

Organismal Responses to Coastal Acidification Informed by Interrelating Erosion, Roundness and Growth of Gastropod Shells

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Current understanding of how calcifying organisms respond to externally forced oceanic and coastal acidification (OCA) is largely based on short-term, controlled laboratory or mesocosm experiments. Studies on organismal responses to acidification (reduced carbonate saturation and pH) in the wild, where animals simultaneously interact with a range of biotic and abiotic circumstances, are limited in scope and interpretation. The present study aimed to better understand how gastropod shell attributes and their interrelations can inform about responses to coastal acidification. We investigated shell chemical erosion, shell roundness, and growth rate of *Planaxis sulcatus* snails, which are locally exposed to acidified and non-acidified rocky intertidal water. We tested a new approach to quantifying shell erosion based on the spiral suture length (EI, erosion index) and found that shell erosion mirrored field acidification conditions. Exposure to acidification caused shells to become rounder (width/length). Field growth rate, determined from apertural margin extension of marked and later recaptured snails, was strongly negatively related to both shell erosion and shell roundness. Since different shell attributes are indicative of different relationships—shell erosion is an extrinsic passive marker of acidification, and shell roundness and growth rate are intrinsic performance responders—analyzing their interrelations can imply causation, enhance predictive power, and bolster interpretation confidence. This study contributes to the methodology and interpretation of findings of trait-based field investigations to understand organismal responses to coastal acidification.

Key words: Biomonitoring, Growth rate, Indicators, Ocean acidification, Snails, Shell dissolution, Shell shape

BACKGROUND

Ocean and coastal acidification (OCA) exerts dissolution pressure on the skeletons or shells of calcifying organisms and reduces their ability to allocate energy for growth and reproduction (Orr et al. 2005; Melzner et al. 2011; Gledhill et al. 2015). We largely derive our understanding of organismal responses to externally forced OCA from short- to medium-term controlled laboratory or mesocosm experiments.

These experiments are temporally limited in terms of an organism's lifetime and do not appropriately account for the biotic interactions (feeding, predation, and competition; Nagelkerken and Connell 2022) that naturally impact an organism's energetics simultaneously with acidification. They further inadequately consider the natural variation in abiotic conditions (e.g., salinity, temperature, tidal, lunar, and seasonal effects, and wave action) that influence the activity, mating and performance of coastal animals (Hall-Spencer et al.

2008; Manno et al. 2017; Cummings et al. 2019; León et al. 2020; Clark et al. 2020; Lagos et al. 2021; Mayk et al. 2022b). Field-based studies on the other hand present an alternative or complementary approach to laboratory assessments (Hall-Spencer et al. 2008; Marshall et al. 2008 2019; Bednaršek et al. 2012a b; Duquette et al. 2017; Harvey et al. 2018; Mustapha and Marshall 2021). However, field results from currently naturally acidified conditions are often complicated by other factors that covary with acidification exposures. We studied several shell traits of gastropod snails exposed to field acidification to better understand the information that can be garnered from interrelating traits. In this paper, *acidification* is used synonymously with *acidified seawater* to refer to the conditions of pH reduction below the ambient level (~ 8.2) and environmental aragonite undersaturation ($\Omega < 1$). The term *trait* is used synonymously with *attribute* to refer broadly to an inherited phenotype and its environmental interaction. *Shell erosion* is a general term referring to the loss of outer surface shell, and includes the effects of weathering and chemical erosion (or dissolution).

Studies considering gastropod shell responses to OCA in the field are disparate in their objectives, taxonomic groups and ecosystems. Earlier work that investigated the shells of pelagic pteropods was followed by investigations on benthic volcanic vent and coastal rocky-shore gastropods (Hall-Spencer et al. 2008; Marshall et al. 2008 2019; Hart et al. 2011; Bednaršek et al. 2012a b 2014a b; Manno et al. 2017; León et al. 2020; Mustapha and Marshall 2021; Mekkes et al. 2021; Mayk et al. 2022a b). Acidification exposure generally leads to changes in the shape, size, thickness, density, mineralogy, and degree of surface erosion of gastropod shells. Carbonate dissolution in combination with reduced calcification and compromised physiology under acidification causes gastropod shells to become smaller, thinner, and less dense (Bibby et al. 2007; Bednaršek et al. 2012a; Garilli et al. 2015; Viotti et al. 2019; Barclay et al. 2019 2020; Marshall et al. 2019; Mayk et al. 2022a b). Whereas intrinsic organism responses to OCA, such as behavior, energy intake (feeding) and energy allocation, manifest as changes in shell shape and size, outer shell surface dissolution (beyond the reach of the mantle after primary shell formation) is an extrinsic passive process (Marshall et al. 2008 2019; Bednaršek et al. 2012a b; Marshall and Tsikouras 2023). Gastropod shell erosion in coastal environments is influenced by multiple factors, including heating, cooling, wetting, and drying, and although this kind of weathering can be significant in some instances, in other cases it can easily be distinguished from chemical erosion (Marshall et al. 2008 2019; Nienhuis et al. 2010; Schönberg et al. 2017).

The quantification of chemical erosion of gastropod shells has been investigated for rocky intertidal species with a view to biomonitor coastal acidification (see Marshall et al. 2019; Mustapha and Marshall 2021).

Gastropod shell trait responses to field acidification are usually considered individually, with less information available for attribute interrelationships (shell erosion, shell roundness and growth rate). Several studies report on gastropod shell growth responses to natural and anthropogenic environmental stressors, including acidification (Nienhuis et al. 2010; Bednaršek et al. 2014b 2021; Garilli et al. 2015; Barclay et al. 2019; Leung et al. 2020b; Slama et al. 2021). Snails expectedly grow slower under more stressful conditions as energy allocation prioritizes servicing an elevated metabolic demand (Nienhuis et al. 2010; Garilli et al. 2015; Barclay et al. 2019; Leung et al. 2020b), resulting in populations comprising smaller individuals (Garilli et al. 2015; Harvey et al. 2018). Although gastropod shell roundness varies relative to environmental stress (Marshall et al. 2008 2019; Saura et al. 2012; Harayashiki et al. 2020; Lahbib et al. 2022), the relationship between shell roundness and growth has been poorly investigated under both field and laboratory circumstances. A field study demonstrating this relationship found that littorinid snails that grew faster (when feeding opportunities improved) also produced rounder shells (Kemp and Bertness 1984). However, there is little consensus on the way shell roundness varies in response to environmental stress, with some studies suggesting that stress increases shell rounding whereas others suggest the opposite effect of shell elongation (see Bibby et al. 2007; Marshall et al. 2019; Mayk et al. 2022a b; Martin et al. 2022).

Coastal environments are prone to acidification through multiple different sources (Yao et al. 2022; Zhang et al. 2022). We investigated an Indo-Pacific snail, *Planaxis sulcatus* (Born, 1791), that occurs abundantly along the Brunei coastline (northwest Borneo, South East Asia). Habitats along this coastline become acidified by groundwater discharge, originating from pyritic geological formations (Marshall et al. 2008; Proum et al. 2018; Gödeke et al. 2020). Naturally-acidified boulder beaches occur near seawalls that are disconnected from groundwater infiltration, resulting in the juxtaposition of acidified and non-acidified intertidal habitats (Marshall et al. 2019; Mustapha and Marshall 2021; Fig. S1). Because other shore features (slope, aspect, wave action) are similar along this linear coastline, these differently-acidified sites represent a natural laboratory for investigating organismal responses to coastal acidification and for testing biomonitoring applications (Marshall et al. 2019; Mustapha and Marshall 2021). Differences in

habitat acidification were found to cause different degrees of gastropod shell erosion, enabling researchers to document the distinctions between shell erosion caused by chemical erosion and weathering as opposed to erosion caused by weathering alone (Marshall et al. 2019; Mustapha and Marshall 2021). An approach to semi-quantifying the erosion of *Planaxis* snail shells and showing how this can be used as an organismal biomarker of OCA is demonstrated in Marshall et al. (2019).

