Exploring Shell Shape Variation and Environmental Influences in *Littorina littorea* and *Littorina saxatilis*

Submitted by:

Marion Billy (marion.billy@imbrsea.eu)

Laura-Marie Dehne (laura.marie.dehne@imbrsea.eu

Gabriel Ecker-Eckhofen (gabriel.eckhofen@imbrsea.eu)

Saeesh Mangwani (saeesh.mangwani@imbrsea.eu)

Nadine Plata Klingler (nadine.plata.klingler@imbrsea.eu)

Under supervision of:

Marta Mega Rufino

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Summary

This study aimed to investigate the shell morphology in *Littorina* snails across different coastal habitat types. The research addressed two primary questions: (1) Whether observable shell shape differences exist in *L. littorea* across contrasting environmental conditions. (2) Whether these shape patterns would differ between *L. littorea* and *L. saxatilis* across habitat types. Our analysis revealed that distinct patterns of shell shape variation were evident in both *Littorina* species across the two studied sites. Differences in average shell shape were not only significant across sites and species but also in the interaction between them, highlighting that the nature of differences in mean shape between sites varies between the two species, suggesting unique strategies for environmental adaptation. Visual comparisons and dispersion analysis further provided limited evidence for greater shape variability in *L. littorea*. This finding contributes to the debate regarding the relationship between high-dispersing species and increased levels of phenotypic plasticity, as higher variability in shape form may be related to a more capacity for plasticity as a strategy for environmental adaptation. The study also summarizes the sources of shell shape variation using two morphometric analysis techniques, helping characterize which shape features are driving differences across species and environmental types. This may offer a starting point for future work investigating the sources of such variation, elucidating the potential benefits of diverse strategies in responding to environmental pressures. Offer findings thus offer a minor contribution to the growing understanding of adaptive potential of *Littorina* snails, particularly focusing on the importance of shape variation in responding to diverse abiotic and biotic pressures in coastal ecosystems.

Abstract

The dynamic relationship between organisms and their environment drives an ongoing process of adaptation and evolution. A particularly pressing challenge for adaptation, especially for sessile benthic organisms, is evolving an optimal body shape for success in an evolving environment. This study examines dynamics of shell shape variation in *Littorina* snails across distinct coastal habitats. Focusing on exposed and sheltered rocky shores, our research addresses two key questions: (1) Can we identify observable differences in shell shape within high-dispersing species *L. littorea* across two tidal habitats? (2) Do patterns of shell-shape variation differ between *L. littorea* and the low-dispersing species *L. saxatilis*? Using two techniques of shape analysis grounded in geometric morphometrics, we examine shell shapes across species, sites, and tidal levels. Our findings unveil distinct shell shape patterns within both *Littorina* species across the sites, reflecting variations not only between sites and species but also in their interplay, highlighting distinct strategies in response to shared environmental pressures. We also find limited evidence for a broader range of shell shape variations in *L. littorea* demonstrates a broader range of shell shape variations, offering some support to the theoretical link between dispersal range and higher phenotypic plasticity. Our investigation also provides a descriptive account of how shell morphologies in the two species vary based on site exposure, opening up questions for future work examining the sources of this variation.

Introduction

Local adaptation has long intrigued evolutionary biologists, posing questions about how populations achieve tailored adjustments within their specific environments and how divergent selection might ultimately pave the path to speciation (Savolainen et al., 2013). A remarkable example of local adaptation is witnessed in the realm of *Littorina* snails (Johannesson, 2003; Rolán-Alvarez, 2007). Sympatric communities of *L. saxatilis* have been shown to contain very distinct morphotypes, depending on the type of selective pressure they are subjected to (Johannesson et al., 1993; Le Pennec, 2017). These species also exemplify the importance of body shape as a critical trait for adapting to complex environments, which can vary based on several factors including sex, predation pressure, dispersal capacity, and environmental stressors (Zelditch et al., 2004). In this species, morphological variation has been shown to indicate a selective pressure potentially driving speciation. This is due to the fact that *L. saxatilis* is characterized by very limited dispersal, likely a consequence of a direct-development reproductive strategy. This trait, combined with the steep inclines characteristic of the rocky intertidal, have been proposed as the reason for driving strong divergences in shell-shape (Janson, 1983; Hollander et al., 2006a).

Despite covering a similar environmental range, body shape variation in response to environmental pressures has remained relatively unexamined in the closely related species *L. littorea.* This species utilizes a distinct reproductive strategy characterized by a planktonic larval stage (Kyle and Boulding, 2000). It can thus achieve a much larger range of dispersal in the water column, enabling it to explore broader habitats and potentially increasing its capacity for gene flow across varying coastal environments (Johannesson, 1988). The impact of environmentally-driven selective pressure on these individuals is likely to vary as compared to *L. saxatilis,* as its larger dispersal capacity may diminish the importance of locally relevant selective pressures in favor of a more generalist body plan, suitable for a more diverse range of environments (Hollander et al., 2006b). To investigate this hypothesis through a field survey of body shapes, our study aims to answer the two research questions: Are there discernible shell shape variations within *L. littorea* across contrasting environmental conditions in Saltö island's coastal landscape? Can we observe different shape variation patterns between *L. littorea* and *L. saxatilis*?

We analyzed shell shape variation in *L. littorea* and *L. saxatilis* using two techniques grounded in geometric morphometry (GM). In so doing, our study aims to contribute to a broader understanding of shell shape variation by comparing patterns of variation of a low and high dispersal marine gastropod. Patterns of shape variation between these two species have already been investigated through experimental work (Hollander et al., 2006b). We aim to complement and compare these findings with the results from a field survey, examining whether experimental findings are replicated in natural conditions. Second, we utilize two distinct techniques in geometric morphometrics for analyzing shape in our sampled populations, aiming to offer a robust, descriptive account of any shape variation between these two species on Saltö island. We expect that our study will reveal distinct patterns of shell shape variations in *L. littorea* and *L. saxatilis* across environmental factors. Additionally, we anticipate *L. saxatilis* to show more pronounced shape disparities, due to its limited dispersal and direct-developmental reproductive strategy leading to clearer disparities between sites at our chosen spatial scale, as compared to *L. littorea*, whose dispersal capacity may improve its ability to spread between different coastal habitats (Behrens Yamada, 1987).

