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Article

Gastropod Shell Dissolution as a Tool for Biomonitoring Marine Acidification, with Reference to Coastal Geochemical Discharge

David J. Marshall[1]*, Azmi Aminuddin[1], Nurshahida Atiqah Hj Mustapha[1], Dennis Ting Teck Wah[1] and Liyanage Chandratilak De Silva[2]

[1]Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, BE1410, Bandar Seri Begawan, Brunei Darussalam;
[2]Faculty of Integrated Technologies, Universiti Brunei Darussalam, Jalan Tungku Link, BE1410, Bandar Seri Begawan, Brunei Darussalam;
david.marshall@ubd.edu.bn (DJM); ultraazmi17@gmail.com (AA); shahidaatiqah88@gmail.com (NAM); dennis.ting@ubd.edu.bn (DTT); liyanage.silva@ubd.edu.bn (LCD)

Abstract: Marine water pH is becoming progressively reduced in response to atmospheric CO_2 elevation. Considering that marine environments support a vast global biodiversity and provide a variety of ecosystem functions and services, monitoring of the coastal and intertidal water pH assumes obvious significance. Because current monitoring approaches using meters and loggers are typically limited in application in heterogeneous environments and are financially prohibitive, we sought to evaluate an approach to acidification biomonitoring using living gastropod shells. We investigated snail populations exposed naturally to corrosive water in Brunei (Borneo, South East Asia). We show that surface erosion features of shells are generally more sensitive to acidic water exposure than other attributes (shell mass) in a study of rocky-shore snail populations (*Nerita chamaeleon*) exposed to greater or lesser coastal geochemical acidification (acid sulphate soil seepage, ASS), by virtue of their spatial separation. We develop a novel digital approach to measuring the surface area of shell erosion. Surficial shell erosion of a muddy-sediment estuarine snail, *Umbonium vestiarium,* is shown to capture variation in acidic water exposure for the timeframe of a decade. Shell dissolution in *Neripteron violaceum* from an extremely acidic estuarine habitat, directly influenced by ASS inflows, was high variable among individuals. In conclusion, gastropod shell dissolution potentially provides a powerful and cost-effective tool for rapidly assessing marine pH change across a range of spatial and temporal frameworks and coastal intertidal environments. We discuss caveats when interpreting gastropod shell dissolution patterns.

Keywords: ocean acidification; acid sulphate soils; calcification; molluscs; snails; tropical

1. Introduction

The importance of pH in marine ecosystems has been elevated with the realization that anthropogenic CO_2 emissions can radically alter the chemistry of the oceans. However, acidification of coastal marine environments is ubiquitous and can derive from numerous alternative processes originating in adjacent land masses and sediments. Non-atmospheric marine acidification may occur naturally and often arises from geochemical discharge, such as that from pyrite-rich (FeS_2) soils or sediments [or acid sulphate soil (ASS) [1]. Globally, approximately 17 million ha of land contains ASS materials, with most distributed across coastal ecosystems (wetlands, mangrove forests and estuaries). Although natural seepage of ASS materials is prevalent, mismanagement of ASS systems can be especially environmentally hazardous through enhancing acidity generation and the

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mobilization of trace metals, ultimately impacting ecosystem structure and functionality [2-6]. This study aimed primarily to assess an approach for biomonitoring coastal acidification.

A plethora of data-logging approaches is now available for monitoring physicochemical conditions in aquatic systems, however, these are often logistically-constrained and in the case of pH monitoring, financially-prohibitive. Constraints arise from the fact that natural coastal ASS seepage and pH can be extremely spatially and temporally variable, and a vast number of logging stations would be required to capture this variability. For instance, estuarine pH can fluctuate by as much as full units within a tidal (or daily) timeframe [7] with discharges stochastic and seasonally related to flooding across a spatially highly variable topographical and geomorphological landscape. As an alternative, assessing the cumulative effect of exposure to a chemical environmental variable at the scale of the organism (individual animal), by measuring an attribute of that organism, has proved to be very useful, especially in the field of environmental pollution [8-10].

Marine gastropods are particularly suitable organisms for environmental pollution biomonitoring [8-10], though remarkably little information is available for their use in monitoring acidification, despite having shells that dissolve relative to acidity exposure [11]. Although gastropods are increasingly being used to inform about biological response to ocean acidification (related to atmospheric CO_2 elevation) [12-14], most work considering their shell attributes focuses on calcification rates and the energetic implications of building shells in undersaturated carbonate conditions [15-18], rather than attempting to inform about pH exposure level.

In the present study we capitalize on the pervasiveness of acid sulphate soils (ASS) in Brunei Darussalam [7, 19]. Together with aquatic eutrophication, peat swamp leachate and acidic pollutants, ASS discharge lowers the pH of interconnected freshwater, mangrove, estuarine and open-ocean marine systems of the region [19, 20], potentially threatening these ecosystems' rich gastropod diversity [21, 22]. Previous research has focussed on the steep pH gradient of the Brunei estuarine system (BES, Fig. 1) and morphological and physiological responses to variable pH conditions of a single gastropod species, *Indothais gradata* [7, 11, 24]. Recent observations coupling visual evidence of ASS-acidification and gastropod shell dissolution in other marine systems at the fringes and outside the BES stimulated further investigation (see Fig. 1). The objective of this paper was to evaluate how gastropod shell dissolution can be used as a tool to biomonitor pH change, by preliminary assessing (1) the link between shell dissolution and marine environmental acidification and (2) gastropod attributes that best reflect anticipated exposure to acidic water. The study further outlines and evaluates novel approaches to measuring the degree of surficial shell erosion.