This study aimed to improve our understanding about the relationships between shell erosion, shell roundness and growth rate in *Planaxis* snails, in response to acidification exposure. Because these different traits carry different kinds of information about OCA - shell erosion represents an extrinsic effector, whereas shell roundness and growth rate represent intrinsic response parameters - their relationships potentially offer insight into causation. Studying these relationships may allow us to identify acidification as the primary cause of a particular response by excluding confounding covarying factors. In other words, a change in shell shape can more reliably be attributed to acidification exposure if the shell is also heavily eroded and this erosion derives from acidification exposure. Additionally, a good relationship between shell roundness and growth rate suggests that field growth rate can be predicted from shell shape alone, and by extrapolation is affected by environmental acidification. A secondary objective (dealt with first) was to develop and test a novel approach to quantifying *Planaxis* shell erosion. This approach was based on the spiral shell suture length and was intended to improve the precision of measurement of shell erosion.

MATERIALS AND METHODS

Field sampling

Planaxis sulcatus snails were collected from an acidified natural rocky shore (Empire, EM, 4.9683°N, 114.8535°E, $n = 36$ snails) and a nearby non-acidified artificial seawall (near Universiti Brunei Darussalam, UBD, 4.9727°N, 114.8940°E, $n = 20$ snails). During the monitoring period of Apr.–Jul. 2018, pH and salinity at the acidified site varied between 8.6 and 5.9 units and 32.8 and 0.2 psu, respectively, and at the non-acidified (reference) site between 8.6 and 7.9 units, and 33.2 and 20.2 psu, respectively (for details see Proum et al. 2018; Marshall et al. 2019). The acid sulphate soil seepage is a permanent feature at the acidified site (EM) and is visible by an oil microbial sheen on the surface of the pool water throughout the year (Marshall, pers.

obs.; Fig. S1). *Planaxis* shells are nearly completely aragonitic (Marshall, pers. obs.) and snails experience aragonite undersaturated water ($\Omega < 1$) at pH and salinity combinations below 7.5 pH and 25 psu (see Marshall et al. 2019). This means that while snails are frequently exposed to corrosive seawater at the acidified site, they are not exposed to similar conditions causing shell dissolution at the non-acidified site. Snails were collected in Aug 2019 from their uppermost shoreline distributions, ensuring that they experienced similar air-water exposure cycles across localities. Collections were made without bias, though intentionally included a broad shell size range (14–28 mm shell length). Snails were preserved and stored in 70% ethanol.

Shell size and shape

Shell sizes were assessed to validate the comparison between the populations from the different shores. The abapertural shell surface of each individual was photographed (Canon S110, Manual, F8.0) and Olympus CellSens imaging software was used to measure projected shell length (SL) and shell width (SW) to 0.01 mm (Fig. 1; Marshall et al. 2019). Shell roundness was computed as SW divided by SL, and formed a key trait of the primary study objective. Generalized Linear Models (GZLM) for a log-link function and normal distribution were used to compare size and roundness between the shores. Statistical analyses and regressions were carried out using TIBCO Statistica ver. 13, StatSoft, and figures were drawn using Statistica or Sigmaplot ver. 14, Systat 149 Software, Inc., New York, US.

Shell erosion indices (SER, EI, ET, SET)

A novel index for shell erosion was tested and compared with a previously used shell erosion ranking approach. Shell Erosion Ranks (SERs) were based on a method described by Marshall et al. (2019), but differed in that all ranks were created from the abapertural surface (Fig. 1A). Erosion ranks were determined using the four shell whorls, subdivided perpendicularly through the shell apex to give eight shell segments (Fig. 1A). Three different degrees of shell erosion were identified: *no erosion*, where the periostracum covers the outer shell, *moderate erosion*, where the periostracum is lost exposing underlying calcareous shell with visible ridges, and *severe erosion*, where shell ridges are eroded producing a smooth outer surface. The erosion rank corresponds to the value of the numerically greatest segment that had *moderate erosion* exceeding 50% of coverage (Fig. 1A).

The Erosion Index (EI) was determined as the

ratio of the planospiral suture length (mm) for the eroded part of the shell and the entire suture length (Fig. 1B). Spiral suture lengths were determined from two-dimensional apical views of the shell and the natural three-dimensional helicospiral suture length. The helicospiral suture length is important in apically-extended shells but was considered negligible for *Planaxis* snail shells, given the relatively small variation in this aspect of shell shape. EI was based on *severe erosion*, with suture lengths measured using Olympus CellSens imaging software (Fig. 1B). Erosion Time (ET) was estimated by dividing the eroded suture length (mm) by the mean growth rate (mm/day), based on the change

in suture extension at the growing edge. Values were then standardized using $(y - \text{sample mean}/\text{standard deviation})$, and plotted against shell length separately for the acidified and non-acidified snail populations. The data were then corrected for shell size (SL) using $[(\text{mean SL}/\text{individual SL})^{\text{slope}} \times \text{ET}]$, where the slope coefficient was computed from regressions for ET against SL, to get standard erosion time (SET). This was used to compare the two acidification conditions.

Growth rate and interrelating shell attributes

Shell erosion is typically influenced by the

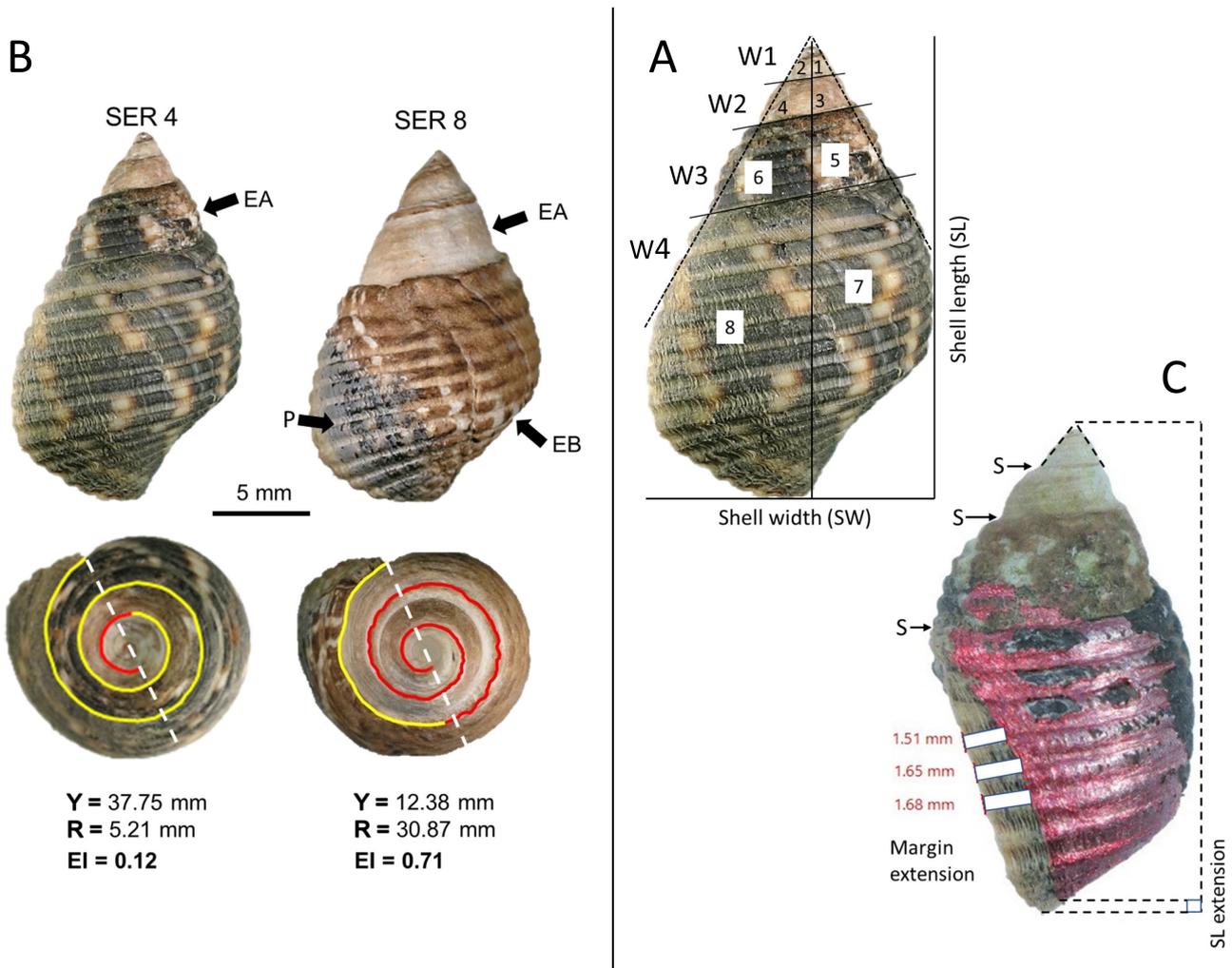


Fig. 1. Methods for determining shell roundness, shell dissolution and growth rate. (A) Shell roundness was assessed from shell width (SW)/shell length (SL). Shell erosion rank (SER) was scored using eight segments, where moderate erosion (ridges still observed) covered > 50% of the numerically greatest segment. The vertical line through the shell bisects the apical angle. By forming the apical angle we could measure projected SL (the intrinsic responder), as the actual SL is influenced by extrinsic apical dissolution in acidified water. (B) Comparison of SER (upper) and Erosion Index (EI) methods (lower). EI was calculated from the spiral suture length of the eroded shell divided by the total planospiral shell spiral length (R/ (Y and R) using *severe erosion* (ridges not observed) determined from apical views (lower images). Upper images show the abapertural surfaces of the same shells, giving their SERs. (C) The growth rate was estimated from the shell margin extension of marked and recaptured snails ($n = 22$). The marginal extension is shown to far exceed shell length (SL) extension. EA, spire whorl, EB, body whorl, S, shell suture, W1-4, shell whorls.

