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TIDAL PERIODICITY IN MICROGROWTH BANDS OF BLOOD CLAM *Anadara indica* (Bivalvia: Arcidae): A POSSIBLE ENVIRONMENTAL SCLEROCHRONOMETER IN THE TROPICS

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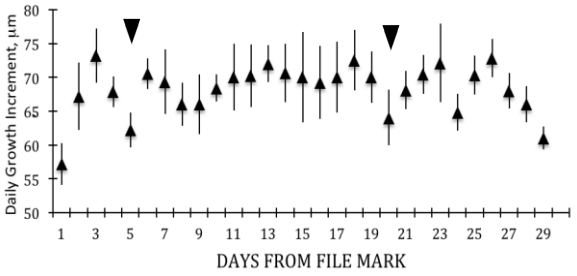
Graphical abstract



Abstract

This study examining the microgrowth periodicity of wild type *Anadara indica* (Gmelin, 1791) being submerged for 29 d in the cage and field plot. Individual clams showed daily increment growth that conforms to semilunar tidal cycle of 14.8 d. The shell therefore provided record about the absolute growth history of the organism, presumably including the environmental conditions under which shell deposition took place. Shell accretion decreased in rhythm to the gonad development and spawning measured as Condition Index. The significantly departed sex ration to 0.25:1 in favour of > 35 mm shell length females indicating the occurrence of male to female sex reversal, likewise its con-specific *A. granosa* (Linne, 1758) and *A. antiquata* (Linnaeus, 1758). It is thought that habitat overlap between *A. granosa* and *A. indica*, combined with irresponsible fisheries of the local people which commercially caught small size male clams caused the shift in population dominance among these two species, i.e., from *A. granosa* to *A. indica*. However, this study showed that *A. indica* might as well thrive in areas close to intertidal region likewise *A. granosa,* where tidal periodicity role as a forcing function to the environment.

Keywords: Condition Index, marine tropical bivalve, microgrowth band periodicity, sex ratio

1.0 INTRODUCTION

The earliest species of *Anadara* existed in the Oligocene period, some 26 × 10^6 year ago. Recent genera are distributed across the southern of Australia, northern Japan, and the Mediterranean Sea. *Anadara* retained its primitive taxodont teeth into present day with little or no changes to most of their morphological attribute [1, 2] at least since the Pliocene period *c.a.* (5.2 to 1.64) × 10^6 year ago [3].

Many species of blood clam are collected for human consumption. In Indonesia, they may be harvested on a subsistence basis or commercially caught from either culture ground or wild populations. This study shows that its daily increment growth provided more evidence on the possibility of using accretion in shell growth of tropical blood clam to assess environmental conditions in their habitat.

Several studies reported the relationship between growth of the shell and body weight changes. Shell

valves increase in size primarily by incremental accretion of calcium carbonate at the valve margins, and once deposited the gross morphology cannot be changed. The analysis of growth patterns within the shell structure of bivalves, a technique originally developed for geological purposes, has proved to be a valuable method to determine the age and growth of many recent bivalves, especially marine species [5–13].

Extrinsic factors influencing microgrowth of the shell, in particular for intertidal species, are water temperature, food supply, light, and many other environmental variables. In subtropical waters, relatively high temperature and abundant food during summer increase growth rates and create wide incremental growth zones. Winter temperature and a decline in food result in a narrowing growth increment, reflecting a reduced growth rate. In the tropical area however, where water temperature that drives food abundance is more or less stable, relatively constant incremental growth of *Anadara* can still be used to assess environmental conditions.

