Ecological responses to fluctuating and extreme marine acidification: lessons from a tropical estuary (the Brunei Estuarine System)

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Abstract

The impact of acidification on marine ecosystems has become a topic of priority research, following realization of unabated ocean acidification (OA), derived from globally-rising atmospheric CO_2. Many coastal and estuarine ecosystems have historically been acidified through various processes, and it is possible that we can learn and make predictions about OA impacts from the ways estuarine species and communities adapt and respond to acidified water. Studying estuarine acidification, nonetheless, aids understanding of the processes that affect ecological structure and functioning, important for coastal conservation and management. A broad-based research programme was implemented at UBD between 2011 and 2015, to investigate how variation in acidity and salinity affected assemblages and species in the Brunei Estuarine System (BES), an ecologically and economically important natural system in the region. This review summarizes studies that investigated for the BES, (*i*) the physical habitats and water physicochemistry, (*ii*) responses of planktonic and benthic microbial and faunal assemblages to exposure to variable and potentially highly acidic water, (*iii*) effects of acidification on ecological processes, such as barnacle dispersal and recruitment, (*iv*) behavioural and physiological mechanisms of organisms to cope with highly fluctuating and extreme pH water, with special reference to the gastropod, *Indothais gradata*, and (*v*) contaminant uptake by *Indothais gradata* under acidified conditions. The significance of the findings to the ecology of the BES, gaps that need addressing, and how estuarine acidification may contribute to predicting responses to OA are evaluated and discussed.

Index Terms: estuarine acidification, ocean acidification, carbonate chemistry, benthic, *Indothais gradata*

1. Introduction

Coastal and estuarine marine systems provide abundant resources, nursery grounds and ecosystem services, yet are increasingly threatened by urbanization and industrialization (more than 60% of the global human population is located along coastlines). The impact on these sensitive ecosystems is exacerbated by anthropogenic climate change, related to increasing CO_2 emissions (IPCC, 2013).[1] There is a direct link between elevated atmospheric CO_2 and warming and acidification of the surface water of the oceans.[2,3] Oceanic acidification (OA) is predicted to greatly adversely affect some of the most biodiverse and functionally significant marine ecosystems (such as the corals), a realization that has motivated innovative research over the past decade.[2,3] Despite such initiatives, referring to gradual pH change (decreasing by fractions of units over decades), our knowledge of

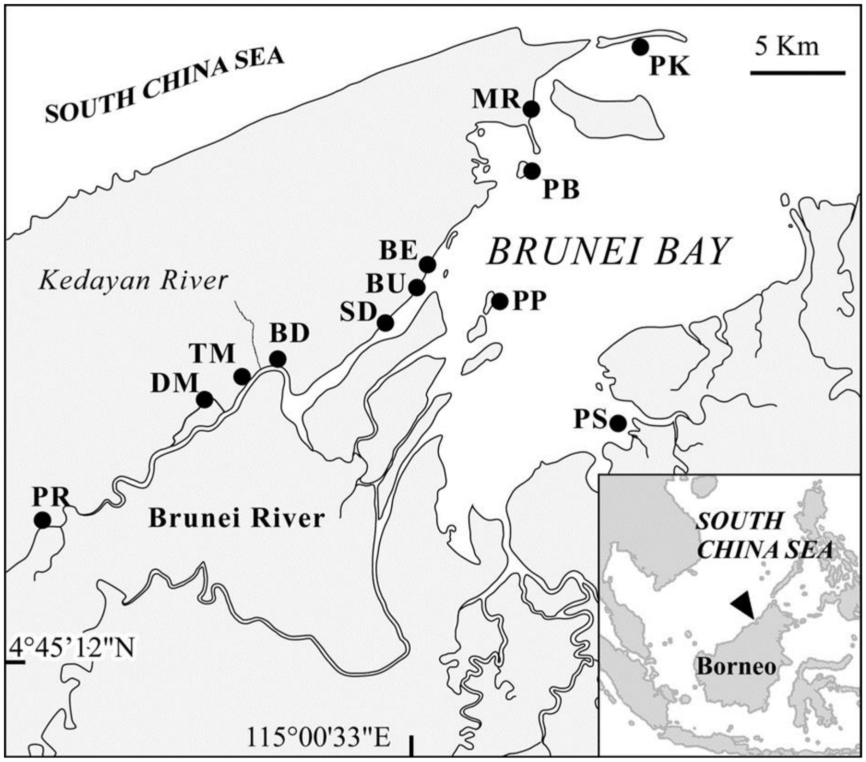

Figure 1. Map of the Brunei Estuary System (BES). Sampling stations for the various studies are shown. Kampong Parit (PR), Damuan (DM), Tamoi (TM), Bandar (BD), Serdang (SD), Sungai Bunga (BU), Sungai Besar (BE), Pulau Bedukang (PB), Muara (MR), and Pulau Keingaran (PK). Also given are locations of Sungai Kedayan, Pulau Pepetan (PP) and P. Selirong (PS).