duration of exposure to corrosive seawater, resulting in the highest degree of erosion near the apex and the least erosion near the aperture margin. Growth rate also influences relative shell erosion, as the same point on two different shells growing at different rates experiences different acidification exposure. We assessed shell field growth rates using a capture-recapture technique for a separate (secondary) population of snails occurring on the lower shore at the acidified site (EM). These snails were readily accessible and mostly experienced conditions similar to oceanic seawater (see Mustapha and Marshall 2021). Around 50 snails were captured, marked with colored nail varnish along the marginal edge (Fig. 1C), and then released back into their intertidal habitat. All the marked snails that were later found, were collected after 14 ($n = 7$), 33 ($n = 4$), and 37 days ($n = 11$), to give a total of 22 recaptured snails. Their shells were fixed in 70% ethanol, and later photographed along the marginal edge. New growth was measured from the extension of three spiral ridges (third to fifth), from the marginal edge to the varnish mark (Olympus CellSens software; Fig. 1C). An individual's shell growth rate was estimated from the average of these three marginal extensions, divided by the number of growing days.

The secondary data set was used to determine the relationship between shell roundness and growth rate, and this relationship was then used to calculate the growth rate from shell roundness for each snail in the primary data set. Both growth rate and shell roundness were then related to the erosion index (EI). These relationships were determined to gain insight into how exposure to acidification (assessed from the extrinsic passive response variable, EI) might affect intrinsic physiological response parameters, such as growth and shell roundness, as well as how shell roundness is affected by growth rate.

RESULTS

Shell size and shape

There were no differences between the populations at the two study sites in terms of primary measures of size (shell length, mm : EM, acidified = 18.75 ± 0.52 , mean and SE; UB, non-acidified = 20.3 ± 0.69 ; Wald Stat. = 3.39, $p = 0.065$; Fig. 2A and shell width, mm : EM = 12.31 ± 0.36 ; UB = 12.74 ± 0.48 ; Wald Stat. = 0.53, $p = 0.466$; Fig. 2B). The snail populations were similarly size-structured, enabling their comparison. Shell roundness (SW/SL) was, however, greater in the EM population (0.66 ± 0.005) compared to the UB population (0.63 ± 0.007 ; Wald Stat. = 11.04; $p < 0.001$;

Fig. 2C). The relationships for shell width against shell length also differed between EM ($y = -0.55 + 0.686x$, $r = 0.97$, $p < 0.001$) and UB ($y = 1.157 + 0.57x$, $r = 0.93$, $p < 0.001$; Wald Stat. = 7.98, $p = 0.005$; Fig. 2D).

Shell erosion indices (SER, EI)

At the acidified site (EM), eroded suture length was positively related to shell size, measured as total suture length ($y = -14.8 + 0.69x$, $r^2 = 0.59$, $p < 0.001$; Fig. 3A) or shell length ($y = -21.8 + 2.11x$, $r^2 = 0.56$, $p < 0.001$; Fig. 3B). In contrast, the eroded suture length of shells from the non-acidified site (UB) was low and not size dependent ($y = -0.17 + 0.104x$, $r^2 = 0.23$, $p = 0.04$ for total suture length; Fig. 3A; and $y = -0.78 + 0.28x$, $r^2 = 0.16$, $p = 0.1$ for shell length; Fig. 3B). Smaller shells of younger snails are exposed for a limited time to corrosive seawater regardless of the acidification level and show site independent responses. The effect of total suture length was expectedly similar to shell length, and these two length variables were strongly correlated (EM: $y = -10.5 + 3.06x$, $r^2 = 0.98$, $p < 0.001$; UB: $y = -16.8 + 3.29x$, $r^2 = 0.98$, $p < 0.001$; Fig. 3C).

The EI (eroded suture length / total suture length) was slightly positively related to shell length at EM ($y = -0.02 + 0.02x$, $r^2 = 0.19$, $p = 0.008$), whereas there was no significant relationship for snails from UB ($y = 0.19 - 0.005x$, $r^2 = 0.085$, $p < 0.21$; Fig. 3D). The EI for an average-size snail was ~ 0.35 at EM and ~ 0.1 at UB (Fig. 3D). The pattern of relationships was similar for the shell erosion rank (SER) against shell length (EM: $y = 4.78 + 0.102x$, $r^2 = 0.13$, $p < 0.03$; UB: $y = 6.7 - 0.095x$, $r^2 = 0.1$, $p < 0.001$; Fig. 3E). The SER for an average-sized snail was ~ 7 at EM and ~ 5 at UB (Fig. 3E). EI was, however, a more sensitive indicator than SER (Fig. 3F). As an example, EIs ranged between 0.3 and 0.6 for a single SER value of 7; similarly, a range of ~ 0.3 on the EI scale of 0 to 0.8 were collected for a single SER value of 8 (Fig. 3F).

Growth rate and erosion time (ET, SET)

Shell margin extension assessed for the secondary population was positively related to the time for growth ($y = 0.08x - 0.34$, $r = 0.65$, $p < 0.01$; Fig. 4A). Mean, maximum, and minimum values for growth rate (mm/day) were 0.06, 0.14 and 0.01 mm/d (Fig. 4B). Shell growth rate was significantly negatively (linearly) related to shell length (SL) ($y = -0.0074x + 0.1817$, $r = 0.53$, $p < 0.01$; Fig. 4B). Snail age, estimated for the primary population by multiplying the reciprocal of the average growth rate by total suture length, was 2.17, 3.3 and 1.5 years (mean, maximum and minimum).

Erosion time (ET, days) determined using a mean growth rate of 0.06 mm/d was positively related to SL, and differed greatly between the acidified ($y = 0.21x - 3.44, r = 0.75; p < 0.001$) and non-acidified snail groups ($y = 0.028x - 1.36; r = 0.39; p = 0.104; F = 86.81, p < 0.001$; Fig. 4C). The median of sized-corrected erosion times (SET) for the acidified site was more than five-fold greater than that for the non-acidified site (Mann-Whitney $U = 44; Z = 5.12; p < 0.001$; Fig. 4D).

Relating growth rate, shell roundness and shell erosion

Field shell growth rate was strongly negatively correlated with shell roundness (growth rate = $0.55 - 0.73x, r = -0.695, p < 0.001; n = 22$, Fig. 5A). For the primary population, shell roundness was positively correlated with erosion index (EI, $y = 0.62 + 0.094x, r = 0.54, p < 0.001$, Fig. 5B), and growth rate derived from

shell roundness was strongly negatively correlated with EI, the environmental acidification exposure marker ($y = 0.097 - 0.069x, r = -0.54, p < 0.001$, Fig. 5C).

