

Carry-over effects of ocean acidification in a cold-water lecithotrophic holothuroid

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METHODS

Lipid Analysis

Briefly, samples were homogenized in a 2:1 chloroform:methanol solution using a Polytron PCU-2-100 homogenizer (Brinkmann Instruments, Rexdale, Ontario, Canada). Chloroform extracted water was used to bring the sample to a methanol:chloroform:water ratio of 1:2:1. Next, samples were sonicated for 5 minutes in an ice bath and centrifuged at 3000 rpm for two minutes. The bottom organic layer was then removed using a double pipetting technique to avoid disturbing the aqueous top layer. Chloroform was then added back into the sample and the procedure was repeated a total of three times. All organic layers were pooled and concentrated using a flash-evaporator (Buchler Instruments, Fort Lee, New Jersey, USA). Final samples were blown down to volume using nitrogen, sealed with Teflon tape and stored at -20°C until measurements of fatty acids and total lipid classes were taken. For fatty acid analysis, 100-200 µL (depending on concentration of original sample) of lipid extract were transferred to lipid clean vials. Fatty acids were extracted using a FAME derivatization; lipid extracts were trans-esterified using Hilditch reagent (1.5H₂SO₄:98.5 anhydrous MeOH) for 1 h at 100 °C. FAMES were analysed on a HP 6890 Series GC system and run for 30 minutes. Chromatographs were compared to a prepared standard and analyzed using Varian Galaxie Chromatography Data System, version 1.93.2 (Agilent Technologies, Colorado, USA).

Total lipid classes, were determined using a series of developing and conditioning sequences routinely used for the separation of aquatic lipid classes on Chromarods (quartz rod covered in silica) (Parrish 1987). Briefly, samples were spotted on Chromarods (x µl-amount will be dependent on original samples) and focused in acetone, then developed twice in hexane:diethyl ether:formic acid (98.95:1:0.05). After drying for five minutes in constant humidity, the rods were scanned using an Iatroscan MK-6 to classify non-polar lipids. The Iatroscan burns samples in a hydrogen flame at a temperature of 800°C; ions are burned off and collected to form peaks for identification. Next, to remove polar lipids two development sequences were used. First, samples were developed in hexane:diethyl ether:formic acid (79.9:20:0.1), dried and scanned. For the last sequence, samples were developed twice in 100% acetone, then developed twice in chloroform:methanol:chloroform-extracted-water (5:4:1) and scanned a final time. Chromatographs were compared to a prepared standard and analyzed using PeakSimple Chromatography Software, version 2.38 (SRI Instruments, California, USA).

Reference

Parrish CC (1987) Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. *Can J Fish Aqua Sci* 44(4): 722-731

