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Journal of Experimental Marine Biology and Ecology 355 (2008) 153–163

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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Growth rate and age effects on *Mya arenaria* shell chemistry: Implications for biogeochemical studies

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Received 9 November 2007; received in revised form 18 December 2007; accepted 20 December 2007

Abstract

The chemical composition of bivalve shells can reflect that of their environment, making them useful indicators of climate, pollution, and ecosystem changes. However, biological factors can also influence chemical properties of biogenic carbonate. Understanding how these factors affect chemical incorporation is essential for studies that use elemental chemistry of carbonates as indicators of environmental parameters. This study examined the effects of bivalve shell growth rate and age on the incorporation of elements into juvenile softshell clams, *Mya arenaria*. Although previous studies have explored the effects of these two biological factors, reports have differed depending on species and environmental conditions. In addition, none of the previous studies have examined growth rate and age in the same species and within the same study. We reared clams in controlled laboratory conditions and used solution-based inductively coupled plasma mass spectrometry (ICP-MS) analysis to explore whether growth rate affects elemental incorporation into shell. Growth rate was negatively correlated with Mg, Mn, and Ba shell concentration, possibly due to increased discrimination ability with size. The relationship between growth rate and Pb and Sr was unresolved. To determine age effects on incorporation, we used laser ablation ICP-MS to measure changes in chemical composition across shells of individual clams. Age affected incorporation of Mn, Sr, and Ba within the juvenile shell, primarily due to significantly different elemental composition of early shell material compared to shell accreted later in life. Variability in shell composition increased closer to the umbo (hinge), which may be the result of methodology or may indicate an increased ability with age to discriminate against ions that are not calcium or carbonate. The effects of age and growth rate on elemental incorporation have the potential to bias data interpretation and should be considered in any biogeochemical study that uses bivalves as environmental indicators.
Published by Elsevier B.V.

Keywords: Bivalve shell; Carbonate chemistry; Element incorporation; Growth rate; *Mya arenaria*

1. Introduction

Marine bivalves have been used successfully as indicators of environmental properties for several decades (e.g. Dodd, 1965; Klein et al., 1996; Boisson et al., 1998). Bivalves are ideally suited for this purpose given their sedentary nature after recruiting to the benthos, high abundances, relatively large sizes, longevity, and hardiness (Phillips, 1977). In particular, bivalve shells have proven useful for environmental reconstructions since shell and ambient water elemental concentrations exhibit a monotonically increasing relationship (Wilbur, 1972). This relationship is sometimes affected by environmental properties such as temperature and salinity; researchers exploit this characteristic to explore environmental conditions that occurred during shell development (e.g. Rucker and Valentine, 1961; Dodd, 1965; Lerman, 1965; Dodd and Crisp, 1982; Pitts and Wallace, 1994; Lazareth et al., 2003). In studies where bivalves are used as indicators of environment, whole shells or portions of individual shells are analyzed for their elemental composition, and then related to spatial or temporal variation in elemental concentrations, temperature, or salinity of the seawater of formation.

Although the relationship between shell composition and water chemistry has been studied in the past, there are no consistent results across different species. For instance, Sr:Ca in molluscan shell has been reported as correlating both positively

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0022-0981/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.jembe.2007.12.022

(Dodd, 1965; Smith et al., 1979; Stecher et al., 1996) and negatively (Zacherl et al., 2003) to temperature, depending on the species examined. These differences likely originate in the physiological underpinnings of shell formation and deposition, such as regulation of extrapallial fluid composition by the mantle, which differs depending on species (Wilbur, 1972). Such contradictory results highlight the need for understanding biological factors that potentially influence elemental composition of shell.

Despite some uncertainty about the physiological processes that drive the monotonic relationship between shell chemistry and water composition, many fields of study take advantage of the relationship to answer questions about the environment and the ecosystem as a whole. For instance, environmental managers use bivalves as bioindicators of metal contamination in coastal habitats by measuring the trace metal content of shells (Lindh et al., 1988; Bourgoin, 1990; Fuge et al., 1993; Pearce and Mann, 2006). Another use of bivalve shell composition is as a recorder of habitat use. For instance, population biologists are exploring the use of geographically variable chemical signatures recorded in larval shell as natural tags to track dispersal (Zacherl, 2005; Becker et al., 2007). Shell chemistry also can be used as a proxy for changes in water properties. Biological monitors of water chemistry, such as bivalves, are especially important in habitats that are difficult to extensively sample on relevant time scales, such as deep-sea hydrothermal vents (Hart and Blusztajn, 1998), or in paleoclimatic studies of salinity or temperature change on millennial time scales (e.g. Bourgoin and Risk, 1987).

