2 | 2558 | 58 | 7 | 2 | 1 | 1643 | 0.878 | nd |
| 2 | 2558 | 58 | 7 | 1 | 2 | 1566 | 0.886 | nd |
| 2 | 2558 | 60 | 7 | 1 | 1 | 1497 | 0.791 | nd |
| 2 | 2558 | 61 | 7 | 1 | 1 | 1635 | 0.882 | nd |
| 2 | 2558 | 61 | 7 | 1 | 2 | 1486 | 0.794 | nd |
| 2 | 2558 | 62 | 7 | 2 | 1 | 1598 | 0.878 | nd |
| 2 | 2558 | 63 | 7 | 2 | 1 | 1546 | 0.881 | nd |
| 3 | 380  | 64 | 8 | 1 | 1 | 2152 | 0.913 | nd |
| 3 | 380  | 64 | 8 | 1 | 2 | 1996 | 0.920 | nd |
| 3 | 380  | 65 | 8 | 2 | 1 | 2271 | 0.900 | nd |
| 3 | 380  | 66 | 8 | 2 | 1 | 2090 | 0.915 | nd |
| 3 | 380  | 67 | 8 | 2 | 1 | 1930 | 0.908 | nd |
| 3 | 380  | 68 | 8 | 2 | 1 | 1698 | 0.919 | nd |
| 3 | 380  | 69 | 8 | 1 | 1 | 2003 | 0.927 | nd |
| 3 | 380  | 70 | 8 | 2 | 1 | 2334 | 0.921 | nd |
| 3 | 380  | 71 | 8 | 1 | 1 | 2381 | 0.911 | nd |
| 3 | 380  | 72 | 8 | 2 | 1 | 1979 | 0.910 | nd |
| 3 | 380  | 73 | 8 | 1 | 1 | 2153 | 0.924 | nd |
| 3 | 380  | 74 | 8 | 2 | 1 | 2085 | 0.927 | nd |
| 3 | 380  | 75 | 8 | 2 | 1 | 2301 | 0.929 | nd |
| 3 | 380  | 76 | 8 | 2 | 1 | 2255 | 0.909 | nd |

|   |     |     |   |   |   |      |       |    |
|---|-----|-----|---|---|---|------|-------|----|
| 3 | 380 | 77  | 8 | 2 | 1 | 2183 | 0.921 | nd |
| 3 | 380 | 78  | 8 | 2 | 1 | 2110 | 0.919 | nd |
| 3 | 380 | 79  | 8 | 2 | 1 | 2198 | 0.912 | nd |
| 3 | 993 | 80  | 8 | 2 | 1 | 2241 | 0.932 | nd |
| 3 | 993 | 81  | 8 | 2 | 1 | 2138 | 0.929 | nd |
| 3 | 993 | 82  | 8 | 2 | 1 | 1851 | 0.913 | nd |
| 3 | 993 | 83  | 8 | 2 | 1 | 2673 | 0.926 | nd |
| 3 | 993 | 84  | 8 | 1 | 1 | 2206 | 0.906 | nd |
| 3 | 993 | 84  | 8 | 1 | 2 | 2067 | 0.910 | nd |
| 3 | 993 | 85  | 8 | 1 | 1 | 2116 | 0.916 | nd |
| 3 | 993 | 85  | 8 | 1 | 2 | 2019 | 0.924 | nd |
| 3 | 993 | 86  | 8 | 1 | 1 | 2228 | 0.923 | nd |
| 3 | 993 | 86  | 8 | 1 | 2 | 1949 | 0.908 | nd |
| 3 | 993 | 87  | 8 | 2 | 1 | 2328 | 0.926 | nd |
| 3 | 993 | 88  | 8 | 2 | 1 | 2391 | 0.927 | nd |
| 3 | 993 | 89  | 8 | 2 | 1 | 2312 | 0.916 | nd |
| 3 | 993 | 90  | 8 | 1 | 1 | 2412 | 0.928 | nd |
| 3 | 993 | 90  | 8 | 1 | 2 | 1930 | 0.912 | nd |
| 3 | 993 | 91  | 8 | 2 | 1 | 2301 | 0.917 | nd |
| 3 | 993 | 92  | 8 | 2 | 1 | 2448 | 0.926 | nd |
| 3 | 993 | 93  | 8 | 1 | 1 | 1950 | 0.915 | nd |
| 3 | 993 | 94  | 8 | 2 | 1 | 2312 | 0.922 | nd |
| 3 | 993 | 95  | 8 | 2 | 1 | 2501 | 0.923 | nd |
| 4 | 380 | 96  | 7 | 1 | 1 | 1266 | 0.928 | nd |
| 4 | 380 | 96  | 7 | 1 | 2 | 1141 | 0.932 | nd |