DISCUSSION

Standard laboratory approaches that compare responses between present-day OCA levels with those predicted for 2100 are limited in their exclusion of natural variations in biotic and abiotic conditions (eg., salinity, temperature, tidal, lunar and seasonal effects, as well as wave action) (Hall-Spencer et al. 2008; Manno et al. 2017; Cummings et al. 2019; León et al. 2020; Clark et al. 2020; Lagos et al. 2021; Mayk et al. 2022b). Field-based studies that compare responses between spatially-separated normal and acidified environments (often at CO₂-enriched submarine volcanic vent systems) can be confounded by covarying environmental factors. Most

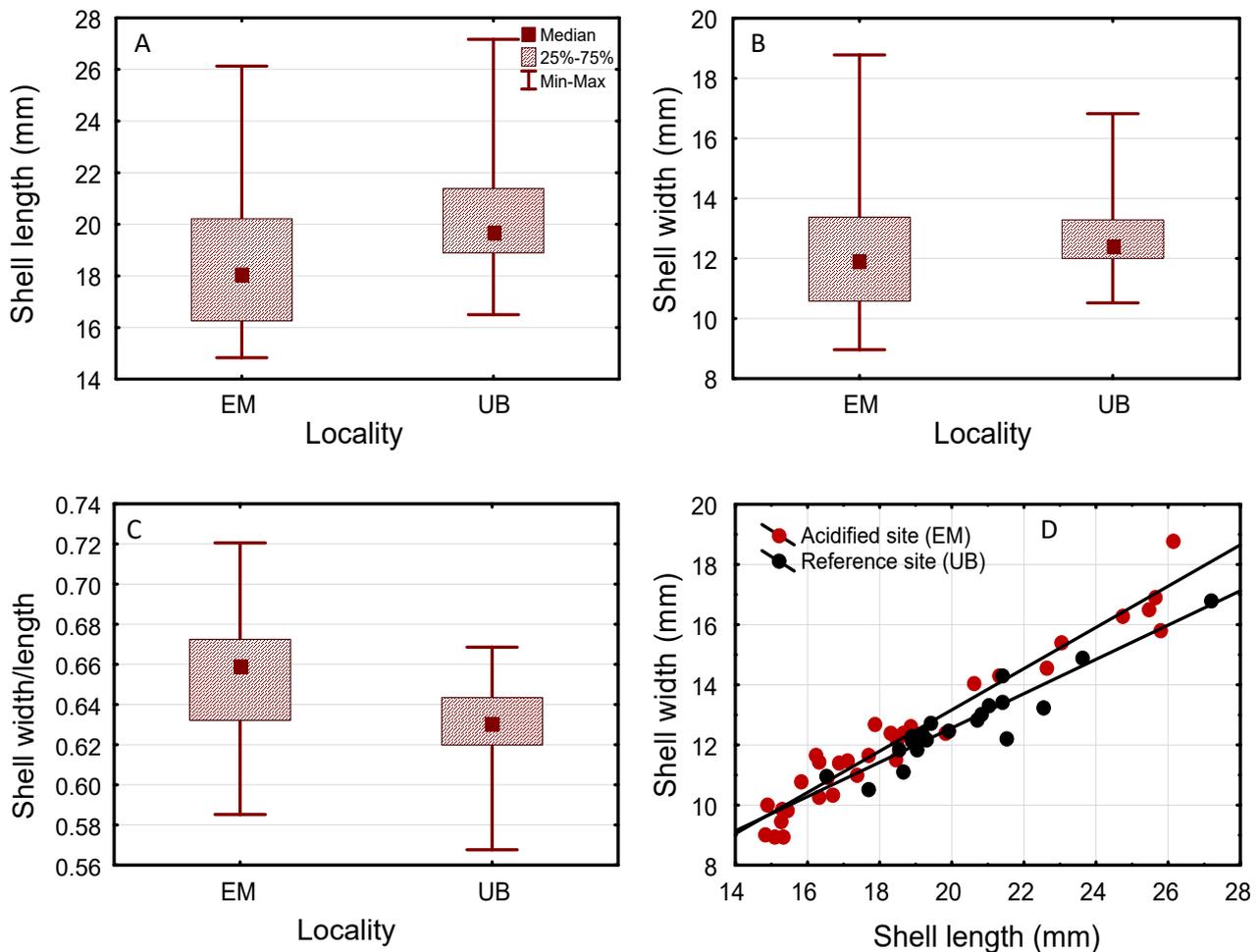


Fig. 2. (A–C). Comparisons between the localities in shell length, shell width and shell roundness (SW/SL). Data are shown as median, 25–75%, min-max (see key). (D) Relationships between shell width and shell length are: EM ($y = -0.55 + 0.686x; r = 0.97; p < 0.001$) and UB ($y = 1.157 + 0.57x; r = 0.93; p < 0.001$). Red circles indicate the acidified locality (EM) and black circles, the non-acidified locality (UB).

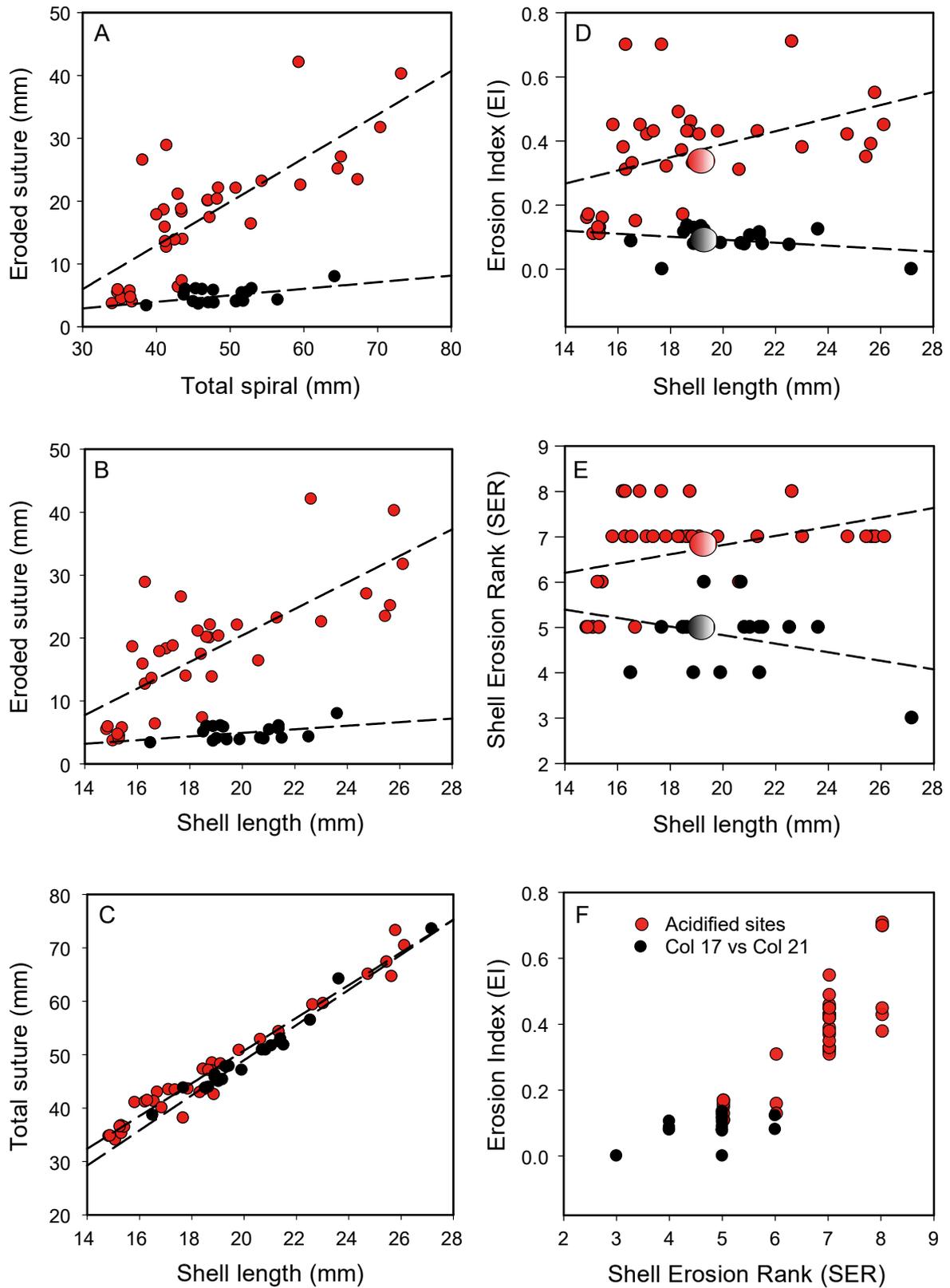


Fig. 3. (A–C) Relationships between total suture length, eroded suture length and shell length for snails from acidified (EM, red) and reference (UBD, black) sites. (D–F) Relationships between erosion index (EI), shell erosion rank (SER), and shell length (SL). Mean values are indicated by large circles. Regression equations and significant differences are given in the Materials and Methods section.

field acidification studies investigate trait responses individually, such that the interrelationships of shell attributes are seldom considered. Here, we reveal that valuable information can be collected by interrelating traits representing different organism-to-environment interactions. For the traits considered separately, we found that acidification exposure caused *Planaxis* snail shells to become more eroded and rounder, and to grow slower.

