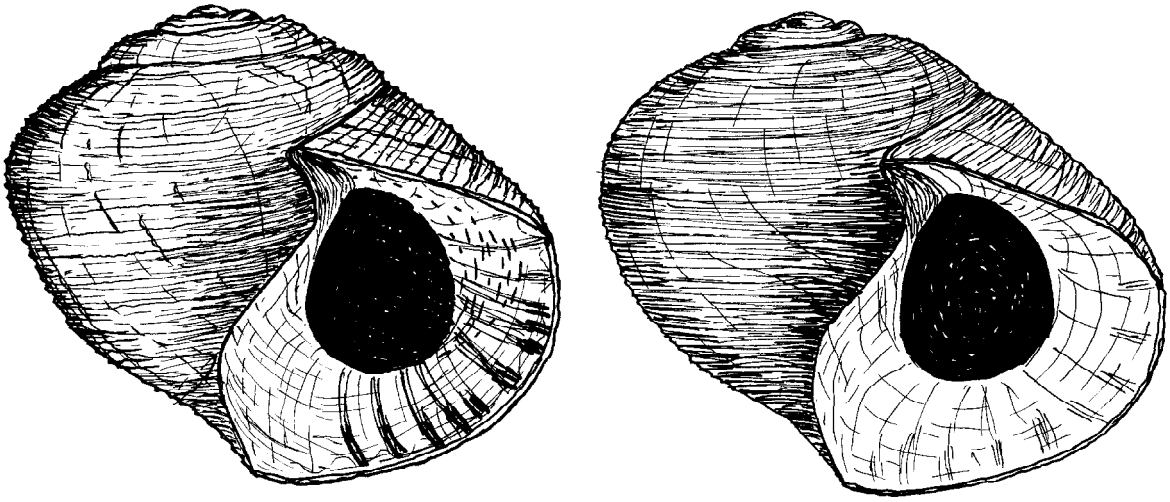
SIZE AND SHAPE POLYMORPHISM ON A MICROGEOGRAPHICAL SCALE IN THE INTERTIDAL SNAIL *LITTORINA FABALIS*



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ABSTRACT

This investigation reveals morphological variation within the snail *Littorina fabalis,* on a microgeographical scale. Differences in size and shape, between snails from sheltered vs. exposed shores, within continuous populations were observed. Snails living at the sheltered shores were smaller than those living at exposed shores (shell height mean ±SD, sheltered 7.4 ±1.4 mm, exposed 9.5 ±1.5 mm). Furthermore, snails from sheltered shores more often had micro-ridges on the periostracum (sheltered 82%, exposed 47%).The sheltered population consisted of two size groups suggesting the presence of at least two age-classes. No such grouping was obvious in the exposed population.

INTRODUCTION

In the Koster Fjord area of the Swedish west cost, populations of *Littorina fabalis* live among macroalgae in the intertidal zone of rocky shores. The eggs develop to snails without any pelagic larval stage. I investigated the microgeographical differences in morphology between populations of snails from exposed and sheltered shores. Pairs of contrasting habitats were chosen from locations with continuous distributions of snails.

There is a difference in the allele frequencies of arginine kinase between populations of the marine snail *Littorina fabalis* living on sheltered and exposed shores (Tatarenkov & Johannesson 1994). Reimchen (1981) described differences between a "large, dark and irregular" and a "dwarf, light and regular" morph of Littorina fabalis. The irregular and regular refers to the micro-ridges on the periostracum. Reimchens study lacks however, statistic evaluation of the results and is thus difficult to interpret. Tatarenkov & Johannesson (1998) conclude that neither assortative mating nor habitat related mortality is present at this locality. The study was, however, unreplicated in time and partly unreplicated in space, the suggestions may be rejected by further studies. There is a need to extend these investigations to include several localities and an objective method of quantifying the ridges to assess morphological variation of this species and of the evolutionary significance of any such variation.

I used nine measurements on the shell as well as total weight, shell colour, and presence or absence of micro-ridges on the shell to assess phenotypic variation. A principal component analysis (PCA) in combination with an analysis of variance (ANOVA) were used to test the hypothesis if snails living in different habitats differed in size and shape.

The purpose of my studie was to analyse size, shape and micro-ridge morphology to test the hypothesis of intraspecific variation which may support the suggestion of two evolutionary lines (Tatarenkov & Johannesson, 1998).

