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Re-evaluation of using rhodolith growth patterns for paleoenvironmental reconstruction: An example from the Gulf of Panama

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ABSTRACT

Rhodoliths are a proxy data source for understanding environment and climate variation in tropical to arctic oceans. A clear understanding of growth pattern and rate is required to interpret time-series proxy data. Variability in growth on multiple-axes of a single rhodolith has not been extensively studied. This investigation compares algal band counting and dating methods found in the literature, and has implications for detecting biases in branch selection for growth and geochemical analyses. Panamanian rhodoliths (*Lithothamnion sp.*) were collected at the Archipelago de Las Perlas, Gulf of Panama (GOP). Alizarin Red S (ARS) stain was used to study growth rates and banding patterns for 10 branched samples randomly selected for microcosm experiments over a 172-day period. DNA analysis was used to help determine the dominant species present in the sample group and reduce the possibility of inter-sample variation. Samples were overturned every 2 weeks for a multi-axial growth test and were grown under ambient seawater conditions except for the addition of LED lighting. Growth post-ARS staining was measured along the long (A), intermediate (B), and short (C) axes. Bisected branches show banding correlated with growth rate while banding was highly variable within an individual and between individuals. Average growth band widths in both the pre- and post-ARS stained growth were approximately the same, suggesting that non-environmental factors, such as overturning or biological influences, can cause the formation of growth increments. Differences in growth pattern and rate suggest that careful inspection of growth formation is needed before pursuing rhodolith-based time series proxy research.

1. Introduction

Annual or sub-annual band validation in non-geniculate coralline red algae (CA; Rhodophyta: Corallinales) is a fundamental step in developing a paleoenvironmental proxy. This process establishes the timeline from which all other parameters can be assessed when attempting to establish a new proxy for areas of research such as paleoclimatology or paleoproductivity. Most age estimations of CA in this context are dependent on establishing the periodicity of observed bands in the algal skeleton. Growth bands are observable in the two predominant, non-geniculate morphological forms of CA: attached living crusts (encrusting) and free-living (rhodoliths). Rhodoliths in particular are in need of more thorough validation studies within the context of proxy research, mainly due to their unique, rounded to sub-rounded shape that allows for frequent overturning on the seafloor while they grow (Marrack, 1999).

Rhodoliths have increasingly become a source of paleoclimate proxy data over the past 15–20 years (McCoy and Kamenos, 2015; Halfar et al., 2000). Like other biogenic marine proxy sources, such as bivalves and corals, rhodoliths have been shown to accrete annual and sub-annual bands of high-Mg calcite (HMC) which can be used to understand fluctuations in ocean geochemistry (Foster et al., 2013; Kamenos et al., 2009; Halfar et al., 2000). However, the controls on the deposition of these bands may vary among different species (Blake and Maggs, 2003). Hypothesized causes of band growth include: seasonality expressed through temperature or salinity flux (Kamenos et al., 2012; Rivera et al., 2004; Adey and McKibbin, 1970), light and dark exposure (Fortunato, 2015; Burdett et al., 2011), tidal and lunar cycles (Moberly, 1968; Blake and Maggs, 2003), and growth cessation caused by rhodolith overturning (Cabioch, 1966 via Blake and Maggs, 2003).

Some of the advantages rhodoliths have for paleoenvironmental analysis are their longevity, diversity of habitat across most low to high latitudes, and lack of ontogenetic effects (Kamenos et al., 2008; Foster, 2001). For example, the rhodolith forming coralline alga *Lithothamnion crassiusculum*, with a reported biogeographic presence

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in the Gulf of California, Mexico and in Italy (van der Heijden and Kamenos, 2015), may live over 100 years based on extrapolation of ^{14}C bomb spike ages relative to the entire length of a rhodolith branch (Frantz et al., 2000). Another example of longevity is found in *Lithothamnion glaciale*, whose distribution extends from the Arctic, throughout the coastal areas of North America (e.g. Alaska, Newfoundland, and Maine), Europe (e.g. Iceland, Ireland, and Norway), Asia (e.g. Japan) and Australia (van der Heijden and Kamenos, 2015). *L. glaciale* has been found to grow for over 60 years using various band counting and geochemical techniques to establish growth models (Kamenos et al., 2012). Additionally, when stacked upon each other on the seafloor under favorable preservation conditions, rhodoliths have the potential to remain relatively in situ for over 20 ky (Adey and Macintyre, 1973), thus possibly providing a long-term paleoclimate record. These attributes make rhodoliths worth investigating in order to generate robust paleo-records.

As outlined by Foster et al. (2013) there are three major steps to developing a proxy, particularly for rhodolith research: (1) determine biological and environmental controls on algal growth, (2) validate the proxy using geochemical analyses of the algae and correlate these data to historical changes in the environment, and (3) apply the algal proxy to environmental change using records that pre-date historical data. Before rhodoliths can be used for environmental proxy development, growth and algochronology must be characterized for the population of interest. This includes being able to identify if growth is continuous and similar on all sides of a rhodolith, determine periodicity of banding, and establish an average growth rate.