Gastropod shell erosion is widely-reported in OCA studies. However, with the exception of a few intertidal species, this is usually not quantified (Marshall et al. 2008 2019; Mustapha and Marshall 2021). Shell erosion is influenced by many different sources and the effect of weathering in intertidal situations, where snails routinely experience heating, cooling, wetting, and drying, can be significant. Studies show that comparing

nearby acidified and non-acidified (or reference) habitats allows researchers to distinguish between the effect of weathering on *Planaxis* shells and erosion caused by dissolution; this was facilitated by a semi-quantitative measure, the Shell Erosion Rank, SER (see Marshall et al. 2019; Mustapha and Marshall 2021). The present study shows that SERs based on eight shell segments for the abapertural surface were greater in snails at the acidified site (EM) compared to the non-acidified site (UB), and were uniquely elevated in the larger shell cohort (> 18 mm SL; Fig. 3D). Because of the effect of weathering of the first two shell whorls, most on the information on shell dissolution using this technique is conveyed by whorls 3 and 4, and because a larger area is covered in assessments of both the apertural and abapertural surfaces, an earlier method to determine SER might be preferential to the method used here

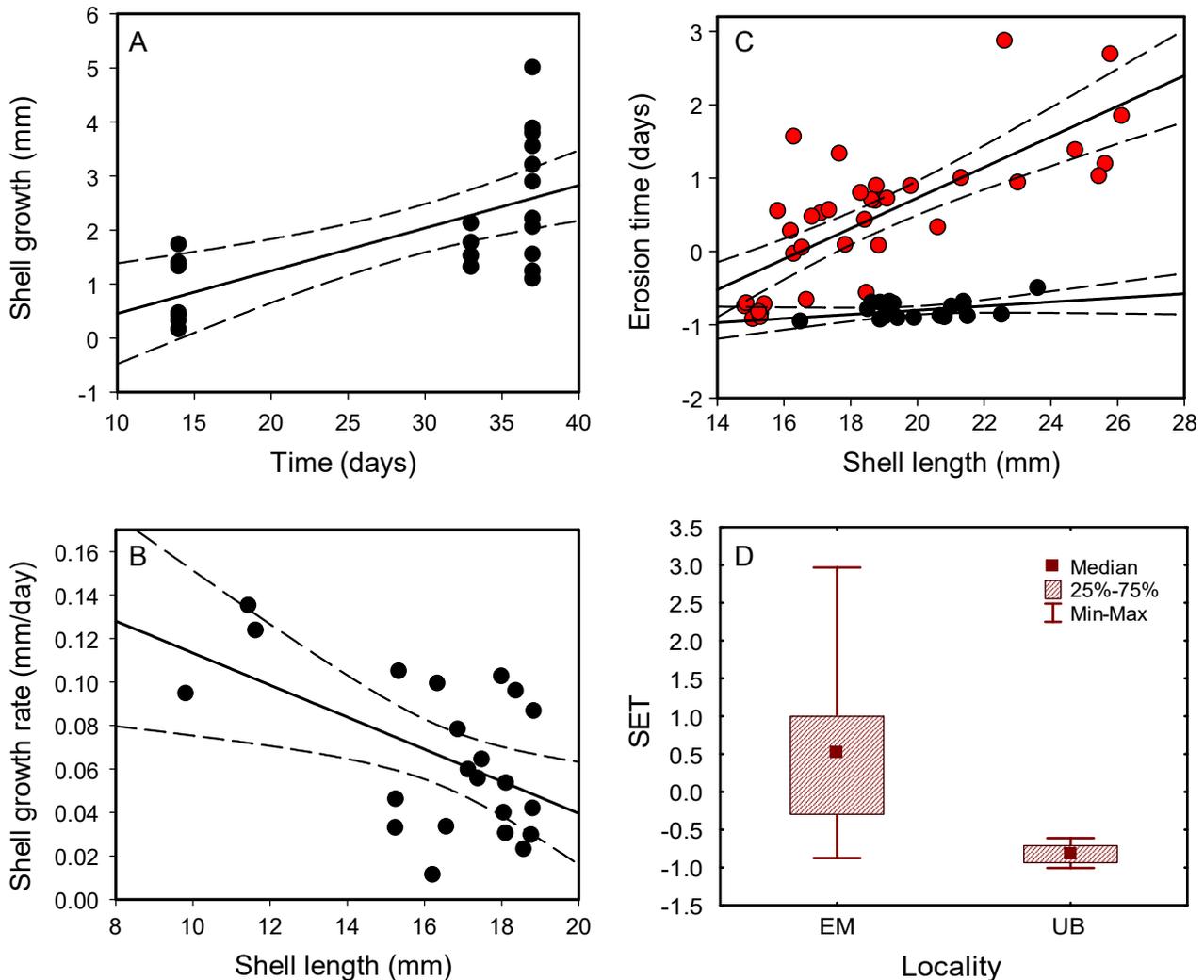


Fig. 4. (A–B) Relationships for shell growth rate and shell size based on the secondary data set. (C) Erosion time (ET) as a function of shell size (SL), and (D) comparison of standardized erosion time (SET) between the acidified (EM) and non-acidified (UB) sites using the primary data sets. Dashed lines represent 95% CI. Red symbols indicate snails collected from the acidified site and black symbols from the non-acidified site.

(Marshall et al. 2019). However, compared to the SER, the newly devised Erosion Index (EI) yielded much greater precision of measurement, such that ranges of EI values coincided with single values of SERs (Fig. 3F). We reveal that the utility of shell erosion metrics can be broadened by converting eroded suture length to a standardized time variable (ET), by integrating suture growth rate. This in turn can be corrected to a standard shell size to give the SET, a metric that was found to readily discriminate between the differently-acidified sites of this study (SET > -0.5 uniquely indicates acidification; Fig. 4D).

Several studies report on gastropod shell roundness in response to environmental stress, including

acidification, marine pollution and food deprivation (Chen et al. 2015; Harvey et al. 2018; Viotti et al. 2019; Marshall et al. 2019; Clark et al. 2020; Mayk et al. 2022b). Other factors influencing shell roundness include food availability (Kemp and Bertness 1984), wave exposure (Kitching and Lockwood 1974) and predator encounters (Palmer 1979). There is, however, disagreement concerning the way the shell shape changes under stress. Some studies suggest that stress causes greater shell rounding, whereas others suggest this leads to shell narrowing and elongation (Chen et al. 2015; Harvey et al. 2018; Viotti et al. 2019; Marshall et al. 2019; Clark et al. 2020; Mayk et al. 2022b). Rounder shells observed in faster growing individuals