species and community responses to ongoing dramatic temporal variation in pH (variable by full pH units over hours) in many coastal and estuarine environments is scarce.[4-6] Understanding the nature of pH variation in coastal systems, and how species and communities respond and adapt to it, potentially provides insights into responses of oceans to acidification, but in any event contribute to the conservation and management of such coastal ecosystems.

This review summarizes the findings of several studies undertaken on the Brunei Estuarine System (BES, ***Figure 1***) that aim to assess biological responses to pH change. These studies represent the culmination of research of UBD graduate students, and local and international collaborators, carried out between 2011 and 2015. The BES is ecologically and economically important to Brunei and the region. Historically, it has played a crucial role in the early establishment of the Bruneian people, through provision of food (artisanal fisheries and aquaculture) and habitation (an original water village still exists at Kampong Ayer; ***Figure 1*** and ***Figure 2***). Being fed by four major nutrient-rich rivers (Sungai Brunei, Limbang, Trusan and Temburong), the BES serves as an extensive nursery ground for fish communities, supports highly diverse, unique and pristine mudflats and mangroves, and offers huge potential for ecosystem services provision and scientific research.

Additionally, the BES represents a suitable model system for testing ecological responses to marine acidification. Although estuarine systems are characteristically acidified by sulphate reduction in muddy sediments, acidification in the BES water column is additionally enhanced by highly acidic groundwater inflows and metabolic activity of planktonic and benthic eukaryotes and microbes. The groundwater is extraordinarily acidified by acid sulphate soils (ASS)[7] and to some extent by peat-swamp leachates, whereas decomposition of high detrital input (mangrove leaf litter) elevates metabolic CO_2 production.

Figure 2. Upper: An artisanal fisherman throw-netting from a jetty at the Sungai Besar (BES) mangroves (photo, Roger Bamber). Lower: Sungai Brunei near Bandar showing Kampong Ayer, and characteristically turbid brown water (photo, David Lane).

Consequently, the water column of the BES is characterized by a steep acidity gradient from highly acidic upper estuarine waters (pH = 5.8 at Bandar, BD) to typically marine pH levels at the open ocean extremity that abuts the South China Sea (pH = 8.2 at Muara, MR) (***Figure 1***).[4] The ecological significance of the BES acidification was first reported from observations of gross shell erosion of the intertidal snail, *Indothais gradata* (Jonas, 1846).[4] Because of its ubiquitous distribution in the BES, this snail species has proven to be a valuable model for investigating organism-level responses to variable acidification.[8]

The review first describes (*i*) the physical environment and water physicochemistry of the BES. Then, it summarizes the results of studies investigating, (*ii*) responses of planktonic and microbial and faunal benthic assemblages to exposure to variable and potentially highly acidic water, (*iii*) effects of acidification on some basic ecological processes (such as barnacle dispersal and recruitment), (*iv*) behavioural and physiological mechanisms of organisms to cope with highly fluctuating and extreme pH water (with special reference to *Indothais gradata*), and (*v*) contaminant uptake by *I. gradata* under acidified conditions.[5,6, 8-15]

2. Ocean acidification versus estuarine acidification

Ocean acidification (OA) is a different concept to estuarine acidification, but there are commonalities, which could help understand both phenomena. Below we summarize (subjectively) the state of the art of the immensely rapidly growing field of OA, and suggest similarities and differences between OA and estuarine acidification (EA).

Essentially, OA refers to increased dissolution of CO_2 in the ocean surface water, as a consequence of anthropogenic atmospheric CO_2 elevation. This has two significant chemical outcomes: (*i*) water pH is lowered and (***Equation 1***) and (*ii*) carbonate dissolution increases (***Equation 3***)