MATERIALS AND METHODS

This investigation of *Littorina fabalis* was performed in the Koster Fjord area (58°52´N, 11°9´E) of the Swedish west coast. The sampling of snails took place in late November. Two islands (Långholmen and Lökholmen) were investigated (fig. 1). On each island two shores were chosen and on each shore two sites. Pairs of one sheltered and one exposed site were chosen. The

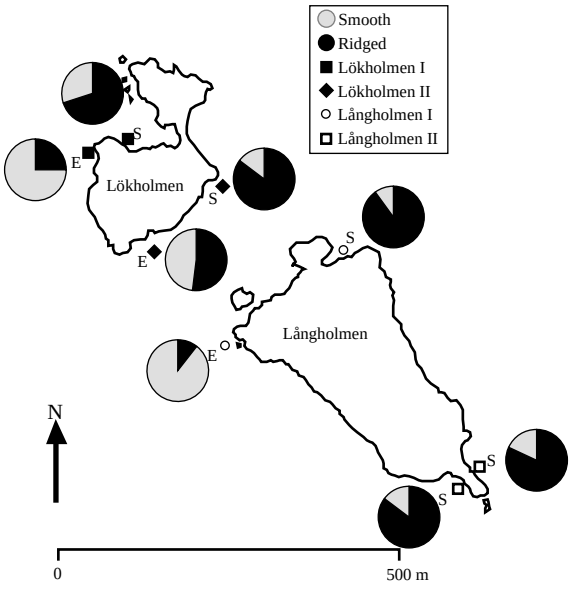


fig. 1
There are more of the smooth morph at the exposed habitats, while micro-ridges are more common at sheltered habitats.
Habitats: E = exposed, S = sheltered.

shores and sites were selected with regard to being part of the same continous population of *L. fabalis* along a gradient from sites sheltered from wave action to those sites considered more exposed. *Ascophyllum nodosum* is considered an indicator of wave-protected sites (Lewis 1972). Accordingly, if *A. nodosum* was present more than rarely, the sites were classified as sheltered, otherwise as exposed. I collected approximately 10 litres of *Fucus vesiculosus, F. serratus* and *A. nodosum* at every site, together with snails hand picked on rocks and stones. The plants were rinsed in freshwater so that all snails loosened their grip and fell off the plants. To avoid the sibling spieces *Littorina obtusata* being mixed with *L. fabalis* I excluded those having black soft parts as these are likely to be *L. obtusata* in this area (Johannesson pers. comm.).

Size and shape

Shell shape and size were assessed using nine measurements (aperture height, aperture width, lip height, shell height, shell thickness, shell width, the width of each of the three whorls) in addition to total weight. Accuracy for all length measurements was ± 0.50 mm, for shell thickness ± 0.001 mm, and for weight ± 0.001 g.

A principal component analysis (PCA) were used to transform the original dataset to a number of principal components. Principal components can be used to explain the variance in decreasing order by projecting the multi-dimensional data set on two-dimensional surface in a way which extracts most of the variance present (Se Manly, 1986 for description of method). PCA tries to present series of principal components as independent data. The first principal component which explains the greatest variance mainly reflects variation in size, whereas the second and third reflect the shape (Reyment et al. 1984). To separate size from shape the original data set was log-transformed and a correlation matrix was used (Reyment et al. 1984).

I used a balanced data set with measurements from 28 snails from each exposure. I ran three sets of ANOVA using PC1, PC2 and PC3 as dependent variables, to test if there were any differences in size and shape between the two sites (exposed or sheltered). A Cochrans test revealed homogenous variances after log-transformation. The three principal components reflect the data in different aspects. The analysis of components PC1, PC2 and PC3 do not include repeated tests of exactly the same hypothesis and therefore Bonferroni adjustments were not relevant.

Micro-ridges and colour

The snails were assigned to two colour groups, one dark and one light. The dark group included black, brown and banded with a dark primary colour. The light group contained the snails with a yellowish colour.

The shell of some snails has small micro-ridges on the periostracum . The original aim was to separate those snails with regular micro-ridges from those with a more or less random sculpturing (Reimchen 1981). A problem that I encountered was that the shell was coated with epiphytes which made it difficult to assess the regularity of the structure in an objective way. Therefor I reduced the observations to presence or absence of micro-ridges.

The colour variation and the presence or absence of micro-ridges were tested separately with chi-square analyses. In the Chi-square analyses I used all the snails from each type of habitat pooled (n=170 for exposed sites, n=224 for sheltered sites).