Materials and Methods

To characterize shape variation in the two *Littorina* species, we sampled individuals from both species in two locations on Saltö island (near Tjärnö, Sweden) between August 18-19, 2023. The shell shape of each individual was characterized using two techniques for morphometric study. The first was traditional geometric morphometrics using landmarks (Rohlf, 2015), henceforth being referenced as "GM". The second was a growth-based model for quantifying shell-shape variation called Shellshaper (Larsson et al., 2020), henceforth "SS". Shape variations across environmental factors were analyzed and compared between both geometric datasets. The sampling protocol and the shape characterization methodology is described below.

Site Selection and Preparation

Two types of sites were compared: an Exposed Rocky Shore (ERS) and Sheltered Rocky Shore (SRS) (Annex 1). Rocky shores are known to host both species, *L. littorea* and *L. saxatilis* in abundance (Johannesson, 2003) and seem therefore suitable for this study. At each site three sections, Upper Intertidal Zone, Intertidal Zone, and Submerged Zone were determined (Annex 2). In addition to the sampling protocol (Annex 2), quadrats were used to measure descriptive environmental features. 5 quadrats (50 x 50 cm) were put at 4 meter intervals along the transect. Within the quadrats, environmental factors such as algae coverage, pebble to boulder ratio and shell density were determined to support the descriptive features of ERS and SRS.

Sample Strategy

Within each site, at each tidal height, we defined a minimum sample number of 15 snails per species. We defined a minimum shell height of 3 mm for sampling individuals since both species are likely to be juveniles below this length, which may imply that morphological distinctions relevant for the ecotype are not yet fully developed (Johannesson et al., 1993). It was made sure that samples were taken, spread along the shoreline. Insufficient numbers of *L. saxatilis* were found in the submerged zone in both sites, which led to leave this species out of our analysis. A preliminary trial phase was conducted to optimize our sampling and preparation methods. This trial informed adjustments to the protocol for seamless alignment with our study's objectives and the unique characteristics of *Littorina* populations.

Sample Labeling and Image Capturing

Each sample was sorted to categorize specimens based on their origin site, species, tidal height, and assigned unique IDs. Images of individuals were then captured by placing them under a stereo microscope with a high-resolution camera. Consistent placement was assured by selecting a standard position and rotation angle, anchored to a fixed position. The shell was fixed on clay, which was colored in bright pink, and therefore offered color contrast to better identify shell shape. A scale paper was used to account for length references.

Image Analysis - Geometric Morphometrics

GM involves the identification of homologous features on images of each individual in the study, which when consistently placed can allow us to compare shape variability between individuals through analyzing change in the relative position of these features. Two methods were implemented. The first was a traditional landmark-based method, which involved identifying homologous points on each shell that either referred to biologically relevant features. Following the landmarks defined by Hollander et al. (2006a), Galindo et al. (2021) and Costa et al. (2020), we defined 29 landmarks on each shell. Nine of these landmarks were fixed landmarks representing anatomically relevant shell features, while the rest were sliding semi-landmarks which captured relevant curves on the shell body (Annex 3). *TPSDIG2 v3.20* (Rohlf, 2015) was used to set landmarks.

The second method approached shell-shape characterization using growth parameters, rather than measuring geometric variations between a set of fixed and sliding landmarks (Larsson et al., 2020). This approach constructs a generalized mathematical model of shell shape, based on a restricted set of landmarks, and uses these to estimate developmental parameters of shell shape, like the growth rate of width and height, as well as the fixed aperture angle. This offers a different approach for shape comparison, as what is compared are parameters that define shell growth rather than direct features of shape. It also offers a more flexible comparison that is more closely tied to a biological interpretation, as shape landmarks in traditional GM cannot be interpreted directly and thus limit our ability to infer biological processes from detected shape variation. The method was employed using a Matlab software provided by Larsson et al. (2020).

Since both methods involved the manual assignment of points to some degree, we divided the task of image labeling between the team. Prior to labeling, we collectively processed several sample images to ensure a consistent labeling protocol, and also tested for potential bias in classification by comparing results across team members classifying the same set of images (Annex 5). To further minimize the possibility of labeling bias affecting the interpretation of effects, shell images were randomly assigned to each person using a stratified randomizer across all of our factor levels. This ensured that everyone was randomly labeled at least some images for every factor level, ensuring that labeling error could be distributed across the set of images, rather than disproportionately affect one factor or another.

Statistical Analysis

Landmark coordinates from the traditional GM method needed to be transformed before statistical analysis, in order to remove variability associated with size, displacement and rotation, isolating differences associated only to shape. This was achieved through a method called Generalized Procrustes Analysis, which superimposes landmarks from all images onto each other in a standardized geometric space, and redefines shape as the degree of displacement from a median landmark position based on least squares adjustment. Alternatively, results from the growth-based landmarking procedure returned a vector of shell growth parameters for each sample, which could be immediately treated as a multivariate dataset describing the shell shape for each individual.

To visually examine the distribution of shape variables, Principal Component Analysis (PCA) was applied and colored based on our grouping factors, helping discern whether clusters of shape data could be detected based on our samples. Significant differences in clusters were then tested using a Multivariate Analysis of Variance (MANOVA), which was applied separately for the GM and the SS datasets. Results were compared for main effects and interaction effects across our factors of site, species, and tidal height. In addition to comparing shape variables via the MANOVA, we tested for the amount of dispersion in shape based on species using a multivariate homogeneity of group divergences test.

Results

Descriptive Results of the Sampling Site

The distribution and characteristics of environmental characteristics at our sampling sites are summarized in Annex 5. They demonstrate conditions that are consistent with exposed and sheltered rocky beaches at Saltö island. Algae coverage was mostly found in the submerged zone, however, the density of its coverage was higher in the exposed site relative to the sheltered site. The amount of boulders (>10 cm) relative to pebbles (< 10 cm) was higher in the exposed site. Patterns of shell density following opposing patterns in each site with respect to the tidal gradient. Overall shell density was higher in the sheltered site than the exposed site (average of 37 shells per square meter, as compared to 26 shells per square meter).