2. Materials and Methods

2.1. Study Area, Habitats and Snail Species

Intertidal zones of Brunei-Muara region are dominated by soft sediment systems (mud to sand) spanning the Sungai Brunei (river), the wave-free Inner Brunei Bay (together make up the Brunei Estuarine system, BES) and the South China Sea (SCS) coastline (Fig. 1). Freshwater inflow to the BES is largely via four major river systems, the Brunei and Temburong rivers (in Brunei) and the Limbang and Trusan rivers (in Sarawak, Malaysia). The water is usually brownish and turbid due to high suspended sediment and organic loading and is significantly acidified [5-7, 20]. Mixed turbid reduced-salinity plumes extend beyond the BES to the SCS during monsoonal rainfall periods. Details of the tidal regimes and the causes and patterns of acidification in the BES are covered in Proum et al. 2018, with all earlier work focussing on an area limited to the Sungai Brunei and Brunei Bay, bounded by Bandar and Pulau Bedukang (Figure 1).

Here we focus on three new localities, (1) a rocky premonitory on the SCS coastline constituting Tungku Punyit and Pulau Punyit (TPUN; 4.975N, 114.849E; see Marshall et al. 2018), (2) mudflats at the mangrove-fringed Pulau Bedukang (Brunei Bay; 4.9795N, 115.0576E) and (3) the low salinity intertide at Sungai Kedayan (4.880N, 114.9339E; see Fig. 1). Bedukang and Kedayan represent the seaward and landward extremes of the BES system not previously considered in any detail.

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Kedayan experiences salinities of mostly below 10 psu and pH below 7, whereas these parameters at Bedukang are mostly above 25 and 7.8, respectively [7]. Notably, in the past four years there has been extensive constructive work ongoing in the BES, with the building of bridges across the bay and river and a riverpark development at Kedayan. Such activities involve widespread dredging and the inevitable release of acidic materials from the underlying ASS sediments, contributing to estuarine water acidification.

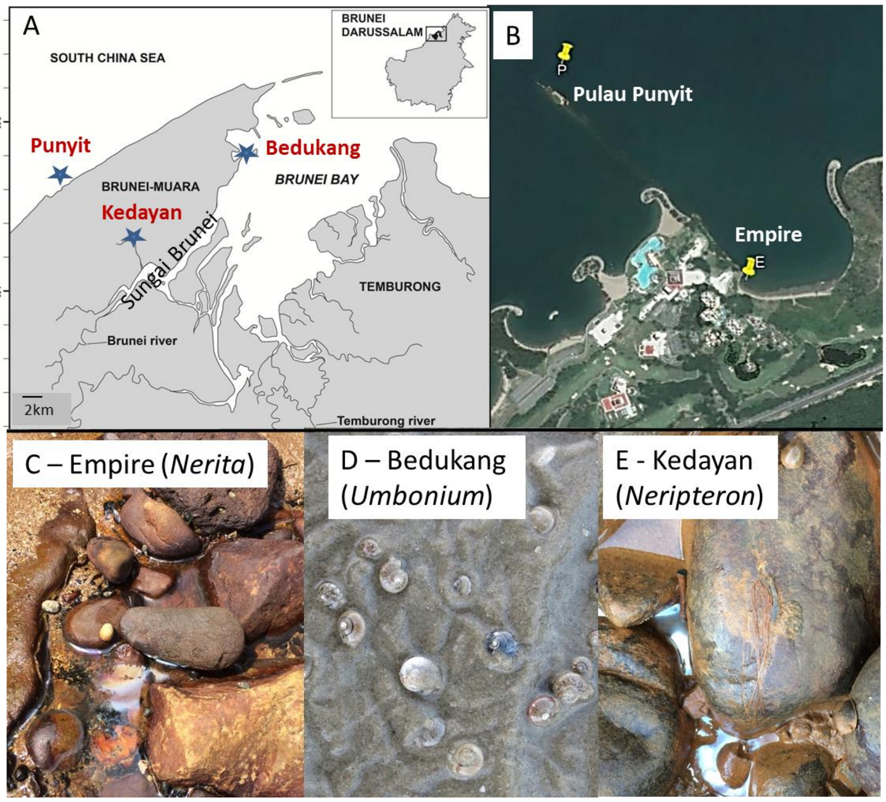

Figure 1. (A) Map showing study localities, Punyit, Bedukang and Kedayan; (B) Satellite photograph showing proximity of Pulau Punyit and Empire; (C-E) Coastal habitats showing snails in ASS-seeps at Empire and Kedayan and mudflat snails at Bedukang.

With regard to habitats and snails species, Tungku Punyit (hereafter referred to as Empire) represents natural coastal mangrove ecosystem, which has been vastly modified following the building of the Empire hotel. A granitic ridge extends from Empire, approximately 1 km offshore to the rocky islet, Pulau Punyit (hereafter Punyit). Whereas there is clear evidence of ASS seepage into the rocky boulder beach habitat at Empire (Figure 1), Punyit supports no historical pyritic sediment formation and there is no evidence of ASS seepage there, such as stained rocks, brown pooled water and glossy biofilms. As such, whereas the boulder beach habitats at Empire are directly infiltrated with reduced pH water, Punyit is devoid of such influence, but rather experiences vigorous circulating open sea current system, presenting overall an ideal study model system to compare biotic responses to different levels of acidic water exposure.

