

Radio- and stable carbon isotopic responses in experimentally-cultured bivalves for the understanding of acidification effect on bivalve nutrient uptake and biomineralization

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The effects of ocean acidification on bivalve growth and calcification have been widely debated in recent years due to potential impacts on the aquaculture sector and thus marine biodiversity. This study aims to elucidate the effects of ocean acidification on bivalve nutrient assimilation into its various components via laboratory culture experiments at six different $p\text{CO}_2$ levels at 25 °C (332, 463, 653, 872, 1137, and 1337 μatm). Radio carbon ($\Delta^{14}\text{C}$) and stable carbon ($\delta^{13}\text{C}$) isotopic analysis will be undertaken to investigate: (1) the proportion of incorporation of carbon from ambient DIC and metabolic CO_2 into bivalve shell and tissues; and (2) effects of changing $p\text{CO}_2$ concentrations on carbon incorporation. Our research marks the first attempt to trace partitioning of nutrients within a single species via application of bomb-pulse radiocarbon principles. By using modern samples in a culture experiment of ocean acidification by using CO_2 gas derived from fossil fuels, differences in contribution from end-members of DIC and metabolic CO_2 can be clearly resolved in high resolution. Combination of radiocarbon techniques with stable isotopic analysis will allow biological contributions to be parsed from a geochemical perspective, providing a comprehensive understanding of bivalve nutrient assimilation.

Specimens of the filter-feeding clam *Scapharca broughtonii* (Bivalvia: Arcidae) were cultured in aquaria for 8 weeks, with a novel high-precision $p\text{CO}_2$ control system which maintained steady CO_2 levels. Bivalve shells and soft tissues, as well as the relevant water and plankton feed samples, were then analysed for $\delta^{13}\text{C}$ in an Isotope Ratio Mass Spectrometer (IRMS) and $\Delta^{14}\text{C}$ in an Accelerator Mass Spectrometer (AMS) to examine variations in end-member contributions to the bivalve components.

Shell carbon was found to be principally derived from seawater DIC in all $p\text{CO}_2$ conditions. Mantle and soft tissue isotopic signatures stayed constant across $p\text{CO}_2$ concentrations and were primarily correlated with the organic carbon signal. A high degree of correlation between $\Delta^{14}\text{C}$ values of bivalve shell and seawater DIC may point to *S. broughtonii*'s suitability as a geochemical proxy of DIC palaeo-concentrations. Using $\Delta^{14}\text{C}$ in concert with $\delta^{13}\text{C}$ allowed separation of isotopic fractionations deriving from effects other than those caused by actual changes in end-member contributions. Shell $\delta^{13}\text{C}$ thus might indicate biologically-related fractionation due to physiological adaptations to cope with effects of changes in ambient pH as these dictated the magnitude of this species-specific effect.

Keywords: Mollusca, Radio isotope, Stable carbon isotope