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \qquad (1)$$

$$H^+ + CO_3^{2-} \rightleftharpoons HCO_3^- \qquad (2)$$

$$CaCO_3 \rightleftharpoons Ca^{2+} + CO_3^{2-} \qquad (3)$$

The destructive potential of OA on marine ecological systems was underscored following observations of shell thinning of pteropod (gastropod) samples during routine oceanic planktonic surveys, in the early 2000s.[2,3] Research that followed centered on key oceanic ecosystems, including corals, and aimed to understand species and community responses. Objectives were largely to identify sensitive species and taxonomic groups, to distinguish ‘winners’ from ‘losers’ for century-long acidification.[2] ‘Calcifiers’, organisms possessing calcified skeletons and structures (including shells), have been repeatedly shown to be more sensitive than moderately calcified or soft-bodied organisms; the latter two generally fair better in high pCO_2 water. Subtle differences in dissolution have been shown to relate to the $CaCO_3$ crystalline form, such that a higher proportion of calcite relative to aragonite offers greater robustness under elevated pCO_2. A plethora of laboratory studies have now investigated behavioural, physiological and morphological responses, in protocols that compare present-day pCO_2 exposures with experimental treatments that mimic pH and pCO_2 levels around year 2100.[17] Elevated pCO_2 does not always have a negative effect; photosynthesizing organisms may benefit from enhanced productivity in high pCO_2 water. Field studies assessing benthic assemblage responses to naturally high present-day pCO_2 have utilized volcanic vent systems.[18] Although oceanic vent studies continue to produce vast insights into the biological impacts of OA, observations are possibly confounded by other gases or metal contamination associated with vents. How organisms and species might acclimate or adapt to pCO_2 change, variable by fractions of units over decades, is considered a major challenge to future OA work. Other areas requiring urgent investigation include, multi-stressor responses (including effects of interacting temperature, nutrients and hypoxia)[19] and the ways in which acidification and elevated pCO_2 affect ecological interactions, such as predation, competition, etc.

OA and EA differ fundamentally in (*i*) acidification mechanism and (*ii*) timeframe and amplitude of pH change. Whereas OA refers to variation in atmospheric CO_2 over decades, EA is dynamic and usually multifactorial involving, (*i*) biological CO_2 generation, as well as, (*ii*) mineral acidification (with each category constituting further multifactor processes). Additionally, coastal systems are strongly influenced by direct anthropogenic acidification (such as, contaminants and acids released directly into the water). Naturally, contributions to the total acidity by either biological or mineral acidification are likely to vary proportionally at different timescales. For example, mineral acidification relating to acidic freshwater inflow is expected to change seasonally in equatorial regions, being greatest during monsoonal flooding, when freshwater inflow is greatest; biogenic acidification is expected to vary tidally, due to mixing of seawater with poorly-buffered, nutrient-rich brackish water near mangroves and mudflats that supports high benthic microbial activity. To our knowledge, no previous published study has assessed whether different mechanisms of acidification have different ecological outcomes. Probably the best known studies linking OA and EA refer to special cases of penetration of acidic, nutrient-rich, productive estuarine waters (for which pH is biogenically-lowered) into the oceans, often extending hundreds of kilometers out to sea (Pearl River estuarine system).[20-22]

3. Brunei Estuarine System (BES)

The Brunei Estuarine System (BES) comprises complex interacting shallow marine water bodies in northern Brunei (Borneo, South East Asia). It is defined here as the Inner Brunei Bay and the major waterways leading into this, including the Sungai Brunei (Brunei River), S.Temburong and S.Limbang (***Figure 1***). This review focuses on the western perimeter of the BES, that edging Bandar-Maura from Kampong Parit (PR) to Pulau Keingarong (PK) (***Figure 1***). Several islands are found within the Inner Brunei Bay, including the proclaimed Brunei protective area at Pulau Selirong (PS), which reputedly contains some of the oldest mangrove stands in the region, and P. Pepetan (PP) and P. Bedukang (***Figure 1***). The BES supports extensive intertidal mudflats, and its perimeter is predominantly mangrove-fringed. The impact of small-scale aquaculture and artisanal fisheries throughout the system is considered low, though shell-fish harvesting on mudflats has potentially damaging ecological effects. Human-derived impact is presently greatest along the Sungai Brunei, which supports widespread urban settlement, particularly towards Bandar and Kampong Ayer. Tributaries, such as S. Kedayang, which enters the system at Bandar, apparently carry polluted water from the relatively densely-populated Gadong and Kuilap (***Figure 1***). Construction and industrial activities in the Brunei Bay (such a charcoal production at Pepetan) are low key, though the situation could change dramatically with the building of the iconic bridge, planned to traverse the BES from Sungai Besar to Temburong.