Shape Analysis

The descriptive analysis of shape clustering patterns using both GM and SS showed a clear clustering of shape types based on species, when considering the primary 2 principal components (Figure 1). In GM, PCA variables are projections of the X/Y positions of the 29 landmarks points in the homogenized shape space. By contrast, the PCA variables in SS are the 7 growth parameters inferred from each shell via fitting the growth model to landmark points.

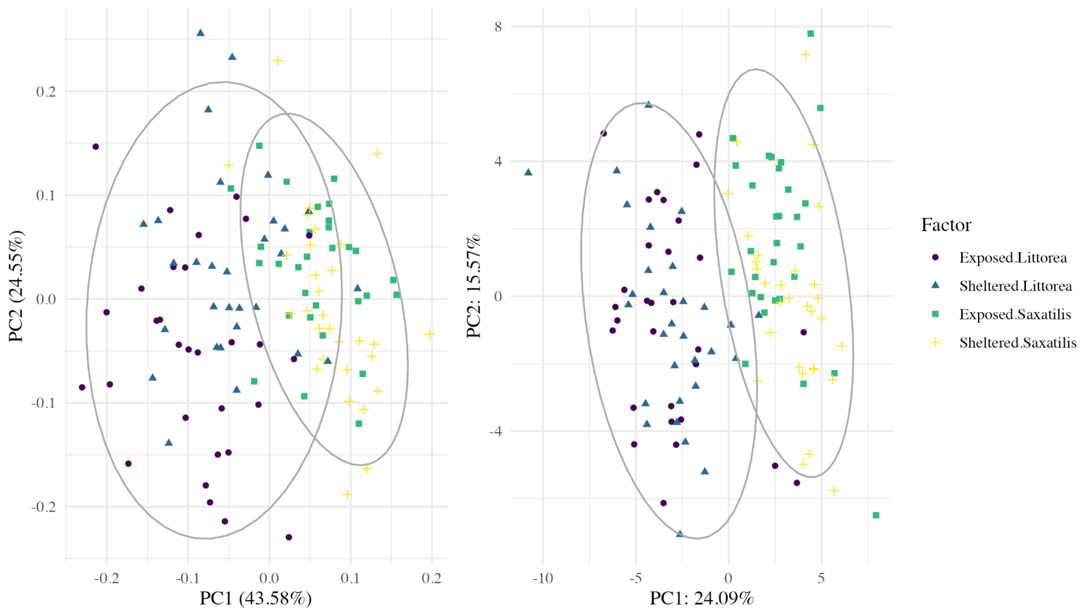

Figure 1: Principal component analysis showing potential clusters between site and species across the 2 main axes of variation. The plot on the left is based on the Shellshaper dataset, while that on the right is based on traditional GM landmarks. Ellipses are drawn around clusters of the same species, while symbols refer to species within each site type.

Patterns of differentiation across our factor levels were tested using Multivariate analyses of variance (MANOVA) (Table 1). MANOVA results from both datasets showed similar patterns, where differences in shape between sites, species and the interaction between site and species were significant. This implies that the pattern of shape differences between species was itself varying between the two sites. The MANOVA on the GM dataset utilized variation based on Procrustes distances, and thus followed a Residual Randomization Permutation Procedure (RRPP) (Collyer and Adams, 2018). It showed no significant effects of any variables involving tidal gradient. The MANOVA from the SS dataset detected a significant main effect associated with tidal height.

Table 1: Summary of results from MANOVA tests run on both the GM and the Shellshaper datasets. Significant effects (p>0.05) and their P-values are colored in red

MANOVA: Shellshaper shape-growth variables						
Term	**df**	**Pillai statistic**	**F-statistic**	**Df**	**Error Df**	**P-value**
Site	1	0.49841	17.72	6	107	**0.00000*** **
Species	1	0.73705	49.988	6	107	**0.00000*** **
Tidal Height	1	0.17185	3.701	6	107	**0.00221** **
Site x Species	1	0.33069	8.811	6	107	**0.00000*** **
Site x Tidal Height	1	0.06039	1.146	6	107	0.34091
Species x Tidal Height	1	0.10266	2.04	6	107	0.06649
Site x Species x Tidal Height	1	0.06037	1.146	6	107	0.34108
Residuals	112					
Procrustes MANOVA with Residual Randomization: GM shape variables						
Term	**df**	**Sum of Squares**	**Rsquare**	**F-statistic**	**Z-score**	**P-value**
Site	1	0.005934	0.02009	3.0029	2.4491	**0.008** **
Species	1	0.056358	0.1908	28.52	6.9775	**0.001** **
Tidal Height	1	0.001703	0.00577	0.862	-0.0513	0.512
Site x Species	1	0.005074	0.01718	2.5678	2.3918	**0.01***
Site x Tidal Height	1	0.001671	0.00566	0.8458	-0.0861	0.547
Species x Tidal Height	1	0.001616	0.00547	0.8179	-0.2129	0.572
Site x Species x Tidal Height	1	0.001696	0.00574	0.8584	-0.1129	0.551
Residuals	112					

Analyses of dispersion relative to group centroids were carried out using permutation-based ANOVA, using Euclidean distances to group centroids. Results of these tests differed between our datasets. GM shape variables showed no significant differences in group dispersion between any factors or their interactions. Effects were also non-significant for all interactions in SS data. However, the SS data did reveal a significant difference in variability between species overall (p=0.002, F=8.1194). Visual investigation showed that *L. littorea* shapes were much more variable around their group centroid as compared to *L. saxatilis* - a pattern that was not repeated in the GM dataset (Figure 2).