In this study, we were most concerned with biological factors that might affect elemental incorporation into shell, specifically the effect of growth rate and age. Declines in elemental incorporation into the shell's crystal latice have been reported to occur with age for Pb in the abalone *Haliotis* spp. (Hirao et al., 1994; Arai et al., 2003) and for Sr and Mg in the bivalve *Mytilus* spp. (Dodd, 1965). In addition to age, growth rate may impact elemental incorporation into shell. Individuals with rapid growth rates have been shown to incorporate higher amounts of elements into carbonate compared to slower-growing individuals of the same species (Wilbur, 1972). This effect has been observed in coral skeletons (Marshall and McCulloch, 2002; Mitsuguchi et al., 2003), fish otoliths (Hamer and Jenkins, 2007), and adult bivalve shells (Stecher et al., 1996; Gillikin et al., 2005; Carre et al., 2006). These two biological factors have the potential to decouple relationships between ambient water properties and biogenic carbonate composition. As such, they must be understood and taken into account during any attempts to reconstruct environmental conditions or habitat use based on shell chemistry.

Although the studies above have explored the effects of growth rate and age on elemental incorporation into carbonate, none have explored the effects of these two biological factors in the same species and within the same study. The goal of this study was to understand the relationships between growth rate and age on shell chemistry in the commercially important softshell clam, *Mya arenaria*. First, we examined the effects of growth rate on shell elemental composition by comparing shells of clams from the same cohort, reared in the same conditions, but with different final sizes and therefore different growth rates. Second, we compared different regions of shell within the juvenile stage to explore the effects of age on incorporation. Previous studies have focused on one or two elements for exploring the relationship between biological factors and elemental incorporation into shell (e.g. Hirao et al., 1994; Gillikin et al., 2005; Carre et al., 2006). Here we look at five elements found to be useful indicators of environmental properties in previous studies. In addition, we used controlled laboratory conditions to distinguish between biological factors and environmental factors that vary in natural settings, such as temperature and salinity. Our results have implications for biogeochemical studies that use bivalve shell elemental composition as an indicator of environmental parameters. If age or growth rate affects incorporation of elements into carbonate, investigators are obliged to take these factors into consideration when interpreting environmental variables based on shell chemistry.

2. Methods

2.1. Clam rearing

Adult *M. arenaria* with ripe gonads were obtained from Cotuit, Massachusetts in April 2006 and transported to the Environmental Systems Laboratory (ESL) at Woods Hole Oceanographic Institution, where they were placed in mesh bags and suspended in a 750 L tank with filtered seawater. Spawning activity commenced approximately 1 h later, and the tank was left undisturbed to allow spawning to complete and for fertilization to take place. After 5 h the adult clams were removed, and the tank contents were filtered through a 35 μm synthetic nylon mesh sieve to concentrate the larvae into a small volume (~20 L). We counted a subsample of larvae and obtained a total estimate of 16 million trochophore larvae.

Trochophore larvae were placed into 12 L high-density polyethylene tanks (three tanks per experiment) at a density of approximately 45 larvae mL^{-1}. Clams whose shells were intended for the growth rate experiment (see explanation of experiments below) were reared in water with salinity ~22.5‰ at 20 °C. Clams whose shells were intended for the age experiment were reared in undiluted seawater (~30‰) at 24 °C. Temperatures and salinities were within the range experienced by this species in temperate tidal estuaries and were chosen because the tanks were in use as part of a larger set of experiments; no comparisons were made between shells of clams reared in different salinities. Tanks were placed into large water baths to maintain experimental temperatures. The 20 °C water bath received a continuous supply of 20 °C water, which was regulated for the facility's seawater supply line by large-volume chillers and heaters. The water bath for 24 °C tanks was maintained using a 120-volt tank heater regulated by a thermostat (Process Technology EasyPlug™ Heater with Digital Controller, 1800 W). Seawater for tanks was obtained from the in-house supply line, which pumps water from Vineyard Sound 100 m offshore at a water depth of 4 m. All