The BES undergoes extreme physicochemical variation. The water is typically brown and turbid, due to high suspended sediment and organic loads, especially during the monsoons (***Figure 2***). Salinity, varying between 4 and 33 psu (Bandar to Muara)[4]**,** is influenced by semi-diurnal tides generated in the South China Sea, stochastic swell forcing, and freshwater inflow. Acidification, as already mentioned, derives from multiple sources, mainly combinations of (*i*) poorly buffered hyposaline water, (*ii*) groundwater permeating acid sulphate soils (ASS), (*iii*) microbial respiration in nutrient enriched waters and (*iv*) direct anthropogenic release of acidic substances.[4] ASS soils are widespread in Brunei[7]; they constitute 'fossilized' pyrite-rich marine sediments (FeS_2) formed during sulphate reduction when the sediments were active marine ecosystems, during periods of global sea-level-rise. Pyrite oxidation, through disturbance of sediments or when flushed, produces iron hydroxide [$Fe(OH)_3$] and sulphuric acid (H_2SO_4). Temporary die-back of local assemblages has been observed along the length of the BES, but communities are seemingly well-adapted to physical extremes and variability[8] (***Figure 3***).

Figure 3. A rich intertidal desmosponge community at Pulau Bedukang was obliterated by extreme sediment loads during January 2014 monsoon flooding.

Although we refer in the review to 'acidification' effects, the BES acidification strongly correlates with other abiotic factors, notably salinity (***Figure 4***), thus, in most cases, the described effects are multifactorial. We use the terms 'acidification' and 'acidity' with reference to the process and the effect, respectively. Both are relevant, as the BES is continually acidified, albeit highly variable in terms of space and time; this differs notably from the linear, slow acidification of the oceans.

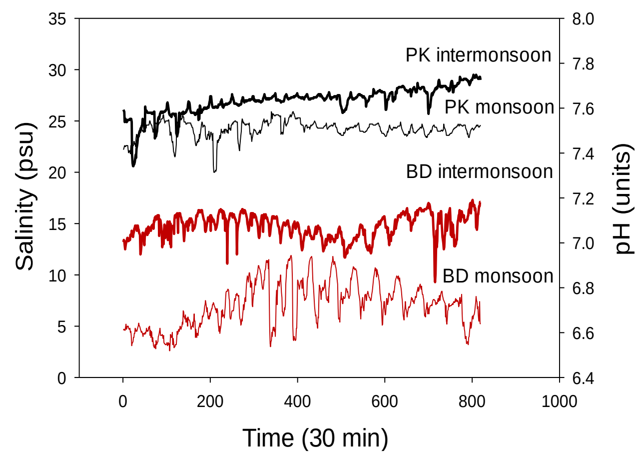

Figure 4. High-resolution temporal recordings of pH and salinity (every 30 min) at Bandar (BD) and Pulau Keingaran (PK) during monsoon (24 Nov -11 Dec 2013, thin line) and inter-monsoon periods (26 Feb – 15 Mar 2014, thick line). Baseline level and tidal amplitude of parameters are shown to vary between stations and seasons.

4. Physical and chemical properties of habitats

4.1 Sediments

Sediment properties of estuarine systems underlie the habitat of functionally-important benthic animals and plants, such as prokaryotes (bacteria), microphytobenthos (benthic diatoms), meiofauna and the macrobenthos (animals with body size <0.05 mm, dominated polychaetes and crustaceans). Hossain et al. studied grain size in the BES.[10] This analysis showed domination of sand fractions in sediments, giving a particle size distribution as follows: sand > clay > silt. Seaward stations yielded a greater sand size fraction and lower organic matter content (< 4 %), compared to the usually low-energy landward stations, which constituted a relatively higher proportion of clay and silt in the sediments, and which were relatively rich in organic content (> 5%). The observed variation in organic matter relative to grain size for the various stations complied with our prediction and with the general trend for tropical estuaries; in the BES, organic content is elevated by input from dense mangrove stands and anthropogenic eutrophication. Despite the general tendency of seaward increase in grain size[10], a finer spatial resolution of sampling in the BES could reveal more within-station particle size variation, given the highly variable hydrological features over narrow spaces at some stations. As an example, strong outflowing currents must prevail at small tributary confluences in the upper Sungai Brunei (near Bandar), producing patches of more sandy and less nutrient rich sediments than those observed in the study.