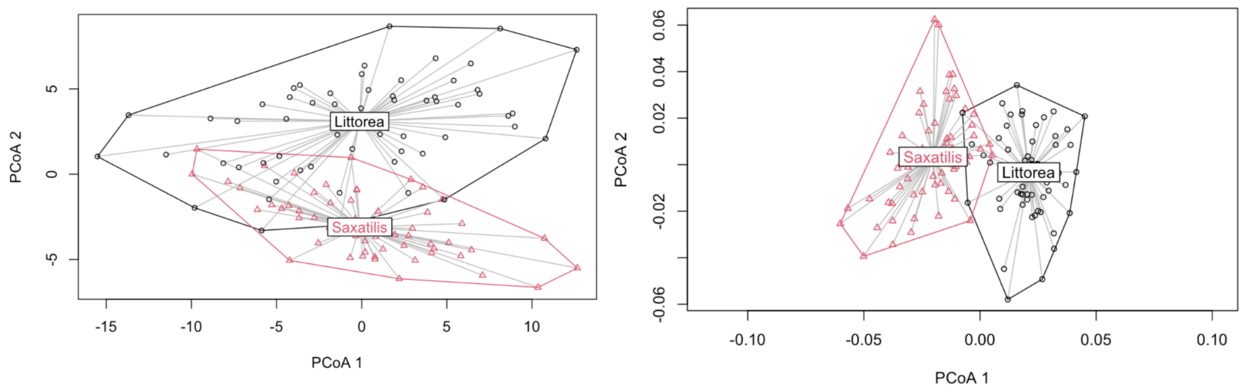

Figure 2: Visual summary of dispersion relative to the group centroid, based on Euclidean distances. The result from the left is based on the Shellshaper dataset, and showed a significantly (p=0.002)higher dispersion in L. littorea. Group dispersion between species from the GM dataset (right) was not significantly different (p=0.776).

Figures 3 and 4 provide a visual summary of the form of shape variation across site and species, as captured by each dataset. Figure 3 shows thin-plate spline deformations, which illustrate the degree of deformity that the average shell in a particular combination of factor levels showed relative to the overall mean shape. Figure 4 highlights the differences in growth parameters across site types (note that we have only included site and species, and not tidal height, to allow comparison with GM). Rather than showing specific deformations to a theoretical shape, these patterns allow us to infer shell shape by examining differences in growth-based characteristics.

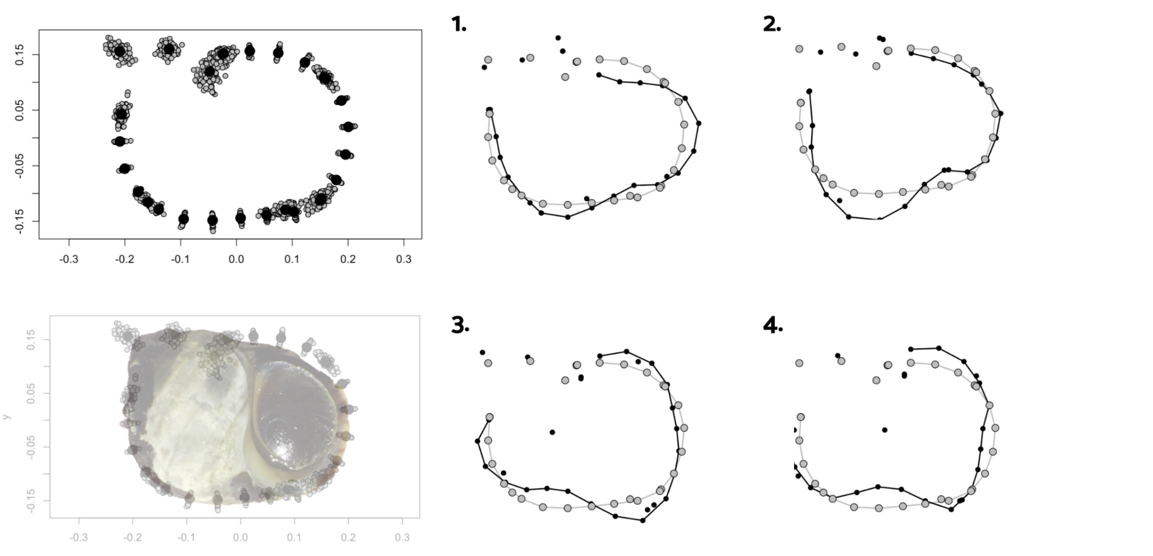

Figure 3: Summarized shape changes captured by GM. The panel on the top-left shows the location of landmarks after GPA was applied. Black points show the mean/consensus landmark position, and surrounding gray points show the variability at each landmark across the samples. The same landmarks are overlaid onto a shell photo on the

bottom-left panel for reference. Panels 1-4 show the average shape for a specific group (in black) as compared to the overall average shape (in gray). Groups are labeled as follows: 1 = Exposed, L. littorea, 2 = Sheltered, L. littorea, 3 = Exposed, L. saxatilis, 4 = Sheltered, L. saxatilis. Differences are exaggerated by a factor of 8 for ease of visualization.