2.0 MATERIAL AND METHODS

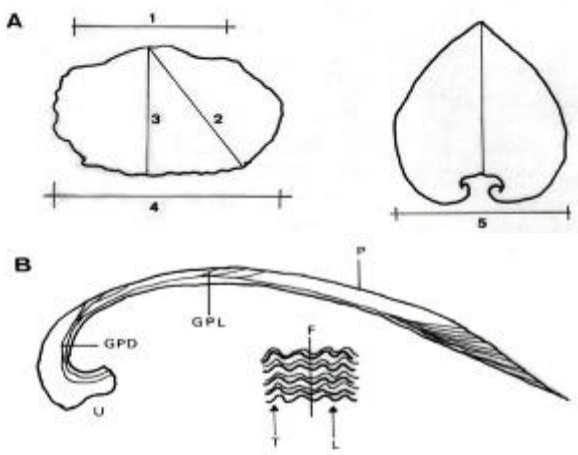
2.1 Field Experiment Plot

As many as 500 individual of wild type *A. indica* of ca. (20 to 30) mm shell length were marked by filing a little notch on the ventro-posterior margin of their shells to interrupt growth, so that all the afterward growth can be related to the duration of the experiment. To keep them from predators (mangrove crabs), they were then distributed into a bamboo-fence plot of 5 m × 5 m at 1 m depth during low-lowest spring tide in the estuary of River Tapak, Semarang in December 1999. In order to ensure recaptured at the end of the experiment, another 80 file-marked individuals were put into a holed-plastic cage of 40 cm × 60 cm × 30 cm on the mud within the plot. At day 29, all living specimen that could be found within the cage and the plot were taken back from the field.

Aside of plot experiment, monthly sample of *A. indica* from the adjacent location to the plot experiment has been conducted for eight more months to construct sex ratios and length frequency distribution of the clam. Only individuals whose sexes can be confidently determined from the external appearance of their gonads were used to build the histograms.

2.2 Shell Replica Preparation

In the laboratory, shells were first washed carefully with mild detergent and running water. They were then boiled individually to separate soft tissues. Particular attention should be paid to not to damage the ventral growing margin of the shells. After being dried in room temperature and manually coded, individual halves of shell valves were embedded in local resin for which the setting time has to be pre-determined to prevent fracturing of the shell due to the process of resin hardening (12 h) and during sectioning. The embedded shells were manually sectioned along the anterior-posterior axis, i.e., from umbo to the ventral edge using a junior hacksaw; as such that the section passed through a trough rather than a ridge on the shell surface (Figure 1).



A. *A. indica* shell measurements
1. Shoulder length/taxodont length
2. Axis of maximum shell height
3. Axis of normal shell height
4. Shell length
5. Shell width

B. Radial section through axis of normal shell height in *A. indica* to measure microgrowth increments. U: umbo, GPD: microgrowth line within inner complex cross-lamellar layer, GPL: microgrowth line within outer complex cross-lamellar layer, P: Periostracum, L: Trough, T: Ridge, F: axis of the radial section.

Figure 1 Schematic diagram and cutting position of the shell of blood clam *Anadara indica*

Acetate peel replicas of the sectioned shells were prepared by grinding, polishing then etching the exposed surfaces in 0.1 M HCl at room temperature [2, 6, 7]. The number of microgrowth bands deposited between the notch and tip of shell margin were counted. A period of 14.8 d, an established standard for semilunar periodicity [14, 15], was used to indicate a fortnightly periodicity of shell formation.

2.3 Microgrowth Analysis of the Shell

Absolute age of bivalve can be estimated either from counts of microgrowth lines in acetate peels replicas of the umbo region [9], prismatic layer of the shell [2] or both [6]. Once the periodicity of the microgrowth lines is established, environmental disturbances occurred during the growth period can be determined.

For these marked blood clams, number of microgrowth bands deposited between the notch and shell margin was counted. In order to examine whether there was a fortnightly spring-lunar variation in its growth rate, the width of individual microgrowth increments, i.e., the distance separating adjacent

bands was measured. Samples were both eight specimens blood clams grown for 29 d in plot experiment along and four individuals grown in a cage within the plot. A calibrated eyepiece micrometer of 1 μm accuracy was used to measure increments of microgrowth lines under the microscope.

3.0 RESULTS AND DISCUSSION

3.1 Periodicity of Microgrowth Lines

File marked *A. indica* in the cage resulted in a low (< 50 %) rate of survival after transplantation into the shallow subtidal for 29 d. At the end, individuals grew within the box appeared to have a thinner, fragile and pale-coloured-shell than those in the fence. Recaptured specimens from the plot observed to have normal shell thickness as well as its colouration. All specimens showed evidence of shell growth after marking.