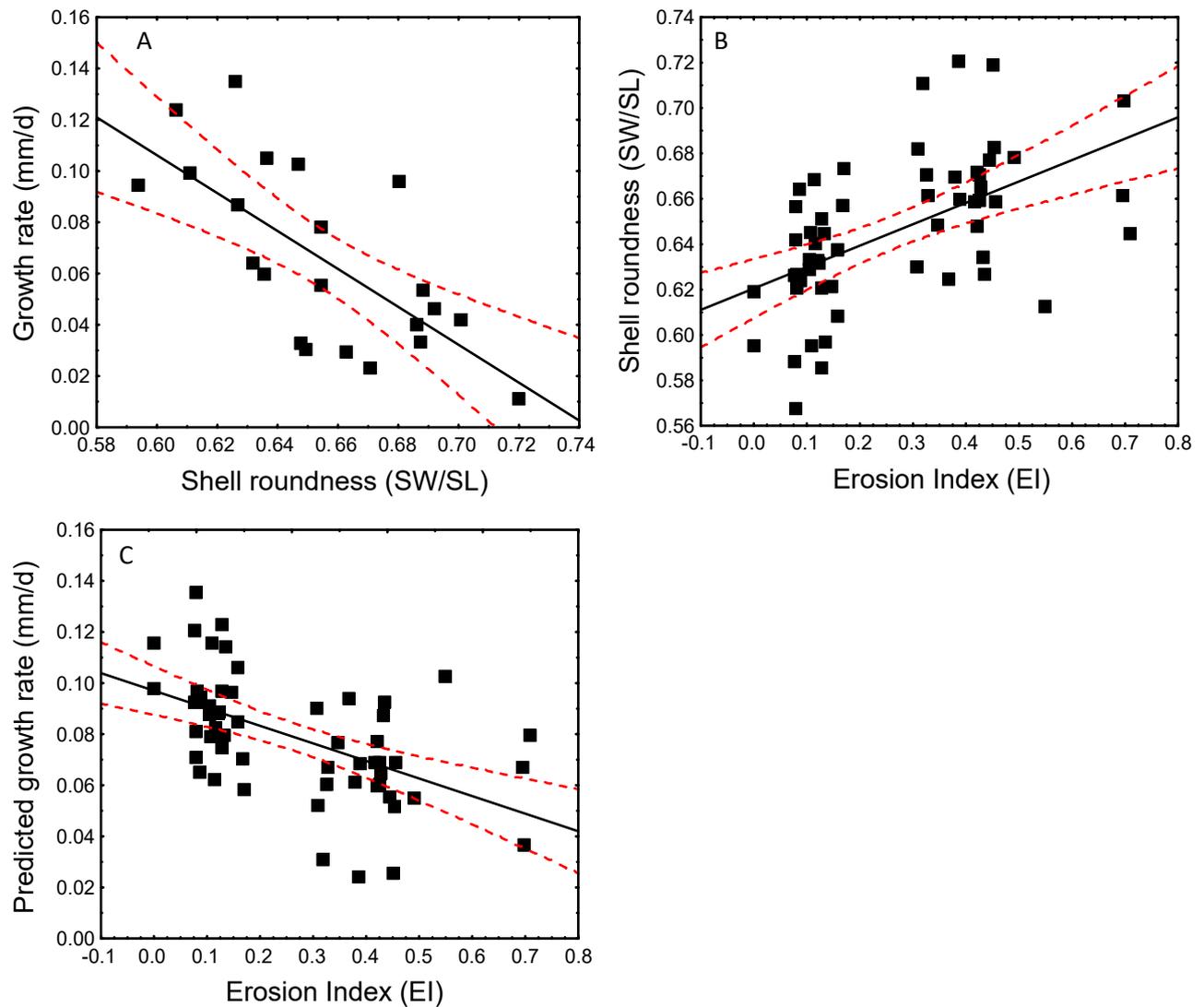


Fig. 5. Relationships for shell growth rate, shell roundness, and erosion index.. (A) Shell growth rate and roundness measured directly for snails from the secondary population. (B) Shell roundness plotted against erosion index for the primary population. (C) Predicted growth rate plotted against the erosion index. Regression equations and significant differences are given in the Materials and Methods section. Dashed lines represent 95% CI.

were thought to be necessary to accommodate a more rapidly increasing body mass and volume (Kemp and Bertness 1984). However, this does not consider the fact that differently shaped shells differ fundamentally in their intrinsic growth priority pattern (Larsson et al. 2020). For example, the internal volume of high-spined shells increases along the apical axis, whereas that of lower-spined (globular) shells increases by broadening of the body whorl (Larsson et al. 2020). We thus postulated that an environmental constraint on growth should compromise the intrinsic growth priority pattern, resulting in the broadening of high-spined shells and the narrowing of intrinsically globular shells. Should this be true, then similar environmental stress conditions potentially lead to different shell roundness responses in different gastropod species. Insight into the intrinsic growth pattern and shell shape can be garnered by comparing the shells of younger and older snails, as younger animals universally grow faster. Our findings were consistent with this hypothesis, by showing that *Planaxis* snails exhibit a shell elongation growth priority pattern (Fig. 5), become rounder in acidified water (Fig. 2), and their roundness is negatively related to growth rate (Fig. 5).

What drives shell roundness and growth rate variation in *Planaxis* snails? Acidification theoretically limits gastropod shell construction (shell extension, thickness and compactness) through two fundamental processes, the uptake and allocation of energy fuels, and environmental availability of calcification substrates (calcium and carbonate ions; Clark et al. 2020). The relative importance of each process on the various shell attributes is poorly understood for gastropods experiencing acidification (but see Leung et al. 2020b). Freshwater gastropods more commonly have thinner shells than marine gastropods (but see Marshall and Tsikouras 2023), implying that calcification substrate availability predominantly impacts shell thickness and compactness (see Leung et al. 2017 2020a for marine gastropods) rather than shell extension (growth rate). However, calcification substrates are only temporally unavailable to *Planaxis* snails, as acidified tidal pool water is inundated with calcium-rich oceanic water during high tide, supporting the assumption that marginal shell extension is more likely controlled by energetics than by shell substrate availability in their case (see also Kemp and Bertness 1984). Thus, the shell growth of *Planaxis* snails in the acidified habitats is more likely limited by reduced energy intake and/or reduced fuel allocation associated with an expected elevated metabolic demand to support compensatory physiology and shell construction (Proum et al. 2017; Marshall and Tsikouras 2023). Some responses are protective and function to resist or compensate for

dissolved shell loss by producing thicker, more compact, and mineralogically more-complex shells (Langer et al. 2014; Peck et al. 2018; Leung et al. 2020a; Mayk et al. 2022a). Whereas severely-constrained growth impacts organismal size, causing individuals exposed to acidification to become stunted (Garilli et al. 2015; Harvey et al. 2018), we found no difference between the study sites in population size structure (Fig. 2). Rather, the observed difference in shell roundness for the populations suggests that roundness represents a more sensitive indicator of environmental or acidification stress (see also Chen et al. 2015; Viotti et al. 2019).

Although individual shell traits of the *Planaxis* snails are themselves informative of acidification exposure, and can potentially be used to indicate local acidification (see Fig. 1C for roundness, and Fig. 3F and Fig. 4D for shell erosion; Marshall et al. 2019; Mustapha and Marshall 2021), their interrelationships offer insight into causation. This is likely the case when different traits form different mechanistic associations with the acidified seawater. Shell erosion represents an extrinsic passive responder to acidification, as this occurs beyond the reach of the mantle after the shell is formed at the growing edge. Shell roundness, on the other hand, represents an intrinsic performance indicator, as this relates functionally to growth rate (Kemp and Bertness 1984; Fig. 5A). A good relationship between these traits means that the performance response (shell roundness) can be interpreted with confidence as being caused by acidification (assessed from shell erosion), rather than some other confounding factor. Such relationships can be especially useful when assessing a performance response along a latitudinal gradient that is not only variable with respect to the carbonate system, but also in terms of temperature and nutrient availability (see Melzner et al. 2011; Ramajo et al. 2015; Clark et al. 2020; Mayk et al. 2022b; Duarte et al. 2022). The interrelationship of the three studied traits (shell erosion, growth, and roundness) indicates directionality and mechanism in the following way: greater acidification exposure, indicated by greater shell erosion, causes reduced growth rate, which in turn causes roundness to vary. Interpretations of correlations in the opposite direction (*ie.*, that shell roundness affects growth rate, that shell roundness affects shell erosion, or that growth rate affects shell erosion) defy all reason. Because these shell trait relationships are grounded in the physical principle that shell dissolution is caused by the seawater carbonate saturation state, they comply with rudimentary mechanistic modeling. There appears to be great potential to expand on such modeling by including other shell traits, such as thickness, compactness and mineralogy. Indeed, a recent review of the current state of organismal attributes and OCA,

not only excluded gastropod shell roundness, but made no reference to trait functionality, or indeed to modeling (Leung et al. 2022).

CONCLUSIONS

This study confirms that the gastropod *Planaxis* represents a good model species for understanding organismal responses to OCA. It shows that relating traits that convey different information about the association of the organism and the acidified environment, enhances the interpretative power of observed responses. Such relationships among traits can also potentially provide information about mechanism. While the techniques refer to Indopacific snails, they nonetheless provide a framework for similar investigations on other gastropods in other global regions. By observing gastropod shell traits, we have the ability to monitor and manage how gastropods respond to changes in the ecosystem, which is becoming increasingly necessary to counter the predicted acidification of the ocean and coasts.