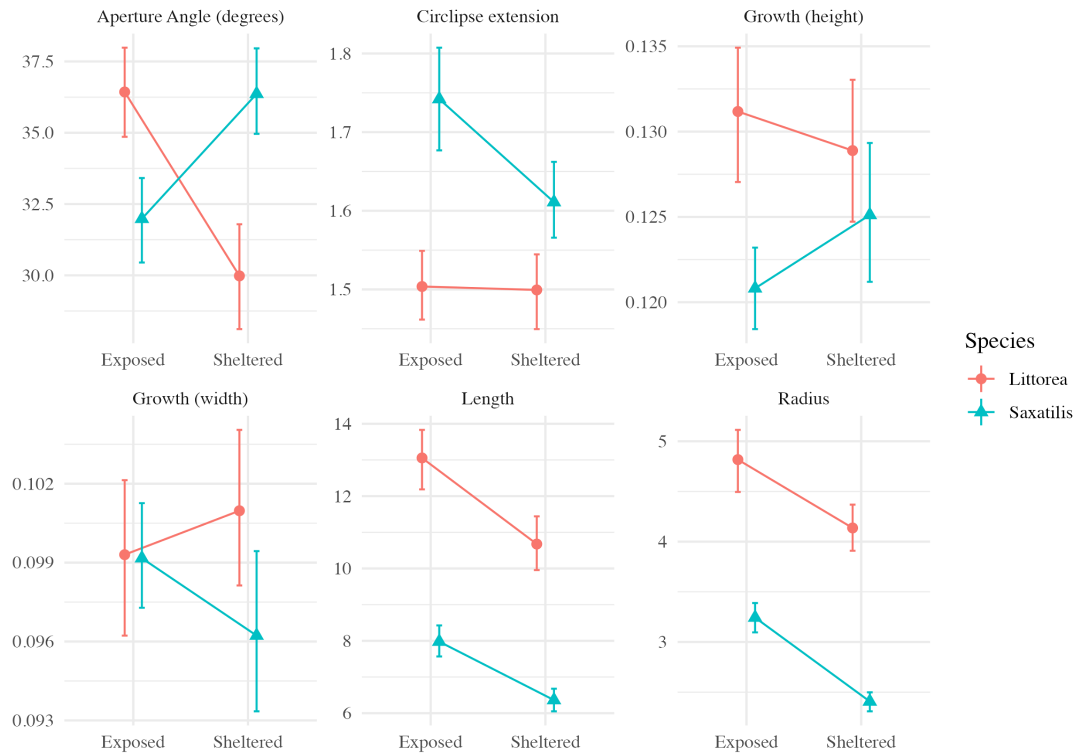

Figure 4: Summary of differences in shape-growth parameters across factor levels, detected by the SS dataset. Points represent estimates of the mean value for each parameter, and error bars show the 95% confidence interval. Lines are drawn to visualize trajectories between sites, showing a differential set of patterns for shape variation between species and across sites.

Discussion

Phenotypic variation is well characterized for species of *L. saxatilis* (Johannesson and Johannesson, 1996), exemplifying the potential and utility of significant shape variation in adapting to different micro-environments. The degree and sources of shape variation in *L. littorea* however, remain contested as studies have found contradicting support for both high degrees of shape variation across spatial scales in widely dispersing species (Behrens Yamada 1987; Parsons, 1997) - suggesting high plasticity - as well as for limited plasticity in favor of a more generalist shape strategy (Hollander et al., 2006b).

Our results offer a contribution to this debate, as we find clear patterns of shell shape variation in both *Littorina* species between the two sampling sites. Differences in the average shell shape, detected by both of our shape analysis methods, were significantly different not only between sites and species, but also in the interaction between them (Table 1). This confirms our initial hypothesis expecting differences in shell shape variation between species. It also reveals that the pattern of differences in mean shape between sites is itself variant across the two species, indicating that they may be utilizing distinct shapes for responding to the same environment.

Visual analysis of the distribution of shapes using PCA shows greater dispersion in shape forms for *L. littorea*. There is also evidence for a less distinct separability in shape types between intraspecific individuals from the same site in *L. littorea*, although this pattern is more evident in the GM result. A significant difference across the tidal gradient was detected only with the SS dataset, and pairwise comparisons showed that this difference was only significant within the exposed site ($p=0.0013$). This may be related to wave exposure forcing a more stratified and differentiated habitat throughout shore elevation levels, potentially leading to higher adaptation and therefore more distinct shape variations (Carro et al. 2019). Differences of observed environmental factors (Annex 5) could support this idea, yet these are not sufficient for an adequate statistical comparison and therefore would require further research.

Statistical tests on the dispersion relative to mean confirmed this visual finding, showing that *L. littorea* had significantly higher dispersion relative to the mean when compared across species, but not within the interaction between species and site levels. This, however, was only observed with the SS dataset. While the lack of consistency limits confidence in these results, this pattern may also be related to the differences in shape features captured by each dataset. GM measures shape patterns directly, while SS aims to model growth parameters, combinations of which can result in a wide variety of shapes. Thus, the detected variability may indeed be the result of a broader combination of growth parameters within *L. littorea*, diverse combinations of which may result in similar geometric forms. For instance, “wide” shells will likely be classified more closely by GM, whereas a “wide” shell shape may result from either a larger width growth rate or a larger circular extension parameter and a steep aperture angle, all of which are very different combinations of growth variables in SS.

The utility of comparing shape variation with two datasets is further emphasized by Figures 3 and 4, which show the observed sources of significant shape variation between the site and species interaction. These results, as well as the results from the MANOVA, are consistent with our expectation that the two species will vary in their responses to similar environmental factors. However, the results contradict our original expectation of more pronounced shell shape variation in *L. saxatilis* as compared to *L. littorea*. Figure 3 reveals that locations of shape variation between sheltered and exposed sites differ considerably for the two species. It shows that variation between exposed and sheltered sites in *L. saxatilis* primarily involves variations in aperture size. This relates to the importance of foot-size, with a much larger aperture and a slightly smaller shell being associated with exposed environments. Indeed, globular shapes, squatter, with larger apertures are considered beneficial to counter the unpredictability characterizing water flows (Cuña et al., 2011; Johannesson and Johannesson, 1996). In contrast, variations in *L. littorea* appear to be primarily focused on the shell, which grows smaller and more elongated in exposed sites as compared to sheltered sites. Shape parameters from SS confirm these patterns (Figure 4), as patterns of change between sites vary between species for each growth-parameter. A larger growth rate of spiral height in *L. littorea* in the exposed site is consistent with the elongation pattern detected by GM, and vice versa for *L. saxatilis.*

While it is challenging to measure the “magnitude” of shape difference without an experimental manipulation, our original hypothesis expected that we would find weaker or non-significant shape differences in *L. littorea* across sites, due to its increased dispersal capacity at the spatial scale of our study (Behrens Yamada 1989; Behrens Yamada 1987). Our results however contradict this hypothesis, finding that phenotypic variation is distinct for both species. We also find limited evidence that the variation in shell morphs is larger for *L. littorea*, as compared to *L. saxatilis.* We can thus consider an alternative explanation, where higher dispersion rates associated with planktonic development in *L. littorea* may necessitate a greater degree of plasticity in shell shape to effectively respond to new and diverse environments (Parsons, 1997; Hollander, 2008). This interpretation needs caution, however, as the association between plasticity and dispersal remains contested both for *L. littorea* (Hollander, 2006b), and in other marine taxa (Bourdeau, 2015). In this regard, our use of two methods may offer some insight for future work. The use of traditional GM, showed no variability in the degree of phenotypic variation between species. However, dispersion was significant when considering growth parameters, which, as discussed above, can interact in multiple ways to produce shapes that may be considered similar within the “Procrustes paradigm” (Adams et al., 2013). We thus advocate for studying plasticity in *L. littorea* from the perspective of growth parameters, where plasticity can be measured as a broader set of combinations between growth variables, increasing a specie's capacity to create a larger variety of body shapes, as opposed to comparisons relating to the shapes directly, which inevitably are limited by environmentally determined stressors.