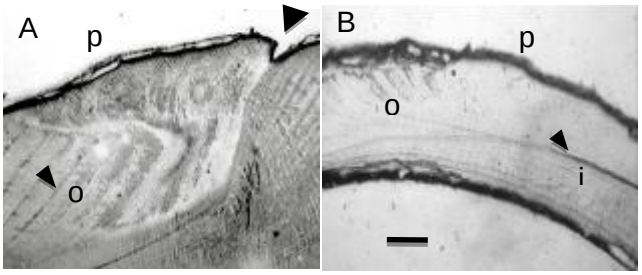


Figure 2 Microgrowth lines in *A. indica*: A) the outer complex cross-lamellar layer (arrow, o), growth direction left to right; B) the inner complex cross-lamellar layer of the shell (arrow, i), growth direction right to left. p: periostracum. Arrow in the cleft: file mark. Scale bar 100 μm

Acetate peel replicas of polished and etched shell sections revealed three shell layers: i) outermost periostracum, ii) outer complex crossed lamellar layer, iii) inner complex crossed lamellar layer (Figure 1B, 2A and 2B). During 29 d of cage experiment, some individuals showed clear definition and relatively similar width of growth band at the outer cross-lamellar layer toward the ventral margin of the shell (Figure 3A and 3B). However, microgrowth bands of these 31.2 mm and 34 mm shell length individuals appeared clumped toward the end of the experiment, yet, in each of them 17 and 18 microgrowth lines can be counted consecutively.

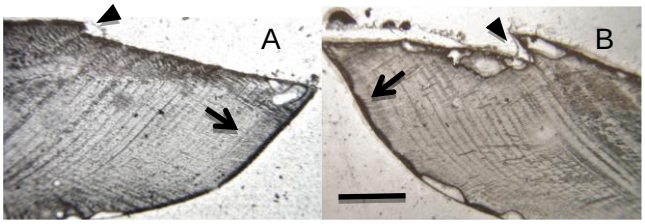


Figure 3 Photomicrographs of acetate peels of shell sections of file marked *A. indica* grown in the cage for 29 d in Semarang estuary, Indonesia. Unresolved microgrowth lines (arrow) of: A) Individual of 31.2 mm shell length, 17 bands, direction of growth left to right. B) Individual of 34 mm shell length, 18 bands, direction of growth right to left. Scale bar 500 μm. Block-arrow showing cleft of file mark

Other individual grows constantly during 29 d plot experiment (Figure 4), indicated daily periodicity of the bands.

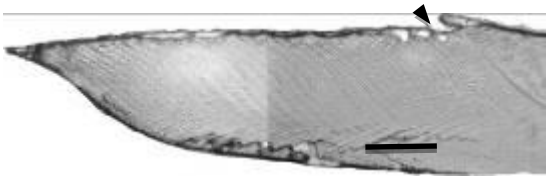


Figure 4 *Anadara indica*, an individual of 15.6 mm shell length showing 29 daily growth bands within 29 d plot experiment. Scale bar 500 μm. Arrow showing cleft of file marks. Direction of growth from right to left

During a semi-lunar tidal cycle from full moon until new moon, tide is characterized by the periodic change in spring and neap tide. The mixed semidiurnal tidal pattern in the area of study has a predominant daily component, so that the number of tides is approximately equal to the number of days. Tidal periodicity of deposition of the microgrowth bands can therefore be referred to, in a general way, as daily bands (2).

Shown in Figure 5, which is the average measurement of eight individuals, the narrowing microgrowth bands (big blue arrow) coincide with the highest reproductive activity noted in Condition Index of the population (Figure 6, 27/12/1999) and the semi-lunar periodicity of the area [2].