| 4 | 380 | 97  | 7 | 2 | 1 | 1566 | 0.915 | nd |
| 4 | 380 | 98  | 7 | 1 | 1 | 1541 | 0.924 | nd |
| 4 | 380 | 99  | 7 | 1 | 1 | 1368 | 0.921 | nd |
| 4 | 380 | 100 | 7 | 2 | 1 | 1082 | 0.914 | nd |
| 4 | 380 | 101 | 7 | 2 | 1 | 1606 | 0.929 | nd |
| 4 | 380 | 102 | 7 | 2 | 1 | 1537 | 0.923 | nd |
| 4 | 380 | 103 | 7 | 1 | 1 | 1385 | 0.919 | nd |
| 4 | 380 | 103 | 7 | 1 | 2 | 1141 | 0.932 | nd |
| 4 | 993 | 104 | 7 | 2 | 1 | 1589 | 0.918 | nd |
| 4 | 993 | 105 | 7 | 1 | 1 | 1506 | 0.906 | nd |
| 4 | 993 | 105 | 7 | 1 | 2 | 1339 | 0.901 | nd |
| 4 | 993 | 106 | 7 | 1 | 1 | 1553 | 0.911 | nd |
| 4 | 993 | 106 | 7 | 1 | 2 | 1413 | 0.903 | nd |
| 4 | 993 | 107 | 7 | 1 | 1 | 1578 | 0.913 | nd |
| 4 | 993 | 107 | 7 | 1 | 2 | 1498 | 0.906 | nd |
| 4 | 993 | 108 | 7 | 2 | 1 | 1515 | 0.921 | nd |
| 4 | 993 | 109 | 7 | 2 | 2 | 1543 | 0.910 | nd |
| 4 | 993 | 110 | 7 | 1 | 1 | 1638 | 0.929 | nd |
| 4 | 993 | 110 | 7 | 1 | 2 | 1579 | 0.914 | nd |
| 4 | 993 | 111 | 7 | 2 | 1 | 1551 | 0.910 | nd |
| 4 | 993 | 112 | 7 | 1 | 1 | 1603 | 0.924 | nd |
| 4 | 993 | 112 | 7 | 1 | 2 | 1904 | 0.906 | nd |
| 4 | 993 | 113 | 7 | 2 | 1 | 1455 | 0.926 | nd |
| 4 | 993 | 114 | 7 | 2 | 1 | 1625 | 0.931 | nd |

|   |      |     |   |    |    |      |       |    |
|---|------|-----|---|----|----|------|-------|----|
| 4 | 993  | 115 | 7 | 2  | 1  | 1520 | 0.931 | nd |
| 4 | 993  | 116 | 7 | 2  | 1  | 1496 | 0.919 | nd |
| 4 | 993  | 117 | 7 | 2  | 1  | 1496 | 0.921 | nd |
| 4 | 993  | 118 | 7 | 1  | 1  | 1606 | 0.933 | nd |
| 4 | 993  | 118 | 7 | 1  | 2  | 1562 | 0.934 | nd |
| 4 | 993  | 119 | 7 | 1  | 1  | 1452 | 0.932 | nd |
| 4 | 993  | 119 | 7 | 1  | 2  | 1516 | 0.918 | nd |
| 5 | 380  | 120 | 7 | nd | nd | nd   | nd    | 52 |
| 5 | 380  | 121 | 7 | nd | nd | nd   | nd    | 75 |
| 5 | 380  | 122 | 7 | nd | nd | nd   | nd    | 73 |
| 5 | 380  | 123 | 7 | nd | nd | nd   | nd    | 73 |
| 5 | 380  | 124 | 7 | nd | nd | nd   | nd    | 72 |
| 5 | 380  | 125 | 7 | nd | nd | nd   | nd    | 69 |
| 5 | 380  | 126 | 7 | nd | nd | nd   | nd    | 56 |
| 5 | 380  | 127 | 7 | nd | nd | nd   | nd    | 67 |
| 5 | 380  | 128 | 7 | nd | nd | nd   | nd    | 67 |
| 5 | 380  | 129 | 7 | nd | nd | nd   | nd    | 68 |
| 5 | 380  | 130 | 7 | nd | nd | nd   | nd    | 72 |
| 5 | 380  | 131 | 7 | nd | nd | nd   | nd    | 66 |
| 5 | 380  | 132 | 7 | nd | nd | nd   | nd    | 74 |
| 5 | 380  | 133 | 7 | nd | nd | nd   | nd    | 70 |
