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Correlations between gastropod shell dissolution and water chemical properties in a tropical estuary

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Abstract

Although poorly reported in the scientific literature, acidic waters characterize many South East Asian estuaries. The observation of shell dissolution in a typically marine gastropod whelk (*Thais*) prompted investigation into determining relationships between shell properties of this whelk and the water chemistry (including pH) of the Sungai Brunei estuary (Borneo) in which it occurs. Shell weight, shell length and topographical shell features were determined for populations of *Thais gradata* distributed along a gradient of pH and salinity ranging between 5.78 and 8.3 pH units, and 3.58 and 31.2 psu. Shell weight varied independently of the co-varying acidity, salinity and calcium levels experienced. In contrast, shell length and a semi-quantitative variable based on shell sculpturing (shell erosion rank, SER) were significantly correlated with these water chemistry variables. This study brings attention to the potential use of estuarine organisms and systems in investigating current marine acidification questions.

Keywords: Acidification; Calcium carbonate; Estuaries; Gastropods; *Thais*; pH

1. Introduction

Acidification of marine systems, notably that relating to elevated atmospheric carbon dioxide (CO₂) and the oceanic carbonate equilibrium, has attracted considerable recent interest (Broecker, Takahashi, Simpson & Peng, 1979; Feely, Sabine, Lee, Millero, Lamb, Greeley et al., 2002; Caldeira & Wickett, 2003; Orr, Fabry, Aumont, Bopp, Doney, Feely et al., 2005). Current prediction of fairly rapid acidification of the ocean surface waters (decades; Orr et al., 2005) is disturbing in the context of our limited understanding of how organisms and ecological systems might respond to this (Seibel & Walsh, 2003; Pörtner, Langenbuch & Reipschläger, 2004; Shirayama & Thornton, 2005). Predictably, organisms with reduced pH tolerance or with calcium carbonate skeletal structures (such as, coralline algae, corals, echinoderms, molluscs) will be most affected (Pörtner et al., 2004; Jokiel, Rodgers, Kuffner, Andersson, Cox & Mackenzie, 2008; Kuffner, Andersson, Jokiel, Rodgers & Mackenzie, 2008; Hall-Spencer, Rodolfo-Metalpa, Martin, Ransome, Fine, Turner et al., 2008), though community cascading is likely to cause local ecological devastation, especially in the case of coral reef systems (Pelejero, Calvo, McCulloch, Marshall, Gagan, Lough et al., 2005). In the above context, investigations into environmental circumstances leading to carbonate undersaturation and consequently to dissolution of shells and skeletons in marine ecosystems are highly relevant and important.

Although the effects of environmental CO₂ and pH change on organismal functions (growth, physiology and toxicity) are becoming better known (Yamada & Ikeda, 1999; Hinga, 2002; Seibel & Walsh, 2003; Michaelidis, Ouzounis, Paleras & Pörtner, 2005; Shirayama & Thornton, 2005; Berge, Bjerkeng, Pettersen, Schaanning & Øxnevad, 2006) information on *in situ* shell dissolution of oceanic organisms is limited to pelagic pteropod (*Clios*) and heteropod molluscs (Orr et al., 2005). In littoral systems,

shell dissolution studies have focused on unicellular foraminiferans which are used to assess current and historical environmental perturbations (Geslin, Debenay, Duleba & Bonetti, 2002; Hayward, Grenfell, Nicholson, Parker, Wilmhurst, Horrocks et al., 2004). While the above is not necessarily all embracing from an ecological perspective of shell dissolution, only a single known study concerns *in situ* dissolution for gastropods in coastal systems (Hall-Spencer et al., 2008).