Diverse gastropod faunas occupy the intertidal zones at both Empire and Punyit, as well as the mangroves/mudflats at Pulau Bedukang [21]. In this study we were highly selective in our choice of species, which was based on abundance and a visible dissolution response to acidification. The primary investigation concerned *Nerita chamaeleon* (Linnaeus, 1758)(< 70 individ) at Empire/Punyit. Further observations are given for *Umbonium vestiarium* (Linnaeus, 1758) (> 100 individs) associated with the mud sediments at Bedukang, and *Neripteron violaceum* (Gmelin, 1791)(< 40 individ), which inhabits various hard-substrata at Kedayan (Figure 1). We comment on *Assiminea* sp. from the Bedukang sediments and *Clithon faba* (Sowerby I, 1836), which inhabits *Nipa* palm fronds at

Kedayan. Reference to *Indothais gradata* was intentionally avoided despite this species occurring at Bedukang and Kedayan, since this species has been and continues to be the subject of ongoing investigation [11, 24, 25]. In all cases, snails were immediately returned to the laboratory in air and fixed and preserved in copious 70% ethanol, in 1L polyethylene lidded jars. The ethanol was replaced the following day and there was never any evidence of snail degradation.

2.2. *Shell and Mass Responses of* **Nerita chamaeleon** *to Acidic Water Exposure*

Shell erosion response was assessed for populations of *Nerita chamaeleon* collected from Empire and Punyit (n=25 per site; Dec 2016). Several morphological and mass attributes were compared to assess their sensitivity to differential acidification at these sites. We considered (1) gross shell loss from relative size (shell length; SL) and relative dry shell mass (SM), and (2) surficial shell dissolution (Fig. 2). Additionally, we determined dry soft tissue mass (DTM) for the same individuals. Length measurements were estimated using Olympus CellSens software, following photography of the apertural and abapertural surfaces of each individual snail using a Canon EOS and uploading photographs to a desktop computer (Figure 2). Shells were then cracked open and shell and soft tissues were separated and dried in an oven at 70°C, for three days. After equilibration to ambient conditions, dry shell and tissue masses were determined using a Mettler balance (accurate to 3 decimal places, g).

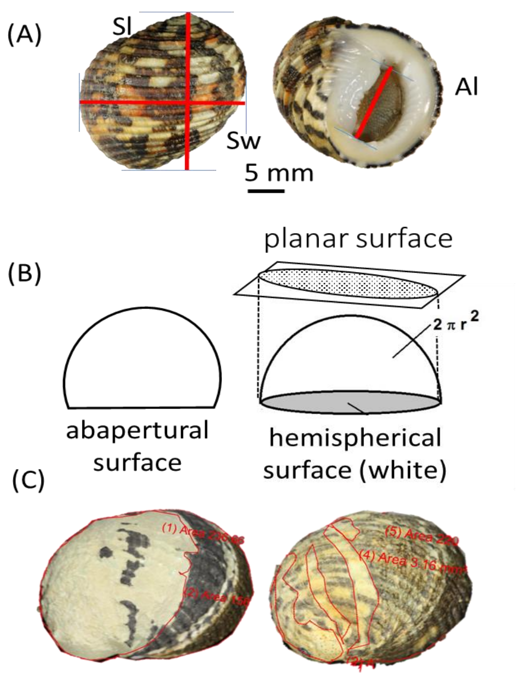

Figure 2. (A) Shell orientation for calculating shell length (SL) and aperture length. (B) The planar area used for dissolution assessment, based on a hemispherical view. (C) Manual surface area mapping from photographs using CellSens software.

To account for a possible collection or natural size bias in the populations from different sites, shell length and mass data were 'size-corrected', by relating to aperture length (AL; Figure 2). We consider aperture length as a conservative estimate of size and possibly age that is not directly affected by acid water exposure or dissolution [11]. Given that these relationships are predicted to vary allometrically, measurements were converted to natural logarithms prior to statistical analysis.

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Ordinary least squares regressions were fitted to the relationships for shell length and masses against aperture length (Sigmaplot ver. 14). The data were then statistically analysed using Generalized Linear Models (GLZM; Statistica ver. 12) to determine the effect of site (Punyit and Empire) on SL, SM and DTM.

2.3 Surficial Shell Dissolution of Nerita chamaeleon

Two methods were used to assess the area of surfical shell dissolution, seen from the planar view of the hemisphere of the abapertural surface (Figure 2). In the first instance, areas that visibly exhibited dissolution were manually encircled and calculated using a drawing tool in CellSens. The total surface area was similarly determined to ultimately give the proportional dissolved area for each shell. In some cases dissolution was very low, so the proportional number of individuals showing no dissolution (< 10% dissolved surface) versus dissolution (> 10% dissolved surface) was assessed for each snail population.

An alternative method to estimate areas was based on a digital approach and pixel analysis. This enabled differential surface coloration of the affected dissolved area (light area) and the original shell surface (dark area). Here we adopted a clustering technique called k-mean clustering. This clustering partitions data into *k* number of mutually exclusive clusters. This technique assigns each observation to a cluster by minimizing the distance between the data point and the mean or median location of its assigned cluster, using Mahalanobis distance measures. Mahalanobis distance is a unitless measure computed using the mean and standard deviation of the sample data, and accounts for correlation within the data. In our analysis we assumed to have only 3 clusters, namely, background (white colour), affected area (light colour) and unaffected area (dark colour). Each cluster in the partition is defined by its member objects and by its centroid, or centre. The centroid for each cluster is the point to which the sum of distances from all colour values in that cluster is minimized. Finally, digital and manual assessments of relative dissolved areas were compared using linear regression.