Ultimately, the broad shape patterns observed in our study provide insight for future studies of shape, in highlighting how different morphological characteristics are involved in adaptations to exposed and sheltered environments in these two species. We find limited evidence to support claims of higher plasticity in *L. littorea* as compared to *L. saxatilis*, but our comparison of two unique datasets offers an avenue for future research to investigate these patterns further. In gathering limited evidence of environmental conditions aside from exposure, we suggest a broader observation in future studies to deepen our understanding. The diverse array of shell shapes could potentially enable these species to effectively exploit a wide range of ecological niches, facilitating their establishment in different environments. Furthermore, the observed plasticity-driven shape variation may play a crucial role in enhancing the resilience of these snail species. Adaptation of their shell morphology to variable conditions could provide a mechanism enabling them to cope with the challenges posed by fluctuating environmental factors, and thus, contribute to their ability to thrive in the dynamic and ever-changing intertidal zone.

Limitations and Challenges

The investigation into local adaptation and morphological variation in *Littorina* species has been met with challenges and limitations. We aimed to reduce the impact of varying weather conditions during sampling by closely monitoring sea levels and conducting collections within similar time frames on two consecutive days. Despite our efforts to mitigate this factor, temporal variations remain one of the limitations of our study. Even though we compared two sampling sites with different environmental conditions, having multiple sampling sites in the same conditions could have increased the robustness of our method and allowed for better statistical analysis. It is further important to note that our sampling strategy may have introduced biases, as complex allometric growth patterns could be present in our sampled individuals that we randomly collected (Starunova, 2021). Incorporating specimens of various life stages, from juveniles to adults, could have added intricacies to our analysis. Further, we address the difficulty to thoroughly characterize the different sampling sites, since although we looked at some factors (algae coverage, shell density and boulder coverage), we could not include other factors due to time constraints. We thus may have unaccounted environmental variables, including temperature and food availability on shell shape variation, which might have provided a deeper understanding.

Future Improvements

Several avenues for refining and expanding the study have been identified, to enhance the comprehensiveness and depth of our research into shell shape variation and environmental influence within *Littorina* species. These proposed improvements encompass both methodological enhancements and the incorporation of additional contextual factors, thus broadening the scope and potential implications of our research.

A larger sample size for shape comparison is worth exploring, as it may enhance the robustness and reliability of statistical analyses. Increasing the number of individuals sampled, per tidal height in particular, may provide a more representative dataset, facilitating more accurate assessments of the relationships between shell shape and environmental factors.

To enrich the ecological context of the study, additional sampling sites characterized by other substrate types, such as muddy and sandy environments, should be included to enable a more complete understanding of how shell shape variation responds to these divergent ecological conditions. Moreover, considering sex as a factor could provide a more comprehensive insight into the adaptive dynamics of shell morphology. This suggestion is supported by Johannesson (2016), who highlights the crucial role of male partner preference in reproductive dynamics. This preference leads to size-based assortative mating and reinforces reproductive barriers between ecotypes, offering valuable insights into the interplay between mate choice, sexual dimorphism and shell morphology responses. Additionally, exploring seasonal variations could unveil adaptive changes in shell shape, as they represent variations in resource availability, predation and environmental stress factors.

Finally, the integration of advanced methodologies, such as three-dimensional modeling and landmarking techniques using Light Detection and Ranging (LIDAR) scans, holds the potential to significantly enhance the precision of shell morphometric analysis by capturing details being provided by adding another dimension (Marquez, 2011).

Conclusion

Our investigation sheds some light on the complex interplay between environmental influences, phenotypic plasticity, and shell morphology variation in *Littorina* snails. Significant shape variations were observed for *L. littorea* across sites, and the pattern of variation did not follow the same patterns detected in *L. saxatilis*. In detecting some evidence of greater variability in shapes among *L. littorea,* our findings offer limited support to the connection between higher dispersal and phenotypic plasticity, although more research is needed to support this observation. The approach of using two different methods provided further incentives to investigate the limitations and advantages of these methods, as the comparison of datasets provides a broader range of information about what factors may be driving differentiation between sites and species. Taken together, they allowed us to provide a clear, descriptive account of shape variation of these two species across a common environmental contrast, offering a starting point for further research that can strengthen our understanding of evolutionary mechanisms such as plasticity and local adaptations using *Littorina* species.