4.2 pH, salinity and carbonate parameter variation

The role of carbonate acidification in estuarine systems that are already acidified by mineral processes has been poorly investigated. This question is addressed in a study by Proum et al.[6] Specifically, variation in surface water pH, salinity, temperature, total alkalinity, pCO2, DIC, calcite saturation and aragonite saturation were studied. Hydrologic characteristics measured at three stations, one from each estuarine region (upper, middle, and lower – BD, SD, PK, respectively, ***Figure 1***), confirmed estuarine-wide rapid seaward increase in pH and salinity. High temporal resolution (30 min) data-logging uncovered tidal fluctuations in pH and salinity that were accentuated upstream (landwards), due to variable mixing of the elevated pCO_2 and acidity in the weakly-buffered hyposaline water there (***Figure 4***). A steep decline in baseline pH was observed during monsoons, presumably due to greater ASS flushing in this season; the baseline decline was again more pronounced in the upper estuary (***Figure 4***, BD). The carbonate system analysis (based in discrete water sampling) showed supersaturation of pCO_2 in the upper and middle BES, indicating that these regions are a net source of atmospheric CO_2, whereas the lower estuarine region appeared more like a net CO_2 sink. pCO_2 levels in the upper BES exceeded values reported for many other estuaries, but were similar to data described for other mangrove or ASS-influenced estuaries. Overall, the study showed complex interactions in the BES between acidification parameters, processes, tides and seasons.[5]

5. Community and organism responses

5.1. Planktonic communities

The planktonic community has been characterized in a study by Majewska et al.[11] Samples were collected at four stations (Chermin (near S. Bunga); BU; Pintu Malim (near BD) and S. Kedayan; Fig. 1) between Aug 2011 to June 2012, covering a range in salinity and (0.4 - 28.5 psu) and pH (5.87 - 8.06). The survey recorded a total of 25 algal families (22 genera of diatoms and seven of dinoflagellates) and one ciliate family. Phytoplankton density varied between 7 and 9107 cells ml^{-1}. Diatoms dominated the communities, with species of *Nitzschia*, *Rhizosolenia* and *Leptocylindrus* having highest abundances. Phytoplankton communities varied seasonally (30% of the total variance) among sample stations (20% of total variation). The interactive effects of pH and salinity, and of pH and temperature, explained 16.7% and 17.5% of the total observed variation, respectively.

5.2. Mudflat bacterial diversity

Bolhuis et al.[5] surveyed the surface intertidal mud bacterial diversity for samples collected at six stations over 40 km along the BES (PR; Damuan, DM; BD; S. Besar, BE; PP; MR; ***Figure 1***). The diversity was compared from community fingerprinting analysis using 16S rRNA gene based denaturing gradient gel electrophoresis, and from 16S rRNA gene sequencing and phylogenetic analyses. Results showed functionally conserved, diatom-dominated communities constituting mainly novel species. Species composition at each station was 50-70% unique. Clustering of the sequences commonly occurred, indicating that proteobacterial diversity related to the acidity/salinity gradient of the BES. On the whole, the diversity (considering all phyla) varied in a predictable way with the physical parameters. Mudflat communities comprised typical functional groups of microorganisms associated with photosynthetic carbon flux, sulphur cycling (Gamma- and Deltaproteobacteria) and decomposition (Bacteroidetes). Structurally, however, communities were discretely distributed along the BES gradient, constituting largely novel bacterial species. This study provides the first insights into patterns of bacterial community structure in a tropical South East Asian estuarine system that experiences highly variable pH and salinity conditions.

5.3. Benthic infaunal communities

Hossain & Marshall[9] investigated infaunal macrobenthic community structure, for samples collected at S. Kedayan, DM, BD, BE and MR (***Figure 1***) between July 2011to June 2012. Sediment pore-water salinity (8.07 to 29.6 psu) was found to decline landwards in accordance with the above-sediment estuarine water salinity (3.58 to 31.2 psu), but interestingly, pore-water pH (6.47- 7.72 units) was remarkably more invariable among stations along the gradient compared to the estuarine water pH (5.78- 8.3 units). The implication is that communities along BES are more strongly influenced by salinity than by pH. Thirty six species were recorded, with neritid polychaetes (*Neanthes* and *Onuphis conchylega*) and a corophiid amphipod dominating. Calcified microcrustaceans (*Cyclopoida* sp. and Corophiidae sp.) were abundant at all stations; there was no clear distinction in BES distribution between calcified and non-calcified infaunal groups. Species richness increased seawards, though abundance (density) showed no distinct directional trend. Diversity indices were largely positively correlated with salinity and pH. Three faunistic assemblages were observed: (*i*) nereid-cyclopoid-sabellid, (*ii*) corophiid-capitellid and (*iii*) onuphid- nereid-capitellid. These assemblages respectively associated with low salinity/pH and muddy sediment, low salinity/pH and sandy sediment, and high salinity/pH and sandy sediment. A multivariate analysis confirmed that species distribution and community structuring are more strongly influenced by sediment particle properties than by water chemistry (either pH or salinity). In conclusion, infaunal estuarine communities are generally well-adapted to cope with highly acidic conditions, and are less likely exposed and probably less vulnerable to estuarine water acidification than epibenthic or pelagic communities.