2.4 Shell dissolution in snails from the Brunei estuarine system

No quantitative analysis was conducted for these snails, but these observations were considered novel and contributing to understanding of the BES acidification, and use of snails to biomonitor acidification. In particular, the photographic data showed a temporal response, which compliments the spatial data presented for the *Nerita* snails. *Umbonium vestiarium* snails were collected from the same site in 2008 (Feb), 2011 (Feb) and 2017 (Dec). Further photographic information is given for *Neripteron violaceum* snails collected from weakly buffered highly-acidic upper estuarine water, infiltrated with an ASS-seep (Jan 2018).

3. Results

3.1. *Shell and mass responses of* Nerita chamaeleon *to acidic water exposure*

Random field collections revealed different size ranges for the populations. The independent variable for size, aperture length (AL), ranged between 11.32 and 5.63 mm for Punyit, but was more contracted for Empire snails (9.47 and 8.1mm; Fig. 3), suggesting that Punyit snails are capable of growing to a larger size. Despite the size differences, all regressions between AL and shell length (SL), shell mass (SM) and dry tissue mass (DTM) were significant for both populations (Figure 3). A significant effect of population/size of size reduction was observed for relative SL and DTM, but no effect was found for relative SM. Empire snails (from the more acidic water) possessed significantly shorter shells and produced relatively less tissue mass compared to the Punyit snails (Figure 3). The slope of relative SL was very close to the predicted slope for a length-length allometric relationship of 1; with regard to SM, negative and positive allometries were observed for Punyit and Empire, respectively, and Empire showed a positive allometry for DTM (predicted slope is 3 for length-mass relationships). However, the Empire allometries are consistently less robust than those of Punyit, given their lower regression values.

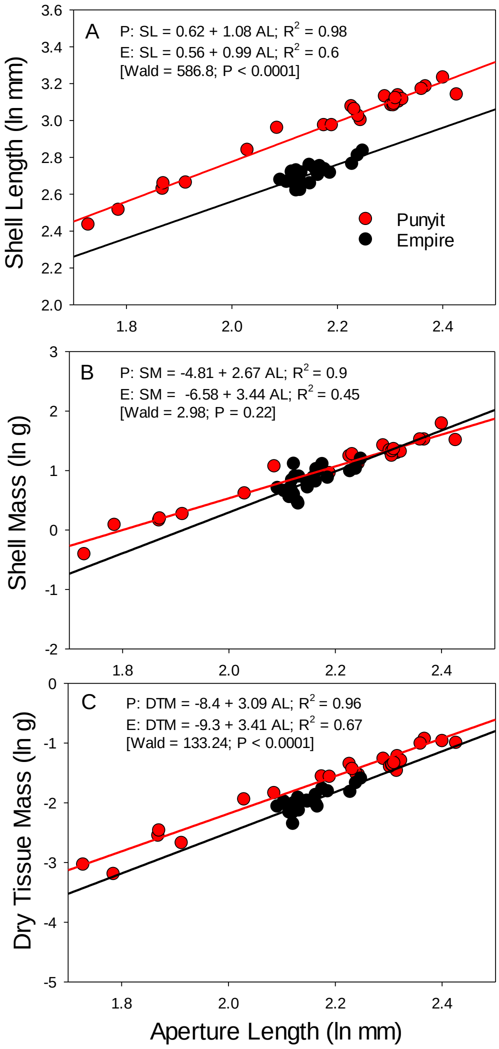

Figure 3. Relationships between aperture length (AL), shell length (SL), shell mass (SM), and dry tissue mass (DTM). Punyit snails, red and Empire snails, black.

3.2 Surficial shell dissolution of Nerita chamaeleon

There were marked differences between the populations with respect to surficial shell dissolution, determined by manually measuring planar hemispherical areas (Figure 4). Compared to Punyit, the total surface area of Empire was reduced, whereas the dissolved surface area and the relative dissolved surface area (proportional to total) of Empire snails were greater than those of Punyit snails. On average the relative dissolved surface of Empire snails was > 40% of the total surface area, compared to < 10% in Punyit snails (Figure 4). The proportional number of snail shells having dissolved area of > 10% versus no dissolution produced an inverse result for these populations; only one Empire snail exhibited no dissolution.

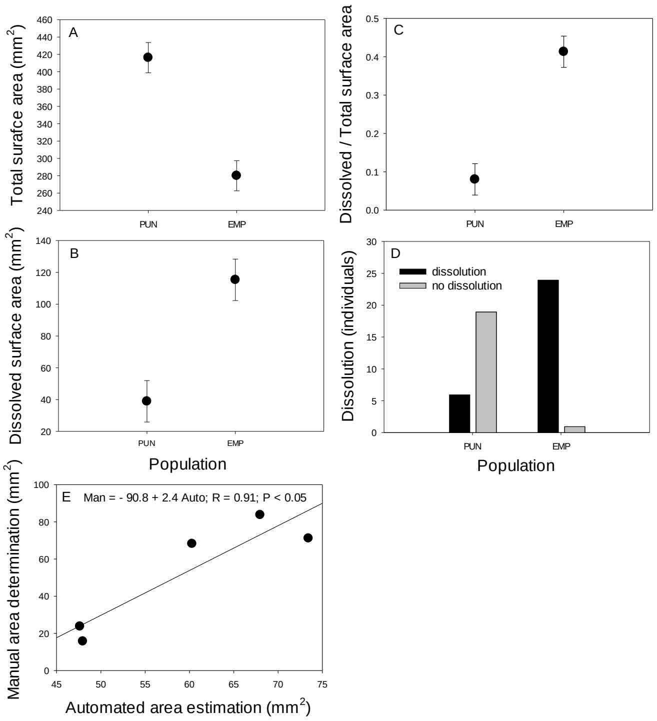

Figure 4. (A-D) Dissolved and total surface area results for Punyit (PUN) and Empire (EMP) snail populations determined manually. (E) Relationship between manually and digitally determined relative dissolution areas.