| 5 | 380  | 134 | 7 | nd | nd | nd   | nd    | 73 |
| 5 | 380  | 135 | 7 | nd | nd | nd   | nd    | 80 |
| 5 | 380  | 136 | 7 | nd | nd | nd   | nd    | 72 |
| 5 | 380  | 137 | 7 | nd | nd | nd   | nd    | 75 |
| 5 | 380  | 138 | 7 | nd | nd | nd   | nd    | 66 |
| 5 | 380  | 139 | 7 | nd | nd | nd   | nd    | 61 |
| 5 | 380  | 140 | 7 | nd | nd | nd   | nd    | 78 |
| 5 | 380  | 141 | 7 | nd | nd | nd   | nd    | 67 |
| 5 | 380  | 142 | 7 | nd | nd | nd   | nd    | 83 |
| 5 | 380  | 143 | 7 | nd | nd | nd   | nd    | 67 |
| 5 | 380  | 144 | 7 | nd | nd | nd   | nd    | 66 |
| 5 | 380  | 145 | 7 | nd | nd | nd   | nd    | 66 |
| 5 | 380  | 146 | 7 | nd | nd | nd   | nd    | 68 |
| 5 | 380  | 147 | 7 | nd | nd | nd   | nd    | 65 |
| 5 | 380  | 148 | 7 | nd | nd | nd   | nd    | 70 |
| 5 | 380  | 149 | 7 | nd | nd | nd   | nd    | 67 |
| 5 | 2558 | 150 | 7 | nd | nd | nd   | nd    | 64 |
| 5 | 2558 | 151 | 7 | nd | nd | nd   | nd    | 83 |
| 5 | 2558 | 152 | 7 | nd | nd | nd   | nd    | 59 |
| 5 | 2558 | 153 | 7 | nd | nd | nd   | nd    | 67 |
| 5 | 2558 | 154 | 7 | nd | nd | nd   | nd    | 55 |
| 5 | 2558 | 155 | 7 | nd | nd | nd   | nd    | 76 |
| 5 | 2558 | 156 | 7 | nd | nd | nd   | nd    | 66 |
| 5 | 2558 | 157 | 7 | nd | nd | nd   | nd    | 70 |
| 5 | 2558 | 158 | 7 | nd | nd | nd   | nd    | 73 |
| 5 | 2558 | 159 | 7 | nd | nd | nd   | nd    | 73 |
| 5 | 2558 | 160 | 7 | nd | nd | nd   | nd    | 64 |
| 5 | 2558 | 161 | 7 | nd | nd | nd   | nd    | 71 |

|   |      |     |   |    |    |    |    |    |
|---|------|-----|---|----|----|----|----|----|
| 5 | 2558 | 162 | 7 | nd | nd | nd | nd | 75 |
| 5 | 2558 | 163 | 7 | nd | nd | nd | nd | 71 |
| 5 | 2558 | 164 | 7 | nd | nd | nd | nd | 69 |
| 5 | 2558 | 165 | 7 | nd | nd | nd | nd | 55 |
| 5 | 2558 | 166 | 7 | nd | nd | nd | nd | 66 |
| 5 | 2558 | 167 | 7 | nd | nd | nd | nd | 73 |
| 5 | 2558 | 168 | 7 | nd | nd | nd | nd | 70 |
| 5 | 2558 | 169 | 7 | nd | nd | nd | nd | 72 |
| 5 | 2558 | 170 | 7 | nd | nd | nd | nd | 64 |
| 5 | 2558 | 171 | 7 | nd | nd | nd | nd | 68 |
| 5 | 2558 | 172 | 7 | nd | nd | nd | nd | 73 |
| 5 | 2558 | 173 | 7 | nd | nd | nd | nd | 76 |
| 5 | 2558 | 174 | 7 | nd | nd | nd | nd | 68 |
| 5 | 2558 | 175 | 7 | nd | nd | nd | nd | 68 |
| 5 | 2558 | 176 | 7 | nd | nd | nd | nd | 61 |
| 5 | 2558 | 177 | 7 | nd | nd | nd | nd | 67 |
| 5 | 2558 | 178 | 7 | nd | nd | nd | nd | 60 |