Acidification is generally overlooked in coastal ecological studies, despite its prevalence in estuarine systems (Howland, Tappin, Uncles & Plummer, 2000; Braga, Bonetti, Burone & Filho, 2000; Abril, Etcheber, Delille, Frankignoulle & Borges, 2003; Lin, Wood, Haskins, Ryffel & Lin, 2004; García-Luque, Forja & Gómez-Parra, 2005). While lowered salinities reduce the buffering capabilities of estuaries, acidification can derive from high natural and or anthropogenic organic inflows through elevated microbial activity (Mirlean, Baraj, Niencheski, Baisch & Robinson, 2001; Abril et al., 2003; Braungardt, Achterberg, Elbaz-Poulichet & Morley, 2003; Lin et al., 2004; Powell & Martens, 2005, Zai, Dai, Cai, Wang & Wang, 2005). Heterotrophic microbial metabolism significantly increases acidity through the production of CO₂ and carbonic acid in the muddy substratum of estuaries, and in some cases, bacterioplankton may account for 60-100% of planktonic oxygen consumption (Jonas, 1997). Acidification is exacerbated in turbid eutrophic estuaries as a consequence of reduced photosynthetic activity and biological withdrawal of CO₂ (Abril et al., 2003). Although the waters feeding the Sungai Brunei estuary (Brunei Darussalam, Borneo, South East Asia) are naturally acidic, biogenic CO₂ production is implied by the combination of extraordinary natural and anthropogenic organic loads, lowered dissolved oxygen levels, elevated dissolved CO₂ levels and highly turbid conditions (Chua, Chou & Sadorra, 1987; DJM, unpublished).

This study stemmed from the observation of shell dissolution in a gastropod whelk (*Thais gradata* (Jonas)) which inhabits the Sungai Brunei estuarine system. We aimed to derive relationships between shell dissolution properties of this whelk and water chemical attributes underlying water carbonate saturation of the estuary (particularly pH, calcium and salinity), and to determine which measurable aspects of the shell were most sensitive to dissolution. This study highlights the use of estuarine organisms and estuaries to address acidification questions relating to a broader marine context.

2. Materials and methods

2.1. Estuary and the snails

The Sungai Brunei estuarine and bay system covers an area of 1380 km², with the inner bay being fed by several large rivers in Brunei Darussalam (Sungai Brunei and Temburong) and Sarawak, Malaysia (Sungai Limbang and Trusan; Fig. 1; Chua et al., 1987). Waterways are fringed with extensive mangrove stands. The estuary is distinctly brown and turbid, and carries a high organic load deriving from the mangroves and urban centres (Bandar, Kiulap and Gadong; Chua et al., 1987). It has been central to the lifestyles of the Bruneian people for the past four centuries, and currently supports one of the oldest water villages in South East Asia (approximately 29 000 people living in stilt houses over the water).

The gastropod whelk, *Thais gradata* (Jonas) is endemic to southern China and the Malay Archipelago (Tan, 1999). Although typically an inhabitant of mangrove trees, in the Brunei estuary it is highly abundant on artificial embankments and piers, built to retain waters and to facilitate small-craft commutation. A congeneric estuarine species, *T. malayensis*, thought to occur in Sungai Brunei estuary, is distributed across South East Asia and northern Australia (Tan & Sigurdsson, 1996).

2.2. Water chemistry

Four recording stations were established along the Sungai Brunei estuary, to cover the range of physicochemical conditions that characterize it (Damuan, D, low salinity, 04°52'05.6"N, 114°54'37.6"E; Bandar, B, low salinity 04°53'09.3"N, 114°56'53.2"E; Kota Batu, K, midrange salinity, 04°55'42.2"N, 115°00'54.3"E and Muara, M, high salinity, 05°01'01.1"N, 115°03'57.8"E; Fig. 1). Water samples were collected from each station on 24 occasions (13 during November 2005 to March 2006 and 11 during August to November 2007). Sampling included a range of tidal conditions and, on any day, all stations were visited in the same order in a 2 h timeframe. Duplicate air-tight samples (allowing no head space) were collected from the upper 1 m column adjacent to *Thais* habitat, using 1000 mL acid-washed, polythene jars. Samples were returned to the laboratory in 1 h of completing the sampling series, and were equilibrated at 28°C for 1 h in a Grant constant temperature water-bath. Field water temperature was relatively constant, varying between 27.9 and 33.6°C (Fluke thermocouple thermometer 54 II). Salinity and pH were determined simultaneously at 28°C; salinity to ± 0.01 psu (YSI Model 85D multi-meter or HACH HQ40d meter and IntelliCal probe) and pH to ± 0.01 units using an NBS scale (Thermo Orion Model 260A or HACH HQ40d calibrated with Mettler Toledo SRM NIST precision buffers). Laboratory measured salinity and pH were invariable to *in situ* field measurements taken on five occasions. For the 2007 samples, 100 mL sub-samples were treated with concentrated nitric acid for calcium analysis. Calcium concentrations of these samples were determined using Shimadzu AA-6701F atomic absorption spectrophotometer in direct injection mode utilising acetylene-air fuel mixture. Total alkalinity was determined for a separate set of samples collected on a single day (27 June 2008, $N = 12$). Determination involved using potentiometric titration and double-indicator titration employing phenolphthalein and methyl orange indicators. A titrant of standard HCl

solution containing 0.7 mol L^{-1} NaCl was used to adjust the total ionic strength to approximate that of the seawater.