RESULTS

Size and shape difference between snails from opposing habitats

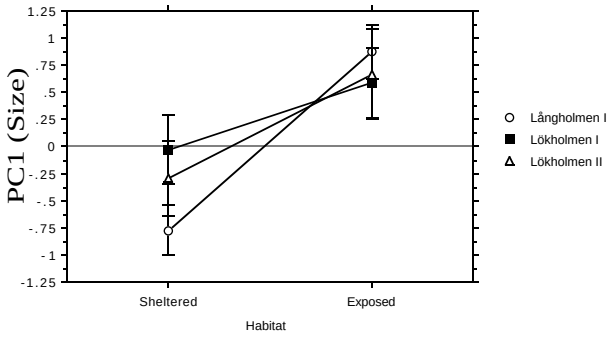
One of the sites at Långholmen II turned out to be densely populated by stands of *Ascophyllum nodosum* and was thus reclassified as sheltered (fig. 1). The data set of the primary ANOVA was no longer balanced (two shores at Lökholmen but only one at Långholmen). The design was of a character which didn´t allow calculation of the error term for the site. There was however, no difference between islands, (P = 0.25) and therefore I could remove island as a factor of variance, and repeat the test including the factor site (tab. 1).

The analys of PC1 which explains 74.4 % of the variance, shows that snails living at the sheltered sites were smaller than those living at

tab. 1

ANOVA with the aposteriori adjustment, because of misjudgement of habitat type at one location.

Source of variation	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Shore	2	1.51	0.76	1.10	0.336	Residual
Site	1	48.37	48.37	12.46	0.072	Shore * Site
Shore * Site	2	7.76	3.88	5.65	0.004	Residual
Residual	162	111.29	0.69			
Dependent: PC 1						



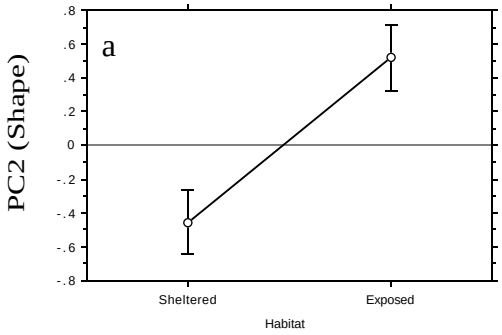


fig. 2

The sheltered population is smaller than the exposed (shell height mean ±SD, sheltered 7.4 ±1.4 mm, exposed 9.5 ±1.5 mm). The interaction indicates a difference between shores due to the interaction lines not being parallel, which is further discussed in the text.

tab. 2

The t-test revealed significant differences between the habitat types exposed and sheltered for all three locations.

Shore	F-Value	P-Value
Lökholmen I	59.143	0.0001
Lökholmen II	66.719	0.0001
Långholmen I	98.683	0.0001

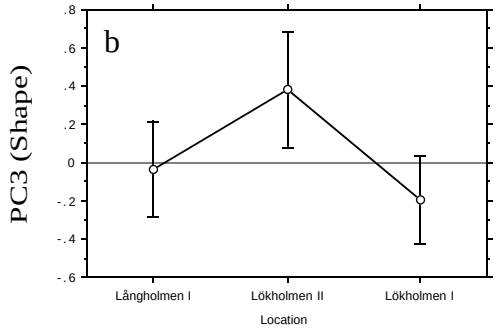


fig. 3

Principal component two and three explains the shape of the snail. PC 2 (a) differs between habitats, but PC 3 (b) differs between shores.

tab. 3

The shape of the snails differs between the habitats (PC 2, P = 0.0108).

Source of variation	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Shore	2	0.917	0.458	0.587	0.5573	Residual
Site	1	40.027	40.027	91.271	0.0108	Shore * Site
Shore * Site	2	0.877	0.439	0.561	0.5715	Residual
Residual	162	126.553	0.781			
Dependent: PC2						

tab. 4

According to PC3 there is a shape difference between shores, but not within shores. No difference between habitat.

Source of variation	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Shore	2	9.929	4.964	5.058	0.0074	Residual
Site	1	0.003	0.003	0.009	0.9319	Shore * Site
Shore * Site	2	0.603	0.302	0.307	0.7359	Residual
Residual	162	159.014	0.982			
Dependent: PC3						

tab. 5
PC1 explains 74%, PC2 9.7% and PC3 6.8% of the variance. All component loadings for PC1 is of the same magnitude and are all positive. PC1 were considered the best estimate for size.

LATENTROOTS (EIGENVALUES)	PC1	PC2	PC3
	7.44	0.97	0.68

COMPONENT LOADINGS	PC1	PC2	PC3
Aperture height	0.98	0.08	0.02
Aperture width	0.97	0.10	-0.01
Lip height	0.94	0.05	0.01
Shell height	0.92	0.02	0.10
Shell thickness	0.91	0.11	-0.00
Shell width	0.87	0.14	0.15
Whorl 1	0.92	0.07	0.02
Whorl 2	0.33	-0.87	0.35
Whorl 3	0.56	-0.39	-0.72
Weight	0.98	-0.01	0.03

exposed sites (shell height mean ±SD, sheltered 7.4 ±1.4 mm, exposed 9.5 ±1.5 mm) (fig. 2). The interaction indicates that there is some difference between shores. The two habitats are separated in size from each other, but on different scales for the three shores. There is no difference between the shores (P= 0.34). To see if the two habitats were separated I ran a t-test on every shore separately (tab. 2). The sheltered and exposed habitats were separated from each other according to size (P= 0.0001) at all three shores.