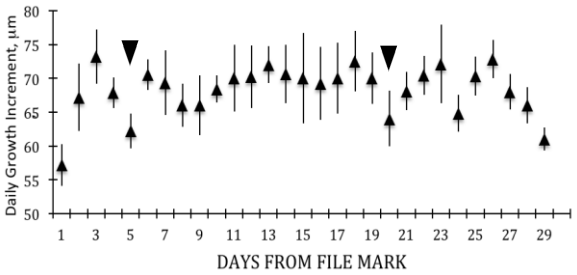


Figure 5 Variation in the average width of daily microgrowth increments (n= 8, ± SD) of file-marked *A. indica* transplanted for 29 days into experiment plot at Semarang estuary, Indonesia (18 December 1999 to 16 January 2000). First big arrow (day 5) is the narrowest increment deposited 1 d after maximum spring tides of semilunar periodicity; second big arrow (day 20) narrowing microgrowth lines coincide with neap tides

Further in Figure 5, at the first day (19/12/1999) of immersion in plot, shell growth rate was very low (57.14 µm), which was probably due to the recovery from filing, followed by the highest rate of growth (73.18 µm, day 3), yet steadily decline afterwards to reach exactly the lowest at day 5 (62.2 µm, range 59.6 µm to 64.8 µm, 23/12/1999) which was one day after full moon (64.84 µm, 22/12/1999). A subsequent semi-lunar cycle depicted that after 15 d of full moon (neap tides), again, reduced growth increments was noticed (64 µm, ranged from 59.9 µm to 68.10 µm, day 20 or 7/01/2000) albeit not as much as the ones during spring tides. Toward the end of the plot experiment, i.e., day 29, subsequent decline occurred (61.22 µm, with a range of 58.62 µm to 63.82 µm, 16/01/2000), which might be related to the discharge commencement of reproductive tissue (Figure 6, 22/1/2000).

3.2 Population Productivity and the Periodicity of Microgrowth Lines

Population productivity stated as Condition Index during 8 mo course of study, which correlating dry shell to dry tissue weight of the specimens (Figure 6) [2, 16] - commenced in July 1999 - attained culmination in December 1999, started to decline in January 2000, and went down to the lowest in March 2000 as individuals within the population discharged their reproductive materials (Figure 6).

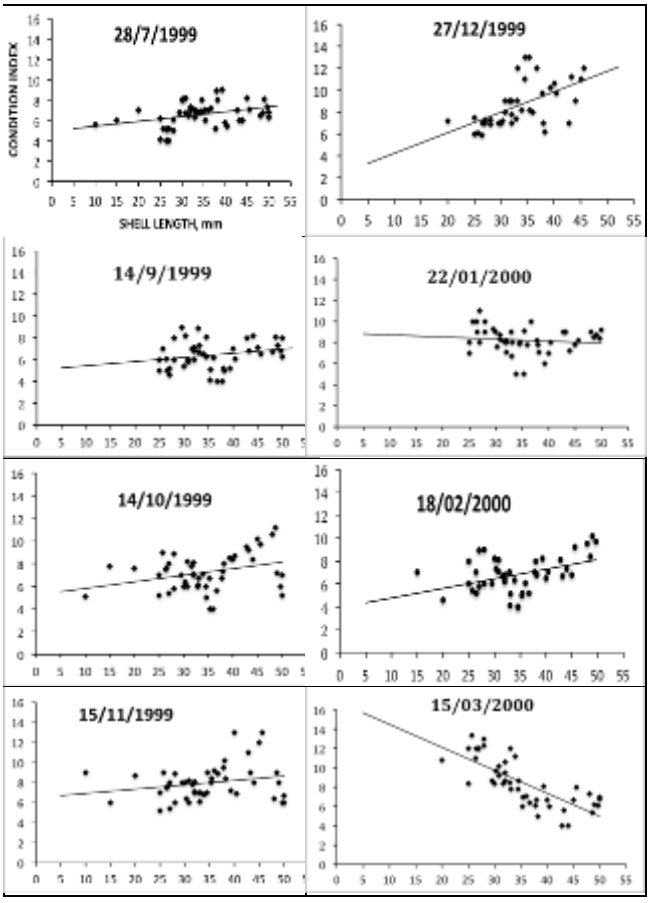
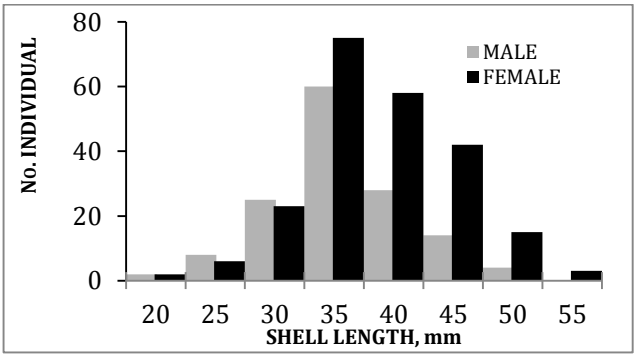
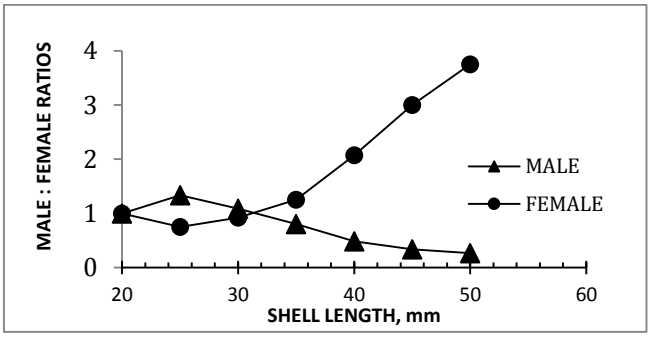