2.3. Shell weight, shell length and aperture relationships

Snails ($N = 377$) were collected from four populations (Tamoi, T; Bandar, B; Kota Batu, K and Pulau Bedukang, P; Fig. 1) during July to December 2005. Populations B and K corresponded with the water sampling stations. However, because T and P were difficult to access rapidly for water sampling, this was undertaken at nearby stations D (where snails occurred at low abundance) and M (where no snails occurred; Fig. 1), which showed similar water conditions respectively to those where snails were collected. Snails were haphazardly collected in a manner to include a broad size range. Shells supporting epizoic barnacles were avoided, except population T which mostly possessed barnacles. Shell scrubbing and removal of epibionts preceded all determinations. To avoid microenvironment effects of mud-bank water, snails were collected from vertical hard substrata, their preferred habitat which drains rapidly during low tides.

Shell length, shell weight, aperture length, and shell structural features were determined in the laboratory. Shell length and aperture length (as a proxy for growth and age) were measured using digital Vernier calipers (precision: 0.01 mm; see Fig. 2). Qualitative features of the shells were assessed by a method described below. Shells were then cracked open with a bench vice to separate shell material from the tissue, and the shell material was dried at 60°C for 3 d to achieve a constant weight, determined to 0.001 g with a Mettler Toledo PB303. We determined how shell weight and shell length were related to aperture length and, whether and how the populations differed with respect to these relationships (General Linear Models, Statistica Version 6, Statsoft Inc.). Because preliminary observations showed that smaller shells elongate more rapidly while

remaining thin, causing a non-linear shell weight to shell elongation relationship, shells having an aperture shorter than 15 mm long were excluded from the analyses. Each model comprised a categorical predictor (snail population with four levels; T, B, K and P), a dependent variable (shell weight or shell length), and continuous variable (aperture). All regressions conformed to assumptions of normality, heteroscedasticity, and homogeneity of variance (Cochran's test).

2.4. Shell erosion rank (SER)

The surface of non-eroded *Thais* shells is characterized by an outer periostracum growing over fine axial riblets which interconnect with spiral riblets to form cancellate patterning (axial riblets and the periostracum are not readily discernible in Fig. 2). Dissolution causes the sequential loss of these features in the above order. Because the apex (being the oldest part of the shell) is always eroded and the outer lip (comprising the most recently deposited shell) is usually intact, these parts were excluded from the shell assessment. Extreme dissolution leads to smoothing of the shell (loss of periostracum, axial riblets and spiral riblets) and rounding of the apex, longitudinal ribs (or spines) and whorl shoulders (Fig. 2c and 2f). Although Tan & Sigurdsson (1996) suggested that the development of spines is individually variable, spines were conspicuous in all individuals of population P (Fig. 2).

Each snail was ranked according to the following criteria. Rank 1 (non-erosion): periostracum and axial riblets distinct on most of the shell, and longitudinal ribs on spire whorls, pronounced (Fig. 2a and 2d). Rank 2: longitudinal ribs on spire whorls, eroded and indistinct; spiral and axial riblets on body whorl adjacent to the parietal lip, distinct (Fig. 2b and 2e). Rank 3: axial and spiral riblets directly adjacent to parietal lip eroded, but conspicuous elsewhere on the body whorl (especially on the surface opposite the

aperture, not shown in figure). Rank 4: axial and spiral riblets eroded on entire body whorl (Fig. 2c and 2f). Aperture length, as an assessment of size, was determined along with the SER for each shell for $N = 33$ for each population. Because very small shells had experienced limited acidic exposure, only shells > 20 mm were used in SER determinations.