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REFERENCES

- Barclay KM, Gaylord B, Jellison BM, Shukla P, Sanford E, Leighton LR. 2019. Variation in the effects of ocean acidification on shell growth and strength in two intertidal gastropods. *Mar Ecol Prog Ser* **626**:109–121. doi:10.3354/meps13056.
- Barclay KM, Gingras MK, Packer ST, Leighton LR. 2020. The role of gastropod shell composition and microstructure in resisting dissolution caused by ocean acidification. *Mar Environ Res* **162**:105105. doi:10.1016/j.marenvres.2020.105105.
- Bednaršek N, Feely RA, Reum JCP, Peterson B, Menkel J, Alin SR, Hales B. 2014a. *Limacina helicina* shell dissolution as an indicator of declining habitat suitability owing to ocean acidification in the California Current Ecosystem. *Proc R Soc B Biol Sci* **281**:20140123. doi:10.1098/rspb.2014.0123.
- Bednaršek N, Naish K-A, Feely RA, Hauri C, Kimoto K, Hermann AJ, Michel C, Niemi A, Pilcher D. 2021. Integrated Assessment of Ocean Acidification Risks to Pteropods in the Northern High Latitudes: Regional Comparison of Exposure, Sensitivity and Adaptive Capacity. *Front Mar Sci* **8**:671497. doi:10.3389/fmars.2021.671497.
- Bednaršek N, Tarling GA, Bakker DCE, Fielding S, Jones EM, Venables HJ, Ward P, Kuzirian A, Leze B, Feely RA, Murphy EJ. 2012a. Extensive dissolution of live pteropods in the Southern Ocean. *Nat Geosci* **5**:881–885. doi:10.1038/ngeo1635.
- Bednaršek N, Tarling GA, Bakker DCE, Fielding S, Feely RA. 2014b. Dissolution Dominating Calcification Process in Polar Pteropods Close to the Point of Aragonite Undersaturation. *PLoS ONE* **9**:e109183. doi:10.1371/journal.pone.0109183.
- Bednaršek N, Tarling GA, Bakker DCE, Fielding S, Cohen A, Kuzirian A, McCorkle D, Leze B, Montagna R. 2012b. Description and quantification of pteropod shell dissolution: A sensitive bioindicator of ocean acidification. *Glob Chang Biol* **18**:2378–2388. doi:10.1111/j.1365-2486.2012.02668.x.
- Bibby R, Cleall-Harding P, Rundle S, Widdicombe S, Spicer J. 2007. Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. *Biol Lett* **3**:699–701. doi:10.1098/rsbl.2007.0457.
- Chen YJ, Wu JY, Chen CTA, Liu LL. 2015. Effects of low-pH stress on shell traits of the dove snail, *Anachis misera*, inhabiting shallow-vent environments off Kueishan Islet, Taiwan. *Biogeosciences* **12**:2631–2639. doi:10.5194/bg-12-2631-2015.
- Clark MS, Peck LS, Arivalagan J, Backeljau T, Berland S, Cardoso JCR, Caurcel C, Chapelle G, De Noia M, Dupont S, Gharbi K, Hoffman JI, Last KS, Marie A, Melzner F, Michalek K, Morris J, Power DM, Ramesh K, Sanders T, Sillanpää K, Sleight VA, Stewart-Sinclair PJ, Sundell K, Telesca L, Vendrami DLJ, Ventura A, Wilding TA, Yarra T, Harper EM. 2020. Deciphering mollusc shell production: the roles of genetic mechanisms through to ecology, aquaculture and biomimetics. *Biol Rev* **95**:1812–1837. doi:10.1111/brv.12640.
- Cummings VJ, Smith AM, Marriott PM, Peebles BA, Halliday NJ. 2019. Effect of reduced pH on physiology and shell integrity of juvenile *Haliotis iris* (pāua) from New Zealand. *PeerJ* **7**:e7670. doi:10.7717/peerj.7670.
- Duarte C, Jahnsen-Guzmán N, Quijón PA, Manríquez PH, Lardies MA, Fernández C, Reyes M, Zapata J, García-Huidobro MR, Lagos NA. 2022. Morphological, physiological and behavioral responses of an intertidal snail, *Acanthina monodon* (Pallas), to projected ocean acidification and cooling water conditions in upwelling ecosystems. *Environ Pollut* **293**:118481. doi:10.1016/j.envpol.2021.118481.
- Duquette A, McClintock JB, Amsler CD, Pérez-Huerta A, Milazzo M, Hall-Spencer JM. 2017. Effects of ocean acidification on the shells of four Mediterranean gastropod species near a CO₂ seep. *Mar Pollut Bull* **124**:917–928. doi:10.1016/j.marpolbul.2017.08.007.
- Garilli V, Rodolfo-Metalpa R, Scuderi D, Brusca L, Parrinello D, Rastrick SPS, Foggo A, Twitchett RJ, Hall-Spencer JM, Milazzo