There are six main methods that have been used to determine growth rates of both encrusting and rhodolith CA. These include: (1) performing *in situ* (i.e. wild) growth experiments on living rhodoliths to determine how natural environmental parameters (e.g. seasonality) influence growth rate (Steller et al., 2007; Adey and McKibbin, 1970); (2) using ^{14}C values to measure pre-and post-bomb growth (Frantz et al., 2005, 2000); (3) using Mg/Ca to count growth bands (Darrenougue et al., 2013; Schäfer et al., 2011; Halfar et al., 2000); (4) counting observed growth bands based on density changes from light to dark cell rows (Freiwald and Henrich, 1994); (5) using Alizarin Red S (ARS) staining and total growth grown after the ARS stain line during an experiment (Darrenougue et al., 2013; Halfar et al., 2008; Rivera et al., 2004); and (6) averaging the band widths along a 2 mm transect with the assumption that bands are annual (Schäfer et al., 2011). However, it has to be kept in mind that a number of rhodolith studies have found sub-annual banding as well (Foster et al., 2013; Darrenougue et al., 2013; Halfar et al., 2000; Frantz; 1999, Agegian, 1981).There is little to no consensus about which method is the most accurate to determine growth rates. In this study we apply methods 4–6, thus allowing for further comparison to previous rhodolith growth studies that only use one method.

Additionally, the GOP offers a unique setting to study long-term change using CA because it is a seasonal upwelling zone that experiences significant shifts in temperature, salinity and nutrient concentration between the upwelling and non-upwelling seasons. It is also a natural system that shows no significant evidence for high nutrient loading from freshwater sources, thus providing a dynamic marine setting to study seasonal nutrient flux and its impacts on productivity (D'Croz and O'Dea, 2007). Given the natural variability in ocean conditions and the potential for rhodoliths to archive ocean chemistry over decadal to centennial time periods, responses to these variables within the algal growth patterns may help in the understanding of productivity over time.

The overall aims of this study were to (1) reduce uncertainty in inter-sample species diversity by conducting DNA and morphological analysis for the sample group, (2) determine band periodicity (e.g. annual, sub-annual) and growth patterns of the GOP rhodoliths, (3) calculate their annual growth rates, and (4) offer further insight into the influences on rhodolith banding and how methodology may influence the interpretation of time series analyses used in paleoclimate applications.

2. Study site

The Archipelago de las Perlas (Pearl Islands) sample site is located approximately 48 km off the southern Pacific coast of Panama in the GOP (Fig. 1). The Archipelago lies within the 50 m bathymetric zone of the shelf, which covers an area of 12,434 km^2. The rest of the shelf is predominately <100 m deep and covers 24,024 km^2 (Schäfer et al., 2011). Panama and the GOP experience a dry, upwelling season (Jan–Mar) and a wet, non-upwelling season (Apr–Dec), with variations in SST (15.6–30.0 °C at 5 m) and sea surface salinity (SSS; 31.7‰–34.2‰ at 5 m) (Smithsonian Tropical Research Institute, STRI, 2012). The experiment was located at the Smithsonian Naos Marine Facility (NMF) near Panama City and the Bay of Panama where semi-diurnal tides range up to 3.65 m (Benfield et al., 2005). This tidal regime is also characteristic of the collection site. The collection site was a rhodolith bed off the western shore of Isla San José (Fig. 1). Based on ten 10 m transects surveyed by two divers during this study, coverage of the bed was 81% rhodoliths, 13% coral, 5% sand, and 1% other macroalgae with a density of 130.4 rhodoliths/m^2. Using the Boscence (1983) classification scheme, the predominant shape of the rhodoliths in the bed ($n = 196$) is spheroidal. No previous surveys or published information are known for this bed.

3. Materials and methods

3.1. Sample collection

Rhodoliths were collected in March 2014 using SCUBA diving in 3–4 m of water from the rhodolith bed collection site. Only live (i.e. visibly red/pink), predominantly open-branched rhodoliths were collected ($n = 250$) for the purposes of the growth experiment. The samples ranged in diameter between 2.4 and 4.4 cm; however, larger (>11 cm in diameter), more rounded rhodoliths do exist at the collection site. The open-branch rhodoliths provided easier access to individual branches within the three axial directions analyzed in this study compared to the denser protuberances characteristic of the larger, more rounded rhodoliths.

Samples were transported in seawater-filled coolers to the NMF where they were placed in covered, outdoor flow-through aquaria with circulation pumps and filtered seawater (Strainrite polyester felt 10 μm pore size filter bag). Samples were placed in the tanks for one week prior to being placed in experimental microcosms. Analogous growth experiments were not able to be performed in the wild due to logistical considerations. Vouchers of the dried samples are housed in the Department of Geological Sciences Microsampling Lab at the University of Alabama.

3.2. Experimental design, staining, and sample preparation

Thirty samples were randomly selected from the live sample collection. If excessive epiphytes resided on the sample, another randomly selected rhodolith was taken from the tank. Samples were stained for 60-h in 10 L of seawater mixed with 15 mg/L of ARS stain (adapted from Darrenougue et al., 2013). Staining took place under continuous LED (Jiangjing; 18W; 170 lx) lightening to help ensure that rhodoliths absorbed the stain. ARS stain was successfully absorbed in previous staining trials and was clearly marked in bisected branches by a bright pink line. This stain was differentiated from all other growth because non-stained growth bands seen in bisection were light or dark shades of white based on differences in HMC density.

Samples were then placed in white plastic bins (13 cm × 24 cm; i.e. microcosms) such that there was one rhodolith per container (Fig. 2). No substrate was added. Each container was fed ambient, filtered

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