3. Results

3.1. Water chemistry determinations

The stations differed markedly in pH, salinity and calcium regime; mean values showed a decreasing trend from the seaward to the landward stations (Stn. $M > K > B \geq D$; Table 1). pH varied between 5.78 and 8.3 units and salinity between 3.8 and 31.2 psu, with lowest and highest values for each variable recorded at stations B and M, respectively (Table 1). The water chemistry was most variable at B and least variable at M (Table 1). Relationships for the water chemical variables are: $\text{pH} = 4.10 + 2.62 \log_{10} \text{salinity}$; $\text{calcium} = 21.25 + 12.65 \text{salinity}$; $R^2 = 0.89$, $P < 0.001$; Fig. 3). The spatial pattern of water chemistry was consistent across stations ($M > K > B \geq D$) on each recording session, but varied temporally on different days in relation to tidal flux and rainfall events (Fig. 3). Preliminary data showed that total alkalinity was related to salinity as follows: $\text{total alkalinity (mmol.L}^{-1}\text{)} = 0.884 + 0.033 \text{salinity (psu)}$, $R^2 = 0.94$, $P < 0.001$.

3.2. Shell weight, shell length and aperture relationships

Relationships for shell weight against aperture length differed significantly among populations; the landward populations generally had heavier shells (Fig. 4a; Table 2). Shell length as a function of aperture also differed among the populations, with shorter shells found in the landward populations, for the range of shell sizes examined (Fig. 4b;

Table 2). The effect of size was removed by scaling data to a standard aperture length (*SAL*) using the slope coefficient (*b*) for the individual regressions (see Fig. 4) as follows (see Packard & Boardman, 1988):

$$\text{Standard shell weight (or shell length)} = (\text{SAL} / \text{aperture length})^b \times \text{shell weight (or shell length)}$$

SAL is the grand mean for aperture length, which for shell weight = 19.1 mm and for shell length = 18.9 mm. Standardized shell weight was not significantly different between populations B, K and P, but T was significantly greater than the others (Fig. 5). However, standardized shell length increased progressively across populations, from landward to seaward (Fig. 5). Population T thus comprises snails possessing not only the heaviest shells, but also the shortest shells (Fig. 5). Significant positive correlations were found for means of standard shell length against estuarine pH and calcium (pH = $-2.25 + 0.36$ shell length, $R^2 = 0.96$, $P = 0.022$; calcium = $-865.32 + 43.09$ shell length, $R^2 = 0.95$, $P = 0.027$; Fig. 5c).

3.3 Shell erosion rank (*SER*)

Shell erosion rank (*SER*) differed strikingly among the populations, with the landward populations (T and B) scoring much higher than the seaward populations (K and P; Fig. 6a). There was no significant difference between populations T and B (Fig. 6a). Shells from population P showed little dissolution effect (Fig. 2). Furthermore, *SER* was independent of aperture length, for shell lengths > 20 mm (Fig. 6b). *SER* was negatively related to estuarine water pH (pH = $8.52 - 0.55$ *SER*; $R^2 = 0.98$, $P = 0.008$) and calcium concentration (calcium = $431 - 66.6$ *SER*, $R^2 = 0.97$, $P = 0.014$; Fig. 6c).

4. Discussion

Acidification has not received the same consideration in estuarine ecology as have processes involving other physicochemical variables (temperature, salinity and hypoxia), despite its implied effect on the structuring and functioning of estuarine systems (Knutzen, 1981; Diaz & Rosenberg, 1995; Burnett, 1997; Ringwood & Keppler, 2002). Relevant studies on estuarine acidification mostly concern spatial and temporal patterns of the water chemistry and not biological effects (Howland et al., 2000; Mirlean et al., 2001; Braungardt et al., 2003; Jennerjahn, Ittekkot, Klöpper, Adi, Nugroho, Sudiana et al., 2004; Lin et al., 2004; García-Luque et al., 2005). We recorded the predictable pattern of a declining landward gradient in pH, salinity and calcium concentration in the Sungai Brunei estuary (Fig. 3). Although the water chemistry at any station was temporally variable in relation to tidal flux and local rainstorm events, shell dissolution in populations of *Thais gradata* correlated with the average pH and calcium concentration experienced (Figs. 5 and 6). While these correlations suggest the potential for using shell dissolution to capture the effects of highly variable estuarine water chemistry, dissolution is caused by carbonate undersaturation, and determination of the water carbonate saturation state (Ω) at the various stations in relation to the measured chemical parameters would be required for a complete interpretation. Saturation state is however expected to closely track the observed variation in pH (see Kuffner et al., 2008).