Figure 6 Productivity of blood clam *A. indica* in Semarang estuary, July 1999 to March 2000 shown as simple regression analysis between Condition Index and Shell Length, n = 49 per month.

As illustrated in Figure 7A, once the clam attained ca. (30 to 35) mm shell length, more female individuals occupy the population. Sex ration of small individuals of 20 mm were 1:1. After being departed to 1.33:1, it returned to 1: in population with individuals of ± 32.1 mm shell length.



A. Size frequency distribution and sex ratios of male (n = 171) and female *A. indica* (n = 194).



B. Sex ratio of blood clam *A. indica* analysed from histogram in Figure A.

Figure 7 Size frequency distribution and sex ratios of male to female *Anadara indica* collected during 8-month period from wild populations in Semarang estuary, Central Java

However, when the animals were > 35 mm shell length and further, sex ratio significantly deviated to become 0.25:1 in favour of females (Figure 7B). This findings indirectly explained the unresolved microgrowth lines in Figure 3 and Figure 4, apart that they have had grown in different condition, i.e. cage versus field plot.

The shell of Arcidae is primarily composed of aragonite, one of three calcium carbonate polymorphs. A vertical section through a valve reveals three layers, i.e., an outermost periostracum, an outer complex crossed-lamellar layer, which is also found in the hinge and the taxodont teeth, and an inner complex crossed-lamellar layer (Figure 2A and 2B) [2, 17]. These zones are a manifestation of the relative proportions of a proteinaceous matrix (conchiolin) and aragonite, which comprise the shell. Annual microgrowth lines and tidal bands in clams shells have been therefore used to estimate age, to investigate the effects of environmental factors such as seawater and air temperature, and spring-neap lunar tidal cycles. However, growth in bivalves is known to vary enormously; even individuals of initially similar size or age, or both, grown under apparently identical conditions can show considerable variations in their growth rates, and this can be related to their genotype [18].

Whereas bivalves from temperate region laid down annual growth lines [6, 13] whilst taking into account some factors such as food supply, thermal tolerance and reproductive processes as possible reasons, this study showed that at least gonad development and spawning affect daily growth rate of shell deposition. Figure 5 and Figure 6 shows that when Condition Index of the tissues was at its highest (Figure 6, 27/12/1999), rate of daily absolute growth of the shell is low (Figure 5, day 4 or 22/12/1999, n = 8), although the lowest (Figure 5, day 5 or 23/12/1999) was one day after full moon (22/12/1999). This finding conformed to those in *A. granosa* ($P < 0.001$) [2], thus suggests that when the clam pacing its generative growth, its vegetative growth reduced or went down to minimum. It is also shown in Figure 3A and Figure 3B that after being immersed for 17 d to 18 d in the cage (17 and 18 counted lines), daily growth bands clumping toward the edge of the shell, as such, that they cannot either be counted or measured. This is perhaps due to the onset of reproductive stages, as the two specimens were mature individual of 31.2 mm and 34 mm shell length. Size at first maturity was 17.9 mm for male and 19 mm for the female in this population. So, young individual in Figure 4 which presumably was a juvenile (15.6 mm) who has yet active in reproduction, grew steadily in plot experiment revealing 29 microgrowth lines. Furthermore, Figure 5 suggests that average daily growth of the shells slowing down from 72.5 μm (4 January 2000, day 17, in Figure 3 shown as unresolved lines) to 61.22 μm (16 January 2000, day 29), indicating that the onset of reproductive discharge might have been started as such, since sample collected in 22 January 2000 (Figure 6) have depicted drastic decrease in Condition Index.