The percentage dissolved shell surface measured using the digital technique was applied to several shell samples (Figure 5). This figure shows how the affected area is replaced with a red colour and the unaffected area with a blue colour, leaving the background white (Figure 5). Comparing the manual calculation with the automated approach produced a good correlation (R = 0.91), though the manual approach underestimates the overall area (Figure 4); this may be the result of difficulty in manually capturing very small areas of dissolution as occurring between the shell ridges/ribs. Due to its simplicity and sufficient accuracy, this automatic segmentation technique can potentially be used for large sample analysis (Figure 4). However, in some cases the shell needs to be visually observed and segment area manually corrected, especially where the colour of the affected area is similar to natural patterns on the shell. Hence, a combination of approaches is best used initially.

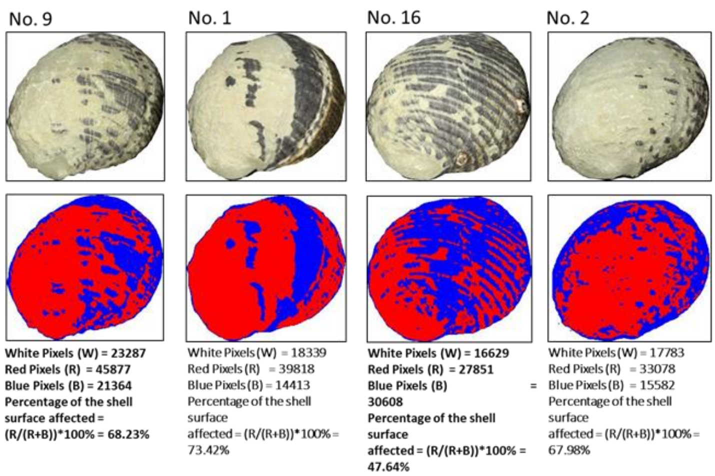

Figure 5. Examples of red-blue colour partitioning in individual shells used in the automated determination of percentage surficial dissolution.

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3.2. *Shell dissolution in snails from the Brunei estuarine system*

Hundreds of *Umbonium* snails have been collected from the same site at Bedukang between 2006 and 2011 during yearly field surveys, and again more recently in 2017. These shells have a varnished outer prismatic calcite shell layer (see specimens of 2008 and 2011 collections; Figure 6). In the 2017 collection, however, approximately 70% of the shells collected showed severe erosion (Figure 6), signifying a change in the pH regime. The coloration and patterning of these low-spired shells present an ideal case for application of the automatic segmentation technique. Manual capturing of surficial dissolution in this case is thwarted by the often very fine erosion lines (Figure 6).

Neripteron violaceum collected within a 1m² area below an ASS-seep at Kedayan, a site characterized by weakly buffered highly-acidic upper estuarine water, showed considerable individual variability in surficial dissolution response. The shells of some individuals were highly deformed, whereas those of others were virtually unblemished (30% of individuals; Figure 7).

Figure 6. *Umbonium* snails collected from the same site at Bedukang mudflats in three different years (month/year indicated).

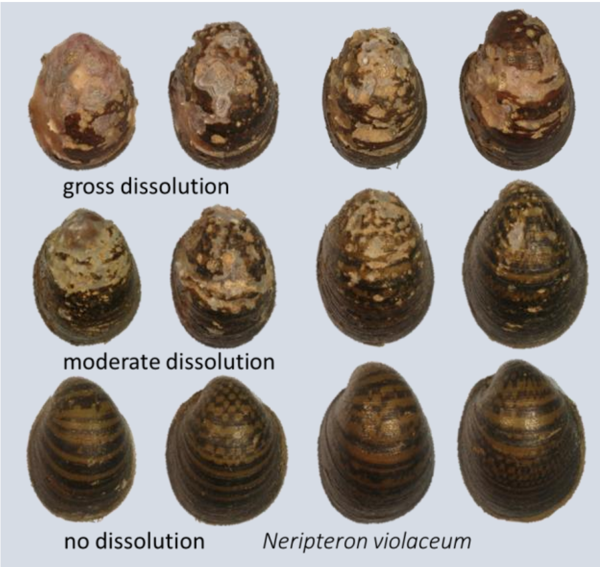

Figure 7. *Neripteron* snails collected from an extremely acidic habitat at Kedayan showing inter-individual varition in shell dissolution.

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4. Discussion

Monitoring of physical conditions in aquatic environments using measures of organismal attributes has been successfully implemented in the field of marine pollution [8-10]. Here we show that similar approach using shell dissolution can be used to monitor marine acidification. We found that gastropod shell dissolution reflects acidic water exposure, and that this potentially can be applied, virtually to the exclusion of conventional approaches, in assessments spanning broad spatial and temporal frameworks and heterogeneous intertidal habitats. Our exploration of several shell dissolution attributes, showed these to vary between nearby individuals to populations separated by kilometers, as well as across populations separated by decades. This cost-effective technology can be further developed, to serve as an early-warning-system of marine acidification, crucial to the context of ocean acidification [24].