Acidification of the Sungai Brunei estuary seems primarily to arise from the combination of natural and anthropogenic eutrophication and heterotrophic metabolism. Although acidic freshwater inflows characterize the system (Chua et al., 1987), significant biogenic carbonic acid production due to mangrove bacterial decomposition must affect the landward stations of the estuary (see Braga et al., 2000; Abril et al., 2003). Similar pH levels at stations D and B, despite the more seaward location of B,

suggests acidic priming from the major urbanized areas via Sungai Kedayang (Fig. 1). Raw and treated sewage enters the system near station B from the water village Kampong Ayer, and downstream of station B from Pintu Malim, the largest sewage treatment plant in the country. Acidification through heterotrophic metabolism is further implied by elevated dissolved carbon dioxide and lowered dissolved oxygen levels at the landward stations (Chua et al, 1987; DJM, unpublished data). Seaward increases in pH results from buffering and increased hardness of the more saline waters (Mirlean et al., 2001; García-Luque et al., 2005). Although water chemistry (pH, carbonate saturation state and alkalinity) commonly varies diel in coastal systems in relation to net CO₂ production by primary producers (Kuffner et al., 2008), we presume that this effect is reduced by the highly turbid conditions that characterize this system and is probably masked by rainfall events and tidal flow patterns.

The three shell variables assessed (shell weight, shell length, and shell erosion rank) differed in sensitivity to the measured conditions underlying dissolution (pH and calcium concentration). While shell length and shell surface sculpturing (SER) varied among populations in a predictable way, shell weight was contrary to the prediction (Figs. 4, 5 and 6). Snails exposed to higher acidity levels sometimes possessed heavier shells (populations $T > B = K = P$; Fig. 5), suggesting that factors other than dissolution influence shell weight loss or gain in field populations. Theoretically, several intrinsic factors may affect shell production or shell weight loss under these circumstances. For example, internal shell dissolution contributes to regulating pH of the extracellular fluids in many shellfish experiencing acidic conditions (Michaelidis et al., 2005). On the other hand, gastropod shell production is affected by nutritional state, organismal growth rate, and even predation pressure. Slower growth rates due to constrained feeding are known to cause shell thickening in congeners of this species (Palmer, 1981), and the possibility

exists for reduced food availability at the more acidic localities. Moreover, congeners are known to induce shell production under predation pressure (Palmer, 1981; Appleton and Palmer, 1988; Palmer 1992), though it is unknown how populations of predatory crabs vary spatially in the Sungai Brunei estuary. It is further possible that snails of the heavily-shelled Tamoi population induce shell production in response to epibiont colonization, or as a consequence of shell thinning detected through proprioception (but see Bibby, Cleall-Harding, Rundle, Widdicombe & Spicer, 2007). In contrast, dissolution of external shell features and erosion of the apex (causing shortening) are largely independent of the intrinsic factors affecting shell weight. Furthermore, the capacity to increase calcification under increased acidity has been recently shown in brittlestars (Wood, Spicer & Widdicombe, 2008).

The absence of previous reports raises the question on how widespread gastropod shell dissolution in estuaries actually is. Does this represent a low incidence of estuarine acidification, is it more typical to the lesser studied tropical estuaries, or is it particular of estuarine-dwelling *Thais* snails? A few published studies, including from South East Asia (Jennerjahn et al., 2004; Zai, Dai, Cai, Wang & Wang, 2005), show that common features of estuarine acidification include high turbidity, eutrophication and heavy rainfall events (Abril, et al., 2003; Paquay, Mackenzie & Borges, 2006). Nonetheless, the structural and mineralogical properties of *Thais* shells suggest a relatively high potential for dissolution. These shells are typically aragonitic and lack the outer slower-dissolving calcitic layer found in some gastropods (Cubillas et al., 2005), notwithstanding other influences of dissolution such as crystal size and the proportion of organic matrix in the shell (Harper, 2000). The global distribution of snails that possess these kinds of shells and live in estuaries characterized by acidification is unknown. We assume that the visible absence of shell dissolution in a variety of littorinid and neritid snails which cohabit with *T.*

gradata in the Sungai Brunei estuary relates to the combination of different shell properties and their more upper-shore distributions, exposing them relatively infrequently to the acidic water.