5.4. Epibenthic faunal communities

Hardsubstrata, such as natural or artificial rocks, concrete or wooden surfaces of mangroves, driftwood, jetties, embankments and bridges, are prevalent in the BES, providing habitat for a variety of animals and plants. Hossain et al.[14] studied variation in species composition, abundance and community structure of BES intertidal epifaunal invertebrates (BD, SD, BU, and PB; ***Figure 1***). Species richness, diversity and abundance were highest at the Bedukang, low in the mid-estuary (Serdang and Bunga) and relatively high again near Bandar. Epibenthic macrofaunal diversity was low (34 in 72 samples), reflecting inadequate taxonomic resolution; however, abundances were high with around 100-300 individuals per 100 cm^2. Three distinct communities were observed: a tanaid-polychaete community in upper estuary, a mussel-dipteran community in mid-estuary and a mussel-amphipod-dipteran dominated community in the lower-estuary. The most seaward station had more than double the abundance of hard substratum macrofauna compared to the other stations. In contrast with infaunal communities, which apparently mainly influenced by salinity variation, the epifaunal communities are likely similarly influenced by both estuarine water pH and salinity. While the seaward diversity probably associates with incursion of typical marine species, the high diversity at Bandar could relate to species favoring nutrient-enriched conditions.

5.5. Ecological processes (barnacle recruitment/settlement on wooden poles)

Barnacles (*Balanus* sp.) are abundant on hard surfaces throughout the BES, settling on natural rocky outcrops, mangrove roots and trunks, and on artificial wooden and concrete walls and jetties. A field study was undertaken by Adam and Marshall[15] to assess whether pH/salinity influenced barnacle recruitment, settlement and early growth in the BES. Wooden poles were embedded into the seabed within the intertidal zone at three locations (DM, S. Kedayan and SD; ***Figure 1***) and barnacle parameters were assessed following collection every month, over 3 months. The study also investigated vertical distribution and microscale hydrological effects around the poles. Interestingly, the results suggested that acidification is not the dominant factor effecting barnacle settlement and early growth along the BES. Recruitment and settlement are apparently effected by the dynamic combination of conducive environmental conditions and high larval supply. Furthermore, nutrient enrichment was suggested to induce more rapid barnacle growth, despite the stressful environmental conditions in the Kedayan area. Further investigation is required to tease apart the confounding influences of larval supply and nutrient enrichment.[15] The wooden pole approach was nonetheless revealing, and showed that several other key taxa (including the serpulid tubeworms, probably *Hydroides*) colonize the poles at the extremely acidic study sites.

5.6. Ecological processes (barnacle size/density on snail shells)

To assess barnacle age/size structure across the pH/salinity gradient, a novel approach was followed, using barnacle settlement on living gastropod shells (*Indothais gradata*) (***Figure 5***).[16] Snails were collected at three stations along the BES (Tamoi, TM, BD, PB; ***Figure 1***), on two occasions, separated by a seven-year period (2005 and 2012). Barnacle growth differed significantly between years and among stations. Barnacle size (standardized to the same shell age) was lower upstream, suggesting an effect of acidification on growth, an observation contrasting with that for early barnacle growth (see above). An interesting unexpected result was the significant difference in surface area of *Indothais* shells between 2005 and 2012; shell surface area was less at 2012. Differences in width-length ratios of shells suggested a cohort effect, relating to local extinction and new recolonization at some stations. Shell surface area decreased landwards (more acidic sites), which might confound the barnacle growth result, due to crowding (competition for space) on smaller shells.

5.7. Physiological and behavioural responses of snails to acidification

Organism-level responses to extreme and rapid coastal acidification were investigated, with special reference to the physiological and behavioral capacities of *Indothais gradata.*[13] Several very novel findings were revealed when comparing laboratory responses between populations from opposite ends of the BES (BD and PB; ***Figure 1***). The pH range for aerobic performance under acutely increasing mineral acidity (appropriate natural rates) was similar for the populations; however, the lower threshold supporting high aerobic function (4.54 - 5.14 units) was much lower than expected, considering the present and past pH levels experienced. CO_2-acidification raised the threshold to 6.3 units, above that of mineral acidification. Population differences were observed for the time course for behavioral and physiological recovery under stable lowered pH and salinity. Seaward populations showed reduced capacity to recover cardiac performance under chronic pH and salinity reductions, but when free to move under these conditions, they quickly became more active and attempted to escape from the water; in contrast the landward population showed little movement. These capacity differences offer novel insights into physiological mechanisms employed by coastal gastropods to deal with highly fluctuating and different sources of acidification (mineral versus CO_2). They suggest that hypercapnia (rather than pH) associated with elevated pCO2 is the key to regulating snail aerobic physiology. This and other physiological observations have consequences for generalizing of effects of marine acidification across taxonomic, physiological and ecological ambits.[13]

