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FULL LENGTH ARTICLE

Periodicity and shell microgrowth pattern formation in intertidal and subtidal areas using shell cross sections of the blood cockle, *Anadara granosa*

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Microgrowth line;
Shell increment;
Shell section

Abstract This study aims to investigate the periodicity of shell microgrowth lines and increments of cockles in the intertidal and subtidal areas. A total of 600 marked specimens of the blood cockle *Anadara granosa* were placed in buried cages that were placed at three sites of differing water levels (Site A: exposed in all low tides, Site B: exposed during spring tides, and Site C: continuously immersed) in Pinang Island, Malaysia. Cockles were collected on a fortnightly basis and the shells were sectioned from the umbo to the ventral margin, before being polished, etched and photographed under a light microscope to observe microgrowth lines and increments. The majority of the cockle shells examined showed that the number of growth lines in shell structures was close to the number of tidal emersions ($P > 0.01$). The number of microgrowth lines observed in shells during the study period was significantly different ($P < 0.01$) from the number of days and tidal changes. Similarly, cockles grown in the laboratory in a simulated tidal regime, deposited a number of microgrowth bands which coincided with the number of tidal emersions. This study shows that shell microgrowth lines are typically formed in accordance with tidal emersions, providing a calendar base for high resolution environmental reconstructions. Endogenous activity synchronised by tidal cues may control rhythmic microgrowth line formation in subtidal areas.

1. Introduction

Anadara granosa is an appropriate species to compare growth information with environmental records due to the high resolution variations that can be observed in their shells (Richardson, 1987). The abundance of this species in intertidal and subtidal zones has enabled a comprehensive evaluation of their ecological response to environmental and physiological factors in their habitat (Faulkner, 2010; Mirzaei et al., 2014). Cockles present regular growth patterns in their shells and provide a variety of growth lines and growth increments that can be considered a calendar with excellent temporal resolution (Goodwin et al., 2001; Poulain et al., 2011). Clearly differentiated and detailed microgrowth lines appear in the inner shell layer, whereas major annual and seasonal patterns can be identified in the outer shell layer (Cerrato et al., 1991). It is clear that the majority of cockle species present periodic lines within

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shell layers that are valuable for environmental studies (Rhoads and Lutz, 1980; Schöne et al., 2003). It has been proven that these random and periodic lines are highly effective for life history and growth pattern studies. Growth bands created from random events (storms, wave action, turbidity) are known as disturbance lines, while bands caused by periodic occurrences (annual, monthly, daily) are identified as periodic lines (Cerrato, 2000; Miyaji et al., 2007; Kanazawa and Sato, 2008; Fan et al., 2011). Disturbance lines arising from random events are not easily distinguished from some growth lines formed in a shell structure, such as the annual growth line. Therefore, over the last two decades, investigations of morphological characteristics within shell layers have developed considerably through additional studies of periodic growth lines within mollusc shells. In addition to providing valuable data in growth pattern studies, the discovery of internal growth lines within the shell layer by Baker (1964) has contributed to extensive interdisciplinary investigations, varying from environmental to palaeoecological research. Periodical growth patterns enable accurate dating, including the identification of day and night (Clark and George, 2005), monthly (Trutschler and Samtleben, 1988) and reproductive (Thompson et al., 1980) cycles. Therefore, shell growth can be investigated to obtain accurate environmental and palaeontological data, once microgrowth lines can be identified and their specific periodicity (daily, monthly and annual) can be verified (Hallmann, 2011). For these reasons, microgrowth line and increment analyses are the preferred method for providing a reliable description of the shell record.

This study investigates the growth bands produced by *A. granosa* placed in intertidal and subtidal areas of Penang Island, Malaysia. The main objectives of this study are: (1) to observe the visibility of microgrowth lines in shell cross-sections of *A. granosa* from intertidal and subtidal areas and (2) to interpret the periodicity of the shell patterns and obtain high-resolution calendar based on the shell structure of the blood cockles collected from different tidal zones.

2. Materials and method

2.1 Field experiment

A total of 600 samples of *A. granosa* (shell length: 10 mm) were obtained from Kuala Juru (N 5° 20′26.44″ E 100° 24′ 32″), one of the main productive mudflats for cockle harvesting in Peninsular Malaysia. At the landing site, the cockles were placed into rotator machine where water was continuously sprayed onto the cockles in order to remove sediments and mud. Samples were then sorted as various size groups and cockles with shell length of about 10 mm were subjected to staining process. Cockles were stained with shell dye (Alizarin Red) at a concentration of 30 ppm before being transferred to the study sites. During the immersion period in alizarin solution, the shells were remained closed causing a minimal disruption of the metabolic activities of the cockles. Therefore, the direction of growth line formation was changed in the shell structure. Stained cockles were placed in fifteen plastic mesh cages (1 m × 1 m × 1.5 m) at three sites (40 individuals × 5 cages × 1 site) in different tidal zones that were easily accessible by boat from Penang Island, Malaysia (Fig. 1). Site A was located at an intertidal site exposed during all low tides. Cockles in Site B were exposed during all low spring tides and cockles at Site C were continuously submerged under water. The relative positions of the cages at the three sites in relation to tidal

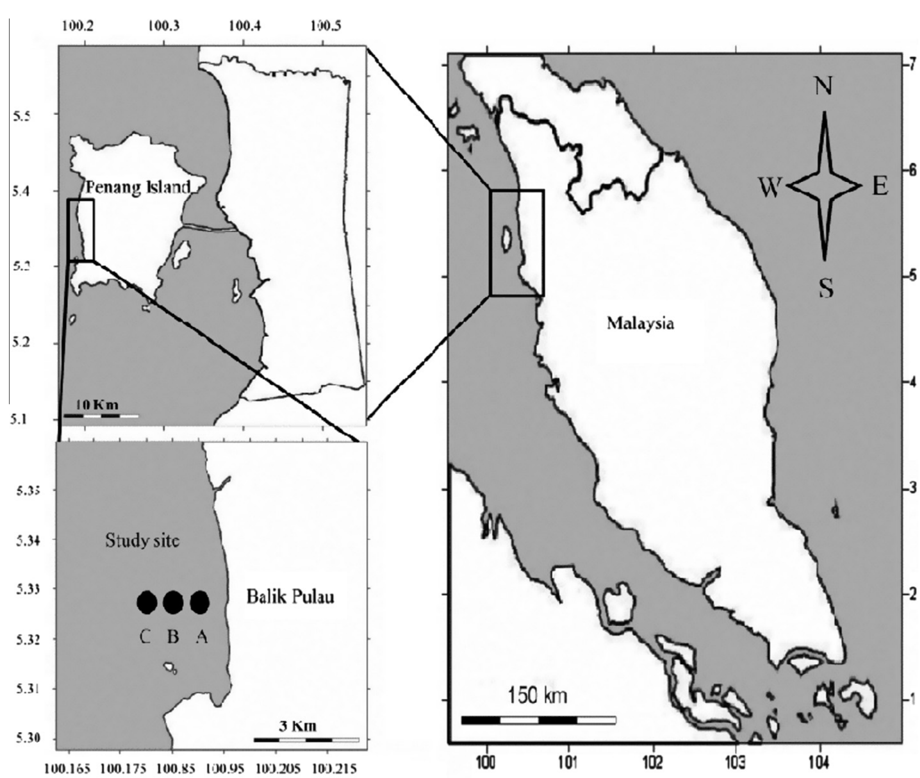

Figure 1 Location of study area and study sites in Balik Pulau, Penang Island, West coast of Peninsular Malaysia.