Aside from their shell properties, *Thais* snails contrast markedly with other marine organisms in physiological ability to tolerate and grow under highly variable and extreme acidic conditions (Hall-Spencer et al., 2008 give recent data on field acidity conditions supporting other coastal gastropods). Studies examining acidity tolerances of marine animals have however mainly concerned plankton and bivalve molluscs (Knutzen, 1981; Bamber, 1990; Yamada & Ikeda, 1999; Hinga, 2002; Ringwood & Keppler, 2002; Michaelidis et al., 2005; Sato, Watanabe, Toyota & Ishizaka, 2005; Berge et al., 2006). Growth rates of bivalves are impeded by increasing acidity down to 6.7 pH units when all growth ceases, and significant mortality occurs at around 6.0 pH units (Bamber, 1990; Michaelidis et al., 2005; Berge et al., 2006). Oceanic copepods are even more limited in their tolerance of acidification and die when exposed to a reduction of only 0.2 pH units (Seibel & Walsh, 2003). In view of the likelihood of their evolutionary historical association with acidic conditions, *Thais gradata* could serve as a model system to understanding mechanisms for survival of elevated CO₂ and acidity, including behavioral isolation, and physiological acclimation and adaptation. This study further highlights the potential for using estuaries in understanding the consequences of marine acidification in general.

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Figure captions

Fig. 1. Map of Brunei bay and the Sungai Brunei estuary. Sampling stations are D, Damuan; T, Tamoi; B, Bandar; K, Kota Batu; P, Pulau Bedukang; M, Muara and *kl*, Sungai Kedayang.

Fig. 2. *Thais gradata* shells from the Sungai Brunei estuary, showing different dissolution effects. Side and apical views of shells from (a, d) Pulau Bedukang (P), (b, e) Kota Batu (K), and (c, f) Bandar (B), respectively indicating shell dissolution ranks (SERs) 1, 2 and 4 (see text for further details). Abbreviations are as follows: *sl*, shell length; *al*, aperture length; *rs*, spiral riblets, *rl*; longitudinal ribs; *pl*, parietal lip and *ol*, outer lip. Scale bar for (a), (b) and (c) indicates 10 mm, and that for (d), (e) and (f) indicates 20 mm.

Fig. 3. Relationships between salinity, pH and calcium concentration in the Sungai Brunei estuary. (a) pH against salinity; Muara (open circles); Kota Batu (closed circles); Damuan (triangles) and Bandar (squares).

Fig. 4. Relationships between aperture length, shell length and shell weight of the four populations of *Thais gradata* from the Sungai Brunei estuary. T, Tamoi; B, Bandar; K, Kota Batu; P, Pulau Bedakang. Regression equations are indicated in figure; $P < 0.001$. (a) Shell weight against aperture length; line B is omitted for clarity. (b) Shell length against aperture length; line T is omitted for clarity. Statistical analyses are given in Table 2.

Fig. 5. Means \pm 95% C.I. for (a) standardized shell weight ($\chi^2 = 13.4$, $df = 3$, $P = 0.038$) and (b) standardized shell length ($\chi^2 = 57.07$, $df = 3$, $P < 0.001$). T, Tamoi; B, Bandar; K, Kota Batu; P, Pulau Bedukang. Number in parenthesis indicates sample size. (c) Mean standardized shell length against means for pH (circles, thick line) and calcium (squares, thin line; see Fig. 4 and Tables 1, 2).

Fig. 6. Means \pm 95% C.I. for (a) shell erosion rank ($F_{3,128} = 89.95$, $P < 0.001$) and (b) aperture length ($\chi^2 = 15.87$, $df = 3$, $P = 0.0012$) using the same snails ($N = 33$) for each population. T, Tamoi; B, Bandar; K, Kota Batu; P, Pulau Bedukang. Significant differences in (a) are indicated by different letters associated with error bars. (c) Mean shell erosion rank (SER) against mean pH (circles, thick line) and calcium (squares, thin line; see Table 1).