5.8 Snails as indicators of metal contamination

Acidification impacts estuarine ecology, but it can also change availability of contaminants (via metal speciation) to organisms. Proum et al. undertook a metal pollution biomonitoring study in the BES, in particular, assessing the accumulation of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in *Indothais gradata.*[12] Because this snail inhabits both hard and soft substrata, (sediments, wooden pillars, rocky outcrops, etc.) it was possible to compare metal accumulation (*i*) between substrata (habitats), (*ii*) between the

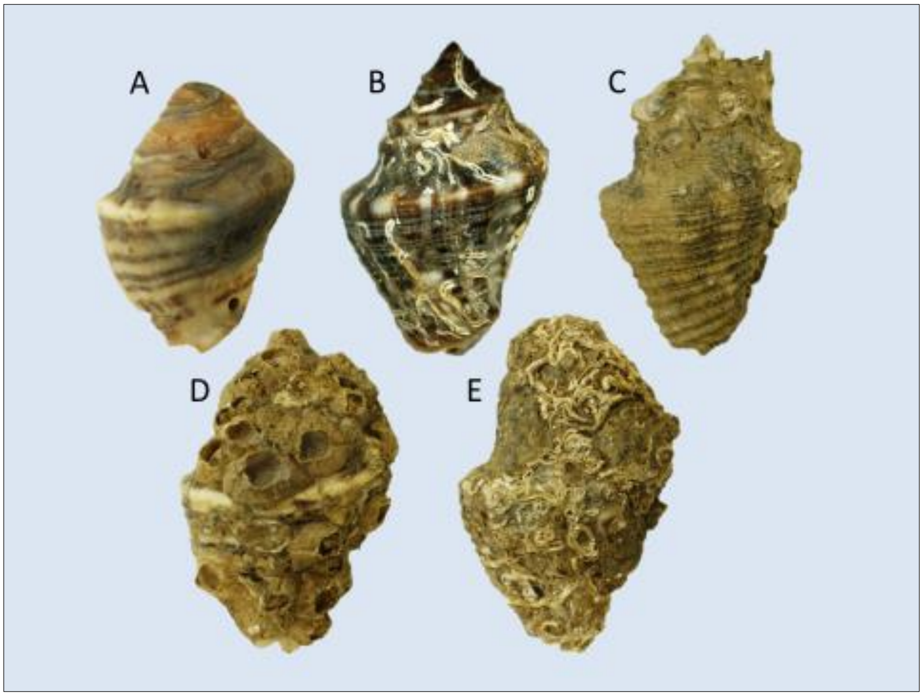

Fig. 5. *Indothais gradata* snails collected from Bandar (A, B), Bedukang (C) and Tamoi (D, E), showing acidic dissolution, epibiont colonization and sediment deposition of shells.

shells and soft tissues of snails and (*iii*) among sample stations along the estuarine gradient. The study found that substratum type had no effect on metal burden. Whereas higher concentrations of essential metals occurred in the tissues relative to the shells, shell metal accumulation was vastly more discriminatory among the sample stations. This and good correlations among different metals, suggest that shells represent better biomonitor tools than tissues for conditions of small estuarine contamination, as in the BES. Domestic pollution was suggested to be most severe, indicated by greatest contamination at Bandar, the entry point of BES tributaries (especially S. Kedayan) feeding from Gadong and Kuilap. Contaminant levels fell off sharply upstream and downstream of Bandar, indicating a limited freshwater mixing or seaward tidal forcing. Fe accumulation in *Indothais* shells was distinctly greater than that of the other metals, consistent with the naturally highly pyritic soils (FeS_2). The effect of acidification in raising the metal burden in the BES, was not clearly shown, when compared with published data for other estuaries; this nonetheless deserves more critical consideration.