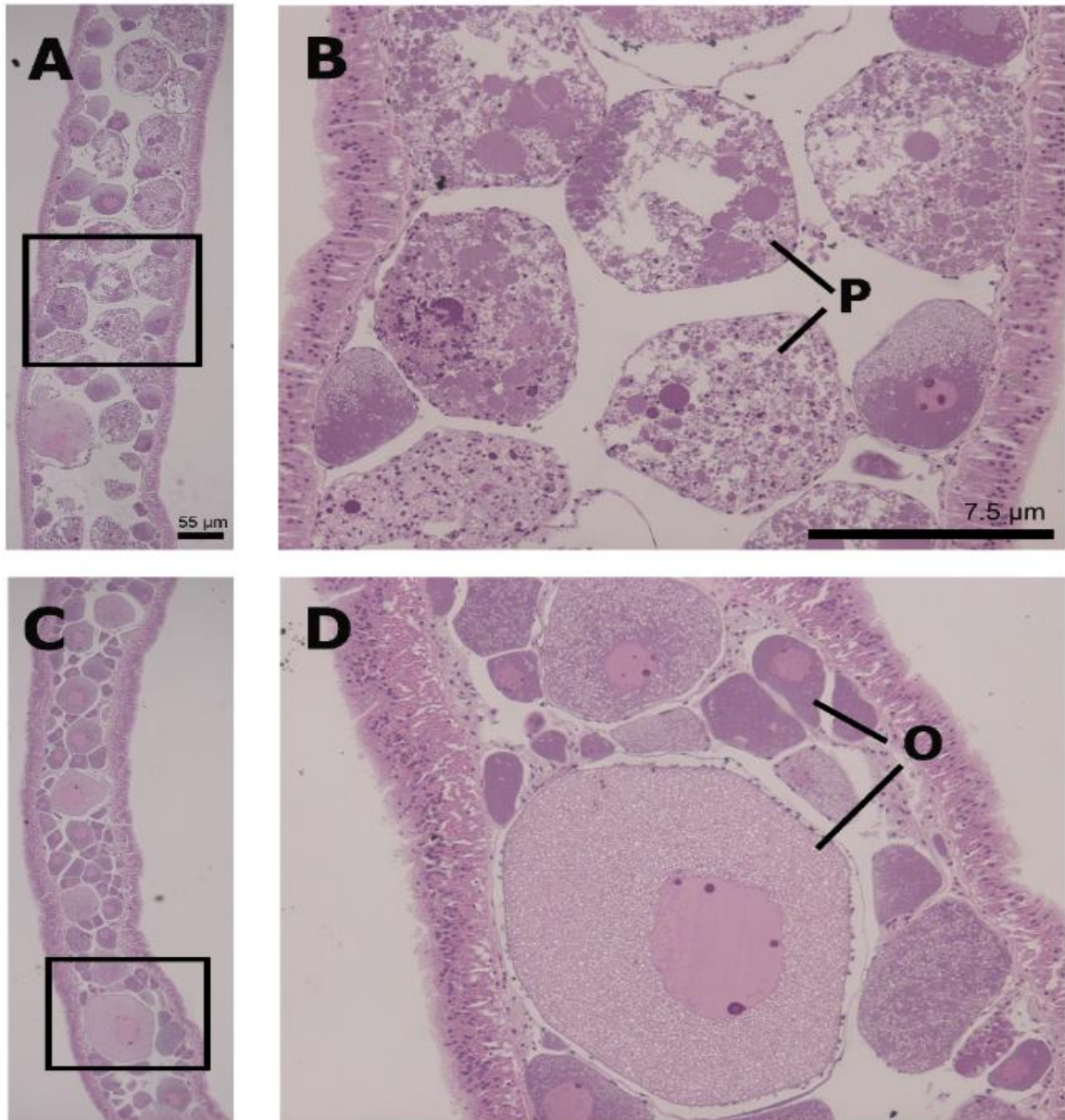


Fig. S1. Histology of gonad tubules of *C. frondosa* (longitudinal sections) at T19 (May 7, 2014) showing difference between treatments. A and B (close up) from ambient pH; C and D (close up) from low pH. P: phagocytized oocytes; O: oocyte

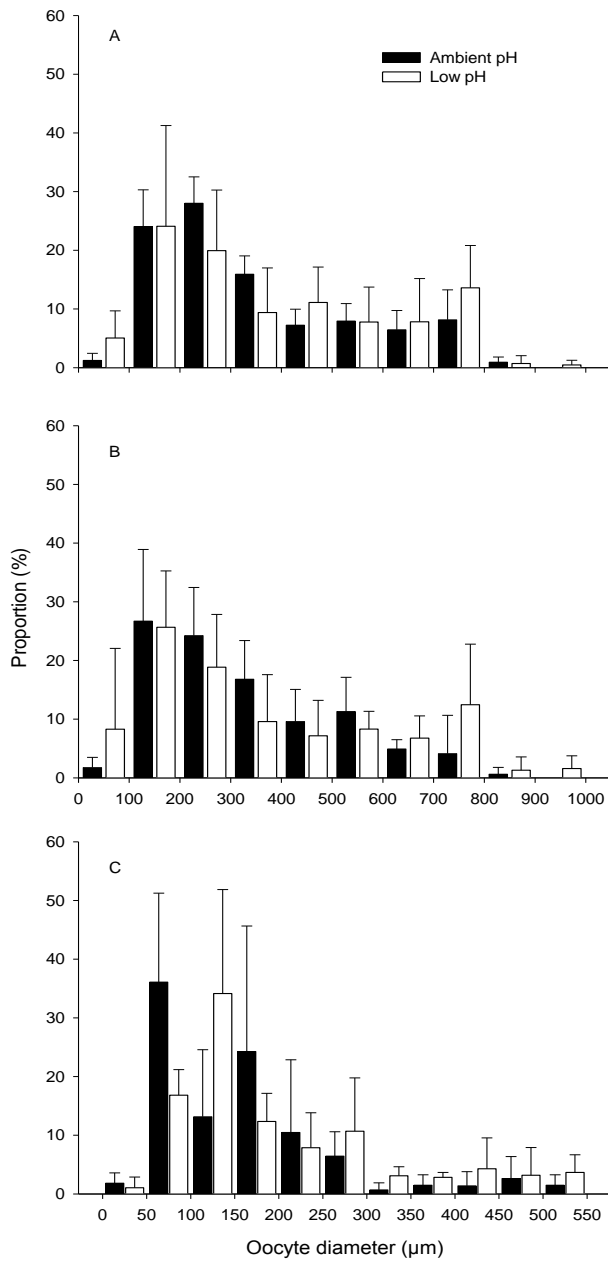


Fig. S2. Oocyte size frequency distributions in *C. frondosa* determined from A) tubule contents at T10 (March 12, 2014), B) tubule contents at T19 (May 7, 2014) and C) histological examination at T19 (May 7, 2014). Data in A and B shown as mean \pm SD (n=5-7). Data in C shown as mean \pm SD (n = 3)