Our study revealed that local impacts of geochemical acidification deriving from ASS-seepage extend across all marine environments in the region, from upper estuarine systems (Kedayan) to oceanic rocky shores (Empire). The rocky boulder beach gastropod, *Nerita chamaeleon*, proved to be excellent model system for evaluating organismal traits for acidification biomonitoring. Surficial shell dissolution was found to be superior in discriminating acidic water exposure than shell mass (loss). Previous reported effects of low pH exposure on shell mass are mixed with some studies showing distinct shell loss and others showing no effect [11-15]. Such different observations are underlain by complex interactive effects of both dissolution and calcification on shell mass, and the fact the calcification rates relate to feeding/energetic status, which is potentially altered by low pH. Stressfully low pH exposures must ultimately cause shell mass reduction by elevating dissolution rate (through carbonate system chemistry; [7]) and limiting calcification. Dissolution alone can cause shortening of the shell through spire erosion [11]. Despite *N. chamaeleon* possessing a low-spired shell, we recorded a shell-shortening response in the more acidic waters at Empire. The reduced dry tissue mass of Empire snails compared to Punyit snails to could result from several possible factors [21], including limited feeding opportunities, or increased energy partitioning towards shell production to counteract dissolution [11].

We show here how surficial shell dissolution can easily be mapped either manually or digitally, that these approaches can be mutually applied, and can be quantitatively assessed (areas) for a sample population or expressed as proportional number of individuals. The shell mass lost through surficial dissolution was not detected gravimetrically, indicating greater sensitivity of the surficial area parameter. Whereas the automative colour analysis used is limited in terms of the intrinsic shell colour contrast, it enables mapping of small areas that would be difficult to incorporate through manual determination. This technique has potential and deserves further consideration.

While no local ASS-seepage is observed at Bedukang, the first observation of shell dissolution in *Umbonium* snails there in over a decade is interesting in its own right. The shell dissolution patterning in the 2017 population is best explained by change in pH regime deriving from a general acidification effect in the Brunei Bay water. Although the Brunei Estuarine System acidification is multifactorial [7, 11, 20], recent published work suggests that aragonite and calcite undersaturation prevails mainly in Sungai Brunei, up to where the river enters the Brunei Bay [7]. Prior observations yielded little indication of significant shell dissolution in *Indothais* (and other) snails occurring seaward of this junction, apart from obvious local effects on mangrove snails in contact with anoxic, H_2S sediments. Shell dissolution effects were also observed for mudflat *Assiminea* sp. snails at Bedukang (Figure 8). The ongoing bridge construction in the vicinity over the past 3 years presents an explanation for a general pH regime shift; massive dredging of ASS sediments as would be associated with such construction is known to vastly raise acidity and metal exposure [25, 26]. Resistance of shells in individuals of some species is implied by the data for *Neripteron violaceum*, which are exposed to extremely corrosive water. Arguably this effect relates to genetic and/or plastic related individual differences in biomineralogy.

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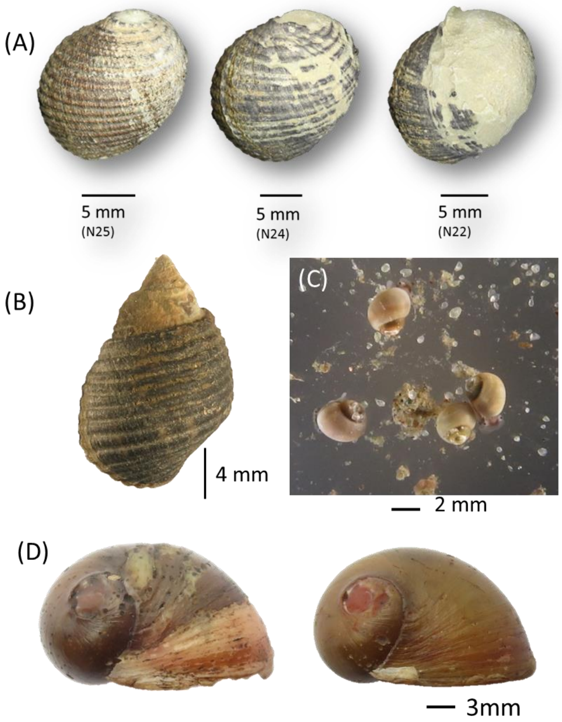

Figure 8. (A) *Nerita* snails (Empire) showing predictable age related dissolution (apex to lip, right to left), expected from acidic water exposure. (B) *Planaxis* snails (Empire) showing excessive spire erosion and (C) small *Assiminea* snails (Bedukang) exhibiting spire truncation. (D) Mating pair of *Clithon faba* collected from high-shore *Nipa* palms (Kedayan); both snails show apical dissolution, whereas the left snail shows unconfirmed shell erosion, pitting and lack of integrity of the growing lip.

5. Evaluation and caveats

Biomonitoring exercises using gastropod shell erosion are complicated by within (individual) and between species differences in response to corrosive water exposure. In all marine habitats considered here, there was a consistently high individual variability in surficial dissolution, which requires analysis of an appropriately large sample size. The selection of species is crucial, and consideration should be given to several general species-specific traits likely to influence biomonitoring outcomes, including growth rate, body size, lifespan and feeding behaviour. Although surficial dissolution features are uninfluenced by a snail's energetics and growth, growth often interrelates with age, which is useful for assessing the rate of dissolution. Additionally, shell traits are critical, such as shape, size, shell thickness and biomineralogy. Mapping of affected areas is readily achieved in low-spired shells, whereas information can be lost in the older shell (the spire) of high-spired shells, due to excessive erosion or truncation (Figure 8). Shell size and thickness generally relate to age, and slow growing, larger snails could be less sensitive biomonitor organisms. Shell mass, an attribute found to be uninformative in the *Nerita* snails, could however be useful for biomonitoring in very small, thin-shelled and fast-growing snails (Figure 8). Carbonate surficial dissolution directly relates to the biomineralogy of the outer shell surface. A confounding factor to interpreting this dissolution could be biomineralogy plasticity; determining whether plastic responses associate with informative or relatively uninformative new shell growth (see Figure 8), appears important.