- M. 2015. Physiological advantages of dwarfing in surviving extinctions in high-CO₂ oceans. *Nat Clim Chang* **5**:678–682. doi:10.1038/nclimate2616.
- Gledhill DK, White MM, Salisbury J, Thomas H, Mlsna I, Liebman M, Mook B, Grear J, Candelmo AC, Chambers RC, Gobler CJ, Hunt CW, King AL, Price NN, Signorini SR, Stancioff E, Stymiest C, Wahle RA, Waller JD, Rebuck ND, Wang ZA, Capson TL, Morrison JR, Cooley SR, Doney SC. 2015. Ocean and coastal acidification off New England and Nova Scotia. *Oceanography* **28**:182–197. doi:10.5670/oceanog.2015.41.
- Gödeke SH, Malik OA, Lai DTC, Bretzler A, Schirmer M, Mansor NH. 2020. Water quality investigation in Brunei Darussalam: investigation of the influence of climate change. *Environ Earth Sci* **79**:419. doi:10.1007/s12665-020-09157-2.
- Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia M. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* **454**:96–99. doi:10.1038/nature07051.
- Harayashiki CAY, Márquez F, Cariou E, Castro ÍB. 2020. Mollusk shell alterations resulting from coastal contamination and other environmental factors. *Environ Pollut* **265**:114881. doi:10.1016/j.envpol.2020.114881.
- Hart M, Dias B, Wall-Palmer D, Smart C, Hall-Spencer JM. 2011. Response of pteropods and foraminifera to changing pCO₂ and pH: Examples from the Mediterranean Sea. *Geophys Res Abstr* **13**:2472–2472.
- Harvey BP, Agostini S, Wada S, Inaba K, Hall-Spencer JM. 2018. Dissolution: The Achilles' Heel of the triton shell in an acidifying ocean. *Front Mar Sci* **5**:371. doi:10.3389/fmars.2018.00371.
- Kemp P, Bertness MD. 1984. Snail shape and growth rates: Evidence for plastic shell allometry in *Littorina littorea*. *Proc Natl Acad Sci* **81**:811–813. doi:10.1073/pnas.81.3.811.
- Kitching JA, Lockwood J. 1974. Observations on shell form and its ecological significance in thaisid gastropods of the genus *Lepsiella* in New Zealand. *Mar Biol* **28**:131–144. doi:10.1007/BF00396304.
- Lagos NA, Benítez S, Grenier C, Rodriguez-Navarro AB, García-Herrera C, Abarca-Ortega A, Vivanco JF, Benjumedá I, Vargas CA, Duarte C, Lardies MA. 2021. Plasticity in organic composition maintains biomechanical performance in shells of juvenile scallops exposed to altered temperature and pH conditions. *Sci Rep* **11**:24201. doi:10.1038/s41598-021-03532-0.
- Lahbib Y, Slama T, Abidli S, Nouet J, Chassefière E, El Menif NT. 2022. Shell alterations in *Hexaplex trunculus* collected in the vicinity of an impacted zone by industrial marine discharges (Gabès, Southern Mediterranean). *J Sea Res* **181**:102178. doi:10.1016/j.seares.2022.102178.
- Langer G, Nehrke G, Baggini C, Rodolfo-Metalpa R, Hall-Spencer JM, Bijma J. 2014. Limpets counteract ocean acidification induced shell corrosion by thickening of aragonitic shell layers. *Biogeosciences* **11**:7363–7368. doi:10.5194/bg-11-7363-2014.
- Larsson J, Westram AM, Bengmark S, Lundh T, Butlin RK. 2020. A developmentally descriptive method for quantifying shape in gastropod shells. *J R Soc Interface* **17**:20190721. doi:10.1098/rsif.2019.0721.
- León P, Bednaršek N, Walsham P, Cook K, Hartman SE, Wall-Palmer D, Hindson J, Mackenzie K, Webster L, Bresnan E. 2020. Relationship between shell integrity of pelagic gastropods and carbonate chemistry parameters at a Scottish Coastal Observatory monitoring site. *ICES J Mar Sci* **77**:436–450. doi:10.1093/icesjms/fsz178.
- Leung JYS, Chen Y, Nagelkerken I, Zhang S, Xie Z, Connell SD. 2020a. Calcifiers can Adjust Shell Building at the Nanoscale to Resist Ocean Acidification. *Small* **16**:2003186. doi:10.1002/sml.202003186.
- Leung JYS, Russell BD, Connell SD. 2017. Mineralogical Plasticity Acts as a Compensatory Mechanism to the Impacts of Ocean Acidification. *Environ Sci Technol* **51**:2652–2659. doi:10.1021/acs.est.6b04709.
- Leung JYS, Russell BD, Connell SD. 2020b. Linking energy budget to physiological adaptation: How a calcifying gastropod adjusts or succumbs to ocean acidification and warming. *Sci Total Environ* **715**:136939. doi:10.1016/j.scitotenv.2020.136939.
- Leung JYS, Zhang S, Connell SD. 2022. Is Ocean Acidification really a threat to marine calcifiers? A systematic review and meta-analysis of 980+ studies spanning two decades. *Small* **18**:e2107407. doi:10.1002/sml.202107407.
- Manno C, Bednaršek N, Tarling GA, Peck VL, Comeau S, Adhikari D, Bakker DCE, Bauerfeind E, Bergan AJ, Berning MI, Buitenhuis E, Burrige AK, Chierici M, Flöter S, Fransson A, Gardner J, Howes EL, Keul N, Kimoto K, Kohnert P, Lawson GL, Lischka S, Maas A, Mekkes L, Oakes RL, Pebody C, Peijnenburg KTCA, Seifert M, Skinner J, Thibodeau PS, Wall-Palmer D, Ziveri P. 2017. Shelled pteropods in peril: Assessing vulnerability in a high CO₂ ocean. *Earth-Science Rev* **169**:132–145. doi:10.1016/j.earscirev.2017.04.005.
- Marshall DJ, Abdelhady AA, Wah DTT, Mustapha N, Gödeke SH, De Silva LC, Hall-Spencer JM. 2019. Biomonitoring acidification using marine gastropods. *Sci Total Environ* **692**:833–843. doi:10.1016/j.scitotenv.2019.07.041.
- Marshall DJ, Santos JH, Leung KMY, Chak WH. 2008. Correlations between gastropod shell dissolution and water chemical properties in a tropical estuary. *Mar Environ Res* **66**:422–429. doi:10.1016/j.marenvres.2008.07.003.
- Marshall DJ, Tsikouras B. 2023. Clay-shielded estuarine gastropods are better protected against environmental acidification than unshielded individuals. *Sci Total Environ* **866**:161367. doi:10.1016/j.scitotenv.2022.161367.
- Martin N, Clusella-Trullas S, Robinson TB. 2022. Predicted changes in temperature, more than acidification, affect the shell morphology and survival of the girdled dogwhelk, *Trochita cingulata* (Linnaeus, 1771). *J Molluscan Stud* **88**:eyac011. doi:10.1093/mollus/eyac011.
- Mayk D, Peck LS, Backeljau T, Harper EM. 2022a. Shell thickness of *Nucella lapillus* in the North Sea increased over the last 130 years despite ocean acidification. *Commun Earth Environ* **3**:158. doi:10.1038/s43247-022-00486-7.
- Mayk D, Peck LS, Harper EM. 2022b. Evidence for carbonate system mediated shape shift in an intertidal predatory gastropod. *Front Mar Sci* **9**:894182. doi:10.3389/fmars.2022.894182.
- Mekkes L, Renema W, Bednaršek N, Alin SR, Feely RA, Huisman J, Roessingh P, Peijnenburg KTCA. 2021. Pteropods make thinner shells in the upwelling region of the California Current Ecosystem. *Sci Rep* **11**:1731. doi:10.1038/s41598-021-81131-9.
- Melzner F, Stange P, Trübenbach K, Thomsen J, Casties I, Panknin U, Gorb SN, Gutowska MA. 2011. Food supply and seawater pCO₂ impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS ONE* **6**:e24223. doi:10.1371/journal.pone.0024223.
- Mustapha N, Marshall DJ. 2021. Tracking coastal acidification from erosion of gastropod shells: spatial sensitivity and organism size effect. *Environ Monit Assess* **193**:690. doi:10.1007/s10661-021-09479-z.
- Nagelkerken I, Connell SD. 2022. Ocean acidification drives global reshuffling of ecological communities. *Glob Change Biol* **28**:7038–7048. doi:10.1111/gcb.16410.
- Nienhuis S, Palmer AR, Harley CDG. 2010. Elevated CO₂ affects shell dissolution rate but not calcification rate in a marine snail. *Proc R Soc B Biol Sci* **277**:2553–2558. doi:10.1098/rspb.2010.0206.
- Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RM,

- Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner G, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig M, Yamanaka Y, Yool A. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**:681–686. doi:10.1038/nature04095.
- Palmer AR. 1979. Fish predation and the evolution of gastropod shell sculpture: experimental and geographic evidence. *Evol* **33**:697–713. doi:10.1111/j.1558-5646.1979.tb04722.x.
- Peck VL, Oakes RL, Harper EM, Manno C, Tarling GA. 2018. Pteropods counter mechanical damage and dissolution through extensive shell repair. *Nat Commun* **9**:264. doi:10.1038/s41467-017-02692-w.
- Proum S, Harley CD, Steele M, Marshall DJ. 2017. Aerobic and behavioral flexibility allow estuarine gastropods to flourish in rapidly changing and extreme pH conditions. *Mar Biol* **164**:97. doi:10.1007/s00227-017-3124-y.
- Proum S, Santos JH, Lim LH, Marshall DJ. 2018. Tidal and seasonal variation in carbonate chemistry, pH and salinity for a mineral-acidified tropical estuarine system. *Reg Stud Mar Sci* **17**:17–27. doi:10.1016/j.rsma.2017.11.004.
- Ramajo L, Rodríguez-Navarro AB, Duarte CM, Lardies MA, Lagos NA. 2015. Shifts in shell mineralogy and metabolism of *Concholepas concholepas* juveniles along the Chilean coast. *Mar Freshw Res* **66**:1147–1157. doi:10.1071/MF14232.
- Saura M, Rivas MJ, Diz AP, Caballero A, Rolan-Alvarez E. 2012. Dietary effects on shell growth and shape in an intertidal marine snail, *Littorina saxatilis*. *J Molluscan Stud* **78**:213–216. doi:10.1093/mollus/ey004.
- Schönberg CHL, Fang JKH, Carreiro-Silva M, Tribollet A, Wisshak M. 2017. Bioerosion: The other ocean acidification problem. *ICES J Mar Sci* **74**:895–925. doi:10.1093/icesjms/fsw254.
- Slama T, Nouet J, Chassefière E, El Menif NT, Lahbib Y. 2021. Monitoring of coastal pollution using shell alterations in the false limpet *Siphonaria pectinata*. *Mar Pollut Bull* **173**:113063. doi:10.1016/j.marpolbul.2021.113063.
- Viotti S, Sangil C, Hernández CA, Hernández JC. 2019. Effects of long-term exposure to reduced pH conditions on the shell and survival of an intertidal gastropod. *Mar Environ Res* **152**:104789. doi:10.1016/j.marenvres.2019.104789.
- Yao H, Wang J, Han Y, Jiang X, Chen J. 2022. Decadal acidification in a subtropical coastal area under chronic eutrophication. *Environ Pollut* **293**:118487. doi:10.1016/j.envpol.2021.118487.
- Zhang Y, Wang X, Xue Y, Zou C, Luo M, Li G, Li L, Cui L, Li H. 2022. Advances in the study of submarine groundwater discharge (SGD) in China. *Sci China Earth Sci* **65**:1948–1960. doi:10.1007/s11430-021-9946-x.

Supplementary materials

Fig. S1. Groundwater discharge into the uppershore of a boulder beach during low tide at the Empire site (EM). *Planaxis* snails occur in and around pools that become acidified, characterized by reduced pH, reduced salinity and undersaturated aragonite (see Marshall et al. 2019). Acidified habitats can be identified by an oily sheen on the surfaces of small pools, and rust-stained sediments are rocks. Points of discharge are indicated by blue arrows, snails by red arrows, and the oily sheen by green arrows. (download)