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Table 1. Descriptive statistics for pH, salinity and Ca for each station

	Station	Mean \pm SD	Max	Min	<i>N</i>
pH	D	6.80 \pm 0.23	7.11	6.33	22
	B	6.83 \pm 0.39	7.51	5.78	24
	K	7.44 \pm 0.43	8.09	6.5	23
	M	8.02 \pm 0.15	8.3	7.73	24
Salinity (psu)	D	12.25 \pm 4.27	19.56	5.32	22
	B	13.50 \pm 5.49	22.7	3.58	24
	K	18.47 \pm 4.89	26.9	9.5	23
	M	27.16 \pm 3.0	31.2	19.6	25
Calcium (ppm)	D	227.9 \pm 55.7	296	111	11
	B	226.2 \pm 85.9	333	43	11
	C	292.5 \pm 50.4	374	209	11
	D	374.6 \pm 47.1	427	280	11

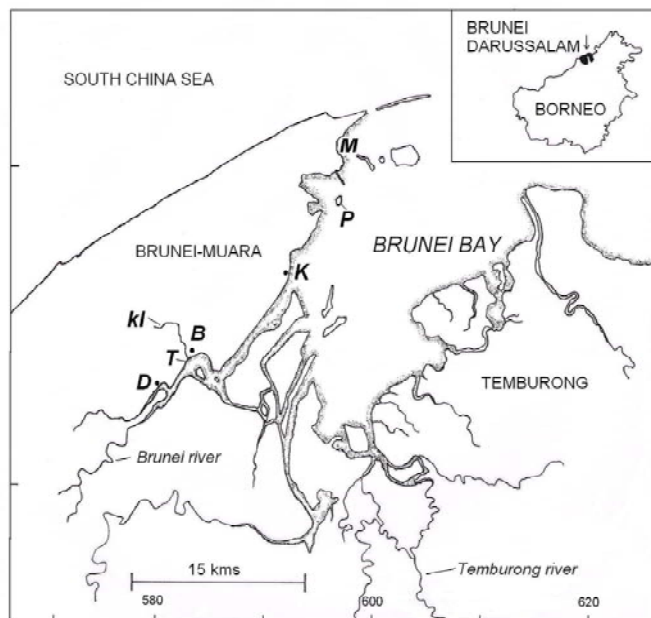
D = Damuan; B = Bandar; K = Kota Batu; M = Muara

Table 2. Statistical analyses of General Linear Models for shell weight and shell length against aperture of the four populations of *Thais gradata* in the Sungai Brunei estuary (see Fig. 4)

	SS	<i>df</i>	MS	<i>F</i>	<i>P</i>
Shell weight versus aperture length (Fig. 4a)					
Intercept	47.3	1	47.3	337.82	< 0.0001
Aperture length	99.8	1	99.8	712.91	< 0.0001
Population	10.9	3	3.6	26.04	< 0.0001
Error	16.2	116	0.14		
Shell length versus aperture length (Fig. 4b)					
Intercept	5.03	1	5.03	4.86	0.028
Aperture length	4113.8	1	4113.8	3974.89	< 0.0001
Population	113.4	3	44.46	42.96	< 0.0001
Error	384.99	372	1.04		

Comparisons of population relationships for shell weight against aperture length are: T = B but \neq K and P; B = K but \neq P; K \neq P; $P < 0.01$ and for shell length against aperture length are: T = B and K but \neq P; B \neq K and P; K \neq P; $P < 0.01$.