6. Concluding remarks

Overall the findings suggest a highly integrated and functional marine ecosystem along the BES to at least Bandar, despite the steep salinity and acidity gradient. Although diversities were relatively low at Bandar, abundances were often high in the case of key marine taxa, presumably largely relating to high nutrient loads. Clearly many taxa are physiologically and behaviourally adapted to the highly dynamically variable salinities and acidities, and supersaturated CO_2 at Bandar and landwards. The fluctuating nature of the physicochemistry suggests periods of amelioration, in which near optimal performance of organisms can be achieved. *Indothais* snails are also well equipped behaviourally to isolate themselves under extreme conditions, reducing exposure. A variety of taxa (mussels, oysters and *Hydroides* – polychaetes that form calcareous tubes) were found to colonize wooden poles at Damuan; whether they are capable of sustaining energetic levels to grow to full size remains to be known. Additional to abilities to behaviorally and physiologically adapt to and secrete calcareous structures under high acidity, communities along the entire BES were found to recover following die-backs during extreme conditions (DJM, personal observation). Generally, these studies have vastly advanced understanding of organism and community responses to the physical stress characterizing the BES, and show that the BES represents an excellent natural laboratory to explore marine acidification impacts.

Nonetheless cognizance should be given to the limitations with working in estuaries. A major limitation is the pH-salinity correlation, which usually makes it difficult to unravel either effect. Other abiotic factors may be correlated with these, such as contamination. Thus, extrapolation to OA situations from EA observations should be made with reservation; however, much knowledge gained from estuarine studies can be integrated with ocean acidification frameworks. There were several commonalities in benthic community structure variation along the estuarine acidity gradient studied and a pCO_2 gradient associated with vent systems.[9,23] To tease out the effect of acidity on estuarine community structure, comparison could be made between similar kinds of estuaries that are either additionally acidified or show high pH waters. In acidified estuaries, comparisons between infaunal (less acidity variation) and epifaunal (more acidity variation) communities may prove to be useful; though care needs to be made to prevent degassing when sampling and measuring the within-sediment pH. Estuarine species which already experience extreme conditions are excellent models for assessing capacities for adaptation in acidified environments. Different physiological responses of *Indothais* warn against approaches based on CO_2 acidification in OA work, which confounds the effects of acidity and hypercapnia in laboratory experiments[13]; while these factors are correlated naturally under OA frameworks, unraveling mechanisms becomes more difficult when they are confounded in studies.

There are omissions in the BES suite of studies of crucial factors relating to estuarine ecology. This is true especially for turbidity and nutrients. These are important to estuarine structure and functioning; localized nutrient enrichment can possibly be inferred in cases where acidity has no effect on distribution. This was found in the plankton and barnacle studies; nutrient enrichment and planktonic blooms probably increase barnacle growth, regardless of acidification level - these situations are ripe grounds for further investigation. There is also a need for long-term, high-temporal-resolution monitoring of the hydrology of the BES and for relating such data to local weather conditions. Greater understanding of impacts on the BES ecology is especially relevant to the context of the bridge construction over this system. The interconnectivity of the BES and nearby coastal systems (which include sensitive coral reefs) could also potentially yield highly-innovative future research.

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***Appendix.* BES studies published in peer-reviewed journals (2011 to 2016)**

1. Proum, S., Harley, C., Steele, M., & Marshall, D.J. (2016). Physiological flexibility and behavioural isolation help snails flourish in fluctuating acidic estuarine waters. *Marine Biology* (in review).
2. Proum, S., Santos, J.H., Lim, L.H. & Marshall, D.J. (2016). Spatial and temporal variation in carbonate chemistry, pH and salinity in the surface water of a pyrite-acidified, tropical estuarine system. *Coastal Shelf Research* (in review).
3. Proum, S., Santos, J.H., Lim, L.H. & Marshall, D.J. (2016). 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). *Regional Studies in Marine Science* (in press).
4. Majewska, R., Adam, A., Mohammad-Noor, N., Convey, P., de Stefano, M. & Marshall, D.J. (2016). Spatio-temporal variation in phytoplankton communities along a salinity and pH gradient in a tropical estuary (Brunei, Borneo, South East Asia). *Journal of Tropical Ecology* (in press).
5. Hossain, M.B. & Lauren, H. (2016). New species *Victoriopisa bruneiensis* and *Apocorophium acutum* (Chevreux, 1908) from Brunei (Crustacea: Peracarida: Amphipoda). *Zootaxa*, 4117, 375-386.
6. Bamber, R.N. & Marshall, D.J. (2015). Tanaidaceans from Brunei, V. The Leptocheliidae (Crustacea: Peracarida: Tanaidacea), with four new species. *Zootaxa*, 3948, 342-360.
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12. Hossain, M.B., Bamber, R.N. (2013). New record of a wood-boring isopod, *Sphaeroma terebrans* (Crustacea: Sphaeromatidae) from Sungai Brunei estuary, Brunei Darussalam. *Marine Biodiversity Records*, 6, e18.
13. Bamber, R. N., & Marshall, D. J. (2013). Tanaidaceans from Brunei III. A New Genus and Two New Species of Shallow-water

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