Erosion patterns are not always the effect of corrosive water exposure, but often relate to bio-erosion and epilithic or endolithic commensalism [27]. This kind of shell erosion occurs randomly across the shell surface, relative to time of colonization. Uniform shell erosion across the entire surface of a dead snail should be ascribed to long-term weathering, which can occur even under normal seawater pH exposures. Nonetheless, assessing the degree of exposure to corrosive water in a snail population requires not only determining the surficial areas, but also ascertaining the spatial pattern of the eroded surface. Dissolution induced by carbonate chemistry experienced during a snail's lifetime should be more intense in the older shell region and moderate in areas where new shell is produced. Of course extreme acidic exposure only later in the lifetime of an individual might be reflected as dissolution at the growing shell edge (the lip). However, this pattern is open to interpretation; it may alternatively relate to a heath issue of the individual snail and inadequacy of calcification (shell deposition).

In summary, we argue that gastropod shell dissolution potentially provides a powerful and cost-effective tool for rapidly assessing marine pH regime change over a range of spatial and temporal frameworks and coastal ecosystems. This is not an exhaustive assessment of acidification biomonitoring, and we have hardly alluded to ecological phenomena. For example, the tidal height occupied by intertidal snails will directly influence the degree of exposure to corrosive seawater. There is also the need for further development and improvement on the methods presented here. Although calibrating rates of surficial gastropod shell dissolution will be difficult, which limits the value of the overall concept, there is little doubt that field observations of shell dissolution provide an early-warning-system of environmental pH change.

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Author contributions: DJM conceived the original idea; DJM and DTTW devised methods and approaches, AA and NAM analysed the shells and captured the data; LCDS developed the automated segmentation technique; all authors contributed to preparing and critically commenting on the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest

References

1. Powell, B.; Martens, M. A Review of Acid Sulfate Soil Impacts, Actions and Policies That Impact on Water Quality in Great Barrier Reef Catchments, Including a Case Study on Remediation at East Trinity. *Mar. Pollut. Bull.* **2005**, *51* (1–4), 149–164, DOI: 10.1016/j.marpolbul.2004.10.047.
2. Amaral, V.; Cabral, H. N.; Bishop, M. J. Effects of Estuarine Acidification on Predator–prey Interactions. *Mar. Ecol. Prog. Ser.* **2012**, *445*, 117–127.
3. Amaral, V.; Cabral, H. N.; Bishop, M. J. Prior Exposure Influences the Behavioural Avoidance by an Intertidal Gastropod, *Bembicium auratum*, of Acidified Waters. *Estuar. Coast. Shelf Sci.* **2014**, *136*, 82–90, DOI:10.1016/j.ecss.2013.11.019.
4. Bolhuis, H.; Schluepmann, H.; Kristalijn, J.; Sulaiman, Z.; Marshall, D. J. Molecular Analysis of Bacterial Diversity in Mudflats along the Salinity Gradient of an Acidified Tropical Bornean Estuary (South East Asia). *Aquat. Biosyst.* **2014**, *10* (1), 1–13, DOI:10.1186/2046-9063-10-10.
5. Hossain, M. B.; Marshall, D. J.; Venkatramanan, S. Sediment Granulometry and Organic Matter Content in the Intertidal Zone of the Sungai Brunei Estuarine System, Northwest Coast of Borneo. *Carpathian J. Earth Environ. Sci.* **2014**, *9* (2), 231–239.
6. Majewska, R.; Adam, A.; Mohammad-Noor, N.; Convey, P.; De Stefano, M.; Marshall, D. J. Spatio-temporal Variation in Phytoplankton Communities along a Salinity and pH Gradient in a Tropical Estuary (Brunei, Borneo, South East Asia). *Trop. Ecol.* **2017**, *58* (2), 251–269.
7. Proum, S.; Santos, J. H.; Lim, L. H.; Marshall, D. J. Tidal and Seasonal Variation in Carbonate Chemistry, pH and Salinity for a Mineral-acidified Tropical Estuarine System. *Reg. Stud. Mar. Sci.* **2018**, *17*, 17–27, DOI:10.1016/j.rsma.2017.11.004.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2018 doi:10.20944/preprints201803.0022.v1