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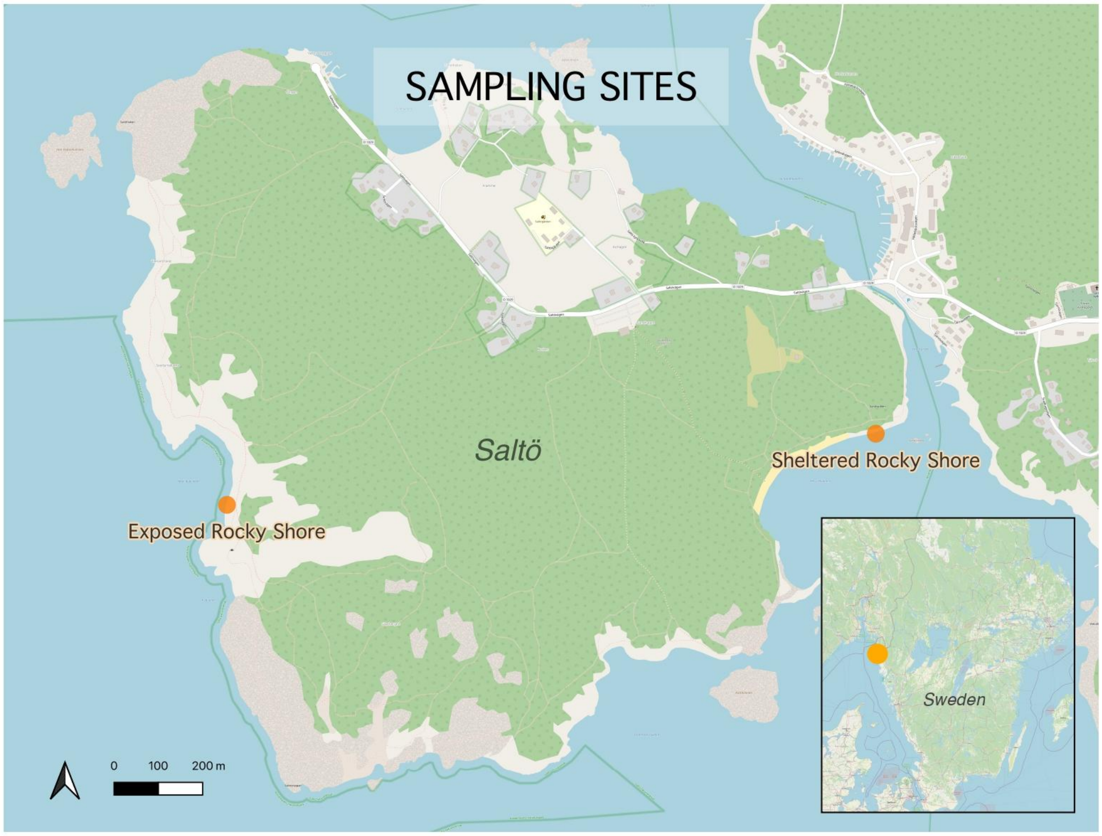
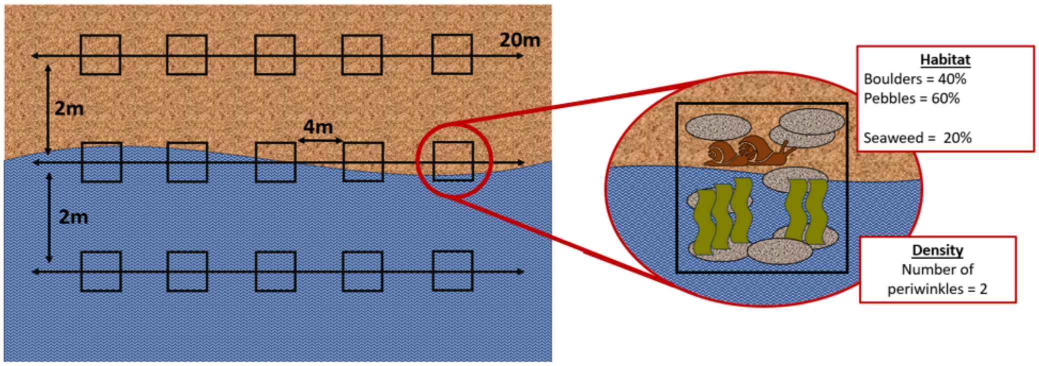
Appendix

Annex 1



Annex 1: Map showing the two sampling sites on Saltö Island near Tjärnö, Sweden: exposed rocky shore (ERS) and sheltered rocky shore (SRS).