Fig.1



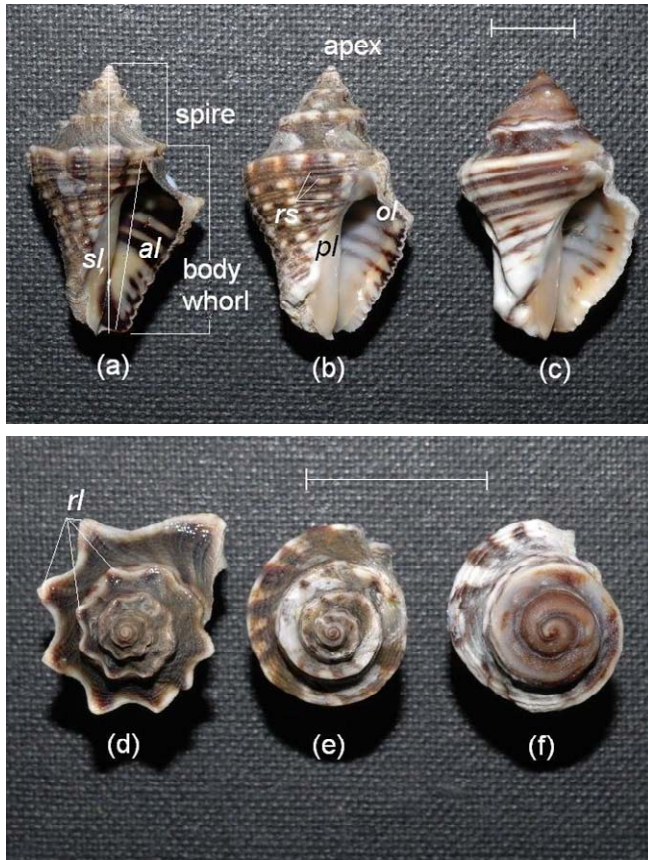


Fig.2

Fig.3

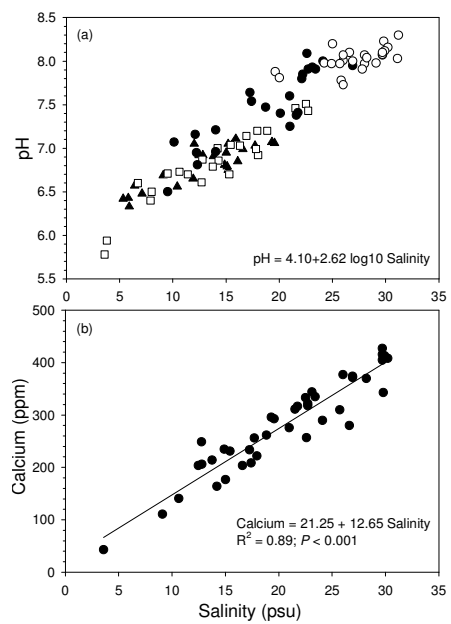
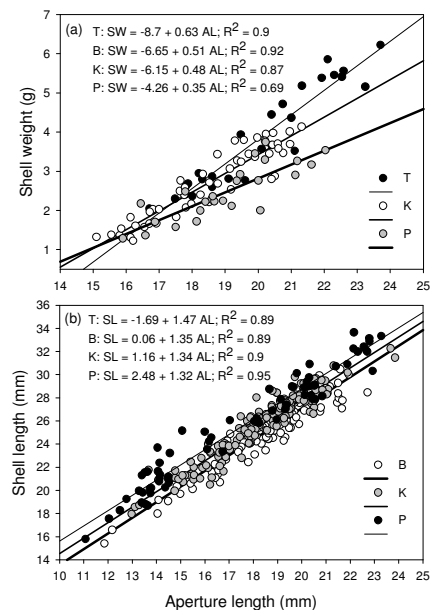


Fig.4



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Fig.5

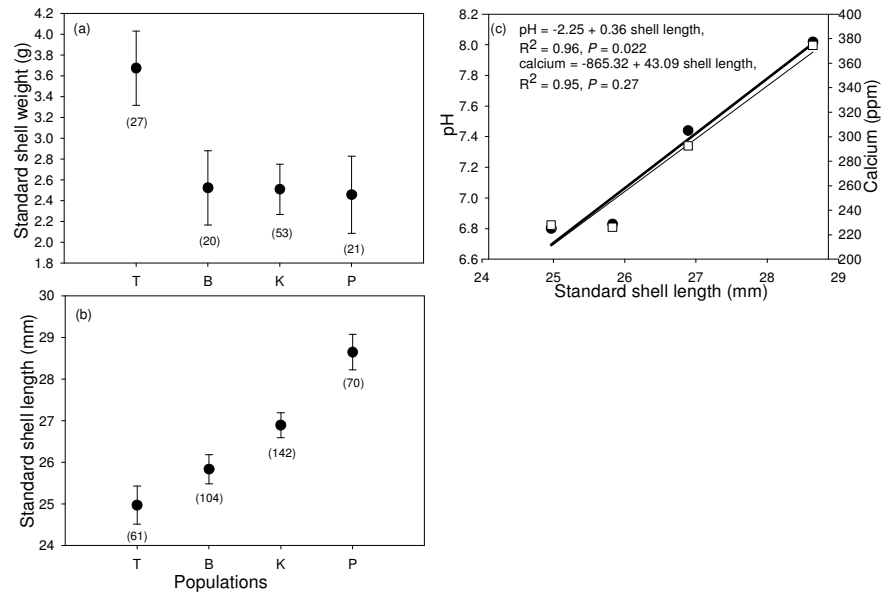


Fig.6