8. Gibbs, P. E.; Bryan, G. W. Reproductive Failure in Populations of the Dog-whelk, *Nucella lapillus*, Caused by Imposex Induced by Tributyltin from Antifouling Paints. *J. Mar. Biol. Assoc. United Kingdom* **1986**, *66* (4), 767-777.
9. Gibbs, P. E.; Bryan, G. W.; Pascoe, P. L.; Burt, G. R. The Use of the Dog-whelk, *Nucella lapillus*, as an Indicator of Tributyltin (TBT) Contamination. *J. Mar. Biol. Assoc. United Kingdom* **1987**, *67* (3), 507–523.
10. Phillips, D. J. H.; Rainbow, P. S. *Biomonitoring of Trace Aquatic Contaminants*; Springer Netherlands: Dordrecht, 1993.
11. Marshall, D. J.; Santos, J. H.; Leung, K. M. Y.; Chak, W. H. Correlations between Gastropod Shell Dissolution and Water Chemical Properties in a Tropical Estuary. *Mar. Environ. Res.* **2008**, *66* (4), 422–429, DOI:10.1016/j.marenvres.2008.07.003.
12. Lardies, M. A.; Arias, M. B.; Poupin, M. J.; Manríquez, P. H.; Torres, R.; Vargas, C. A.; Navarro, J. M.; Lagos, N. A. Differential Response to Ocean Acidification in Physiological Traits of *Concholepas concholepas* Populations. *J. Sea Res.* **2014**, *90*, 127–134, DOI:10.1016/j.seares.2014.03.010.
13. Garilli, V.; Rodolfo-Metalpa, R.; Scuderi, D.; Brusca, L.; Parrinello, D.; Rastrick, S. P. S.; Foggo, A.; Twitchett, R. J.; Hall-Spencer, J. M.; Milazzo, M. Physiological Advantages of Dwarfing in Surviving Extinctions in High-CO_2 Oceans. *Nat. Clim. Chang.* **2015**, *5* (7), 678–682, DOI:10.1038/nclimate2616.
14. Chen, Y. J.; Wu, J. Y.; Chen, C. T. A.; Liu, L. L. Effects of Low-pH Stress on Shell Traits of the Dove Snail, *Anachis misera*, Inhabiting Shallow-vent Environments off Kueishan Islet, Taiwan. *Biogeosciences* **2015**, *12* (9), 2631–2639, DOI:10.5194/bg-12-2631-2015.
15. Harvey, B. P.; McKeown, N. J.; Rastrick, S. P. S.; Bertolini, C.; Foggo, A.; Graham, H.; Hall-Spencer, J. M.; Milazzo, M.; Shaw, P. W.; Small, D. P.; et al. Individual and Population-level Responses to Ocean Acidification. *Sci. Rep.* **2016**, *6* (1), 20194, DOI:10.1038/srep20194.
16. Connell, S. D.; Doubleday, Z. A.; Hamlyn, S. B.; Foster, N. R.; Harley, C. D. G.; Helmuth, B.; Kelaher, B. P.; Nagelkerken, I.; Sarà, G.; Russell, B. D. How Ocean Acidification Can Benefit Calcifiers. *Curr. Biol.* **2017**, *27* (3), R95–R96, DOI:10.1016/j.cub.2016.12.004.
17. Duquette, A.; McClintock, J. B.; Amsler, C. D.; Pérez-Huerta, A.; Milazzo, M.; Hall-Spencer, J. M. Effects of Ocean Acidification on the Shells of Four Mediterranean Gastropod Species near a CO_2 Seep. *Mar. Pollut. Bull.* **2017**, *124* (2), 917–928, DOI: 10.1016/j.marpolbul.2017.08.007.
18. Doubleday, Z. A.; Nagelkerken, I.; Connell, S. D. Ocean Life Breaking Rules by Building Shells in Acidic Extremes. *Curr. Biol.* **2017**, *27* (20), R1104–R1106, DOI:10.1016/j.cub.2017.08.057.
19. Grealish, G. J.; Fitzpatrick, R. W. Acid Sulphate Soil Characterization in Negara Brunei Darussalam: A Case Study to Inform Management Decisions. *Soil Use Manag.* **2013**, *29* (3), 432–444, DOI:10.1111/sum.12051.
20. Marshall, D. J.; Proum, S.; Hossain, M. B.; Adam, A.; Hoon Lim, L.; Santos, J. H. Ecological Responses to Fluctuating and Extreme Marine Acidification: Lessons from a Tropical Estuary (the Brunei Estuarine System). *Sci. Bruneiana* **2016**, *Special Is* (December), 1–12.
21. Marshall, D. J.; Aminuddin, A.; Ahmad, S. Gastropod Diversity at Pulau Punyit and the Nearby Shoreline – a Reflection of Brunei's Vulnerable Rocky Intertidal Communities. *Sci. Bruneiana* **2018**, *17*, 34-40.
22. Ng, T. H.; Kahar, R. S.; Marshall, D. J. Preliminary Checklist of the Freshwater Gastropoda of Brunei. *Occas. Molluscan Pap.* **2015**, *4* (March 2014), 1–5.
23. Proum, S.; Harley, C. D.; Steele, M.; Marshall, D. J. Aerobic and Behavioral Flexibility Allow Estuarine Gastropods to Flourish in Rapidly Changing and Extreme pH Conditions. *Mar. Biol.* **2017**, *164* (5), 97, DOI:10.1007/s00227-017-3124-y.
24. Caldeira, K.; Wickett, M. E. Anthropogenic Carbon and Ocean pH. *Nature* **2003**, *425*, 365, DOI:10.1038/425365a.
25. Proum,S; Santos, J.; Lim, L.H.; Marshall, D.J. Metal Accumulation in the Tissues and Shells of *Indothais gradata* Snails Inhabiting Soft and Hard Substrata in an Acidified Tropical Estuary (Brunei, South East Asia). *Reg. Stud. Mar. Sci.*, **2016**, *8*, 487-497.
26. Demas, S.Y.; Hall A.M.; Fanning, D.S.; Rabenhorst, M.C.; Dzantor, E.K. Acid Sulfate Soils in Dredged Materials from Tidal Pocomoke Sound in Somerset County, MD, USA. *Austral. J. Soil Res*. **2004**, *42*(6), 537-545. DOI.ORG/10.1071/SR03089.
27. Schönberg, C.: Fang, J.F.K.; Carreiro-Silva, M.; Tribollet, A.; Wisshak, M. Bioerosion: the other ocean acidification problem. *ICES J. Mar. Sci*., **2017**, *74*, 895–925, DOI.ORG/10.1093/icesjms/fsw254