Annex 2



+ Pick >15 individuals of each species at each height

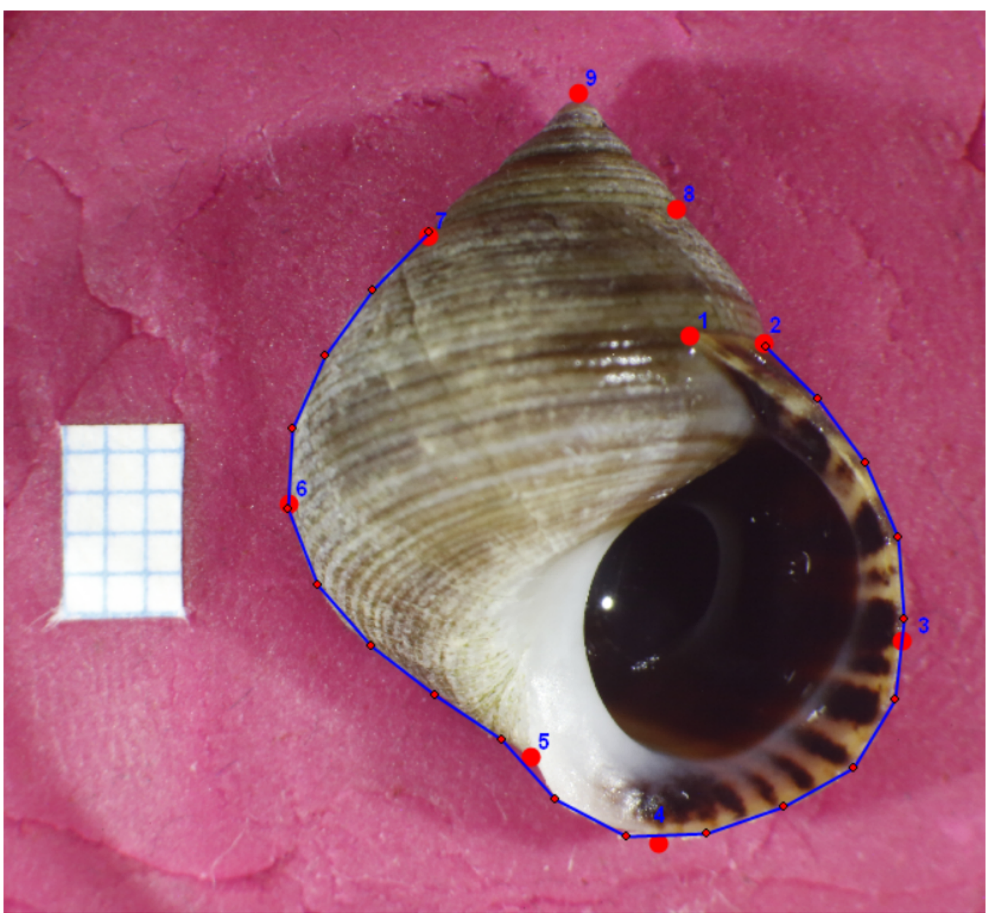
15 x 2 x 3

= 90

x 2 sites =180 total

Annex 2: At the ERS and SRS sites, three sections were determined: upper intertidal zone, intertidal zone and submerged zone. 5 quadrats (50 x 50 cm) were used to measure descriptive environmental characteristics, with 4-meter intervals along the transect. Within each site, at each tidal height, we defined a minimum sample number of 15 snails per species.

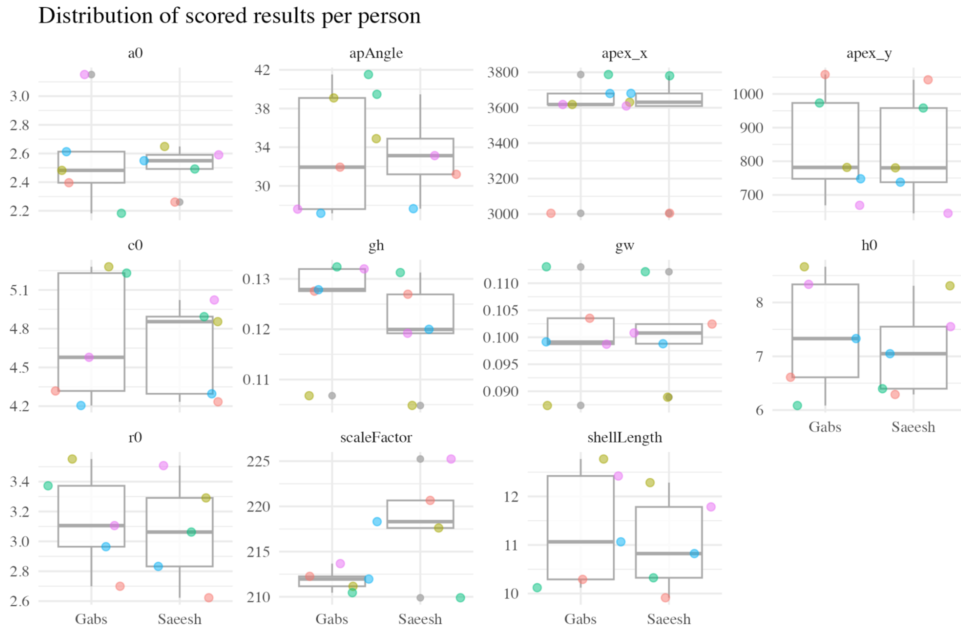
Annex 3



1 & **2:** left & right of the labia
3: most right point
4: most down part of the shell
5: intersection labia & shell
6: most left point
7 & **8**: left & right of the first curvature
9: apex
Curve: 20 sliding landmarks from 2 to 7

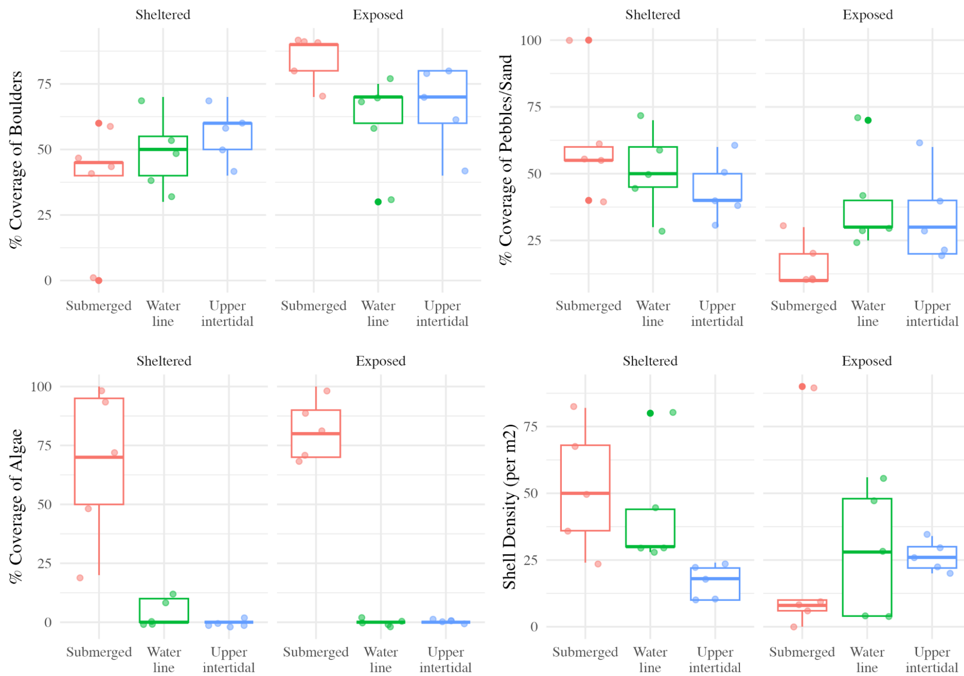
Annex 3: Sample image showing the 29 fixed and sliding landmarks used for our GM analysis. Fixed landmarks are the large red points, while sliding landmarks are the small red points that define the blue curve

Annex 4



Annex 4: Graphs summarizing the results of our bias analysis The top graph shows the distribution of shape parameters returned from the SS model from each of two members who landmarked the same set of images. It shows that overall our classifications were not markedly different from each other, except for the scale factor - which we found and made a correction for in subsequent classification.

Annex 5



Annex 5: Description of sampled environmental conditions at each site type, distributed across the tidal zones.