Moreover, Figure 6 (January to March 2000) indicated that *A. indica* experiencing negative growth during spawning, i.e., when reproductive tissues has been discharged, animal lost most weight of its soft part. However, daily increment of its shell continued lay down albeit very slow, resulting in larger individuals with lighter total weight. Overall, negative growth does not mean negative in individual life cycle, since the larger the animal the more the tissues and the reproductive materials can be produced, means the more successful the existence as species. Aside of that, the always presence reproductive tissues in various stages of development during the course of study, suggesting survivorship strategy of this particular blood clam in assuring year round availability of larvae as new recruit to the population. The same strategy applied by *A. granosa*, but not for their conspecific *A. antiquata* which performing distinct seasonal spawning time [2].

3.3 Tidal Periodicity and the Shift of Population Dominance to *A. indica*

This study is part of a basic preliminary work to reveal the shift of composition of mixed-trawl-caught blood clam in several places in Semarang waters where *A. indica* is landed more than its conspecific *A. granosa* within these recent years. It is assumed that *A. indica* replacing the population dominance of *A. granosa* because it has a more adaptive feature and plasticity in encountering environmental changes.

A. granosa grows mostly in soft muddy sediment of intertidal to marginally subtidal areas that may exposed to air during low tides, environment generally considered to be unsuitable to many bivalve species, and makes them easy to collect manually. Afiati [19] reported that small sizes *A. granosa,* ca. 2 cm shell length are mostly male and have a possible

occurrence of sex changes from male to female. Apparently both species performed a protandric type of development in which a primary male phase precedes the adult stages until both sexes were approximately equally represented (30 mm to 35 mm shell length, Figure 7B), after which sex reversal took place [20]. Local people may deplete shellfish bed of juvenile *A. granosa* (15 mm to 20 mm shell length) for commercial harvesting only within 2 mo to 3 mo after the bed was discovered, for which average density may dropped (from 45 indiv · m^{-2} to 9.19 indiv · m^{-2}) [2]. After some years, sudden decrease of clam density reflects in smaller shellfish bed with fewer individuals compared to the previous season or year. In many cases, people continued gathering small size clams, which apparently are male and sell them for a very cheap price to be boiled for soup broth. Meanwhile, it seemed that female clams discharge their reproductive tissues if the water has ample sperm concentration released continuously by the males (Afiati, Pers. Obs.). This perhaps explained the recent scarcity of *A. granosa* within the landed harvest.

On the other hand, *A. indica* are prolific in marginally sub-tidal depth, this lead one to consider that their physiology as well as their shell shape must have been evolved to aid in their survival as a genus [4]. This study, however, shows that *A. indica* might as well thrive in areas close to intertidal region, where tidal periodicity role as a forcing function to the environment. This fact thus lead to suggest that *A. indica* might become a good aquaculture candidate to meet the rocketing demand for shellfish protein. Although, marginally subtidal areas of the water naturally preferred by *A. indica* allowed it to escape early age catching, and later on superseding the dominance number of *A. granosa* in the haul.

4.0 CONCLUSION

It has been shown that microgrowth bands permanently etched in the shell of *A. indica* was laid down daily and performed a secured dynamic harmony with for example spawning period of the clams. Therefore, besides to estimate their absolute age, microgrowth lines could also be related to the impact of environmental factors, thus providing more evidence on the possibility of using accretion in shell growth of tropical blood clam to assess environmental conditions of their habitat. Considering some similar features and growth strategies followed by both *A. granosa* and *A. indica*, it might be possible that specific habitat occupied by this con-specific explained the dominance shift from *A. granosa* to *A. indica* within this decade.

Acknowledgement

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