PC2 explains 9.7% of the variance and reflects the shape of the snails. There is a difference between the two habitats, sheltered and exposed (p=0.01) (fig. 3a, tab. 3). PC3 which explains 6.8%, shows significant differences between the shores (p=0.007), but no differences between sheltered and exposed habitats (p=0.93), (fig. 3b, tab. 4).

To isolate the characters that provide the difference between snails of different habitats, the component loadings were used (tab. 5). The sheltered population has the most negative values, which according to component loading is correlated with whorl 2 and 3 width. The exposed population has positive values which correlates

tab. 6
Micro-ridges are more common at sheltered habitats (82%) and smooth at exposed (53%). There were no difference in colour between habitats (P = 0.50). Chi-square test for heterogeneity.

	Habitat type Exposed	Sheltered	χ^2	P	df
Ridged	80	184	53.810	<0.001	1
Smooth	90	40			
Light	125	157	0.562	0.500	1
Dark	45	67			

the most with shell thickness and width, aperture height and width.

Micro-ridges and colour

Snails from the sheltered sites, more often had micro-ridges on the periostracum (sheltered 82%, exposed 47%) all samples pooled, χ^2 =53.81, df=1, P<0.001, n=394 (fig. 1, tab. 6). Colour frequencies however, did not differ between snails of the two habitats, χ^2=0.56, df=1, P=0.50, n=394 (tab. 6).

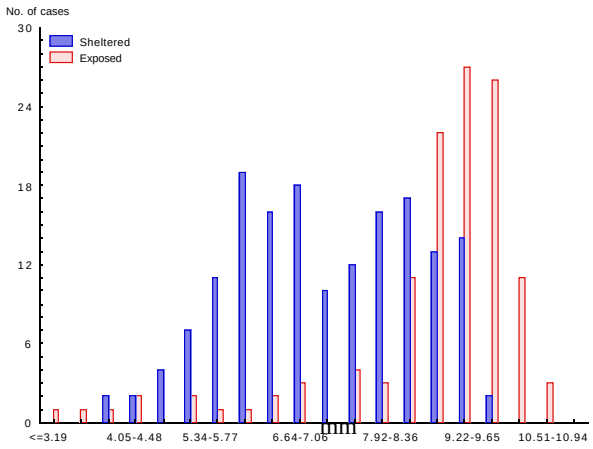


fig. 4
The snails in the exposed populations are bigger than in the sheltered. The sheltered population is subdivided into two size classes. To better visualize the size difference I standardized shell size from a linear regression with shell height as a function of PC1 (shell height in mm = 7.93 + 1.72 · PC1, r = 0.97).

Population structure
The snails at the exposed shores are bigger than at the sheltered ones. The exposed population has a peak approximately near 10 mm, but the sheltered has two peaks, one near 7 mm, and one near 9 mm (fig. 4). Thus the sheltered population may be subdivided in two size classes which presumably corresponds to age classes.

DISCUSSION

Species of benthic invertebrates with planktotrophic larvae have the potential to spread over large distances during weeks to months, and for those with lecitotrophic larvae hours to days. In these species we expect a high level of genetic similarity over distances of tens or thousands of km. With no, or a restricted larval dispersal, a higher degree of diversity between populations separated on different islands, and in semi isolated habitats can be expected. In the marine snail *Littorina saxatilis* there is a size as well as a shape difference within a shore in a depth gradient. Banding as well as ridge depth is genetically inherited (Johannesson et al. 1993). This could be very interesting to test for *L. fabalis*. A crossbreading test for *L. fabalis* is lacking and will be needed in order to draw some conclusions about the heritability, and the influence of habitat on the characters found.

In snails with restricted dispersal the random genetic drift plays a great role because of the restricted gene pool, so stochastisity can give the observed diversity (Crisp 1974). A transplantation experiment can give us an answer to whether there is a habitat related mortality or not. Even though Tatarenkov & Johannesson (1998) did not find this there is reason to repeat the experiment, and include replicate locations. Tatarenkov & Johannesson (1998) did the experiment at one location during a restricted time period. Mortality can strike in every part of the life cykel and during any time of the year, so even replicate times should be included in a repeated experiment.

L. fabalis lacks the pelagic larval stage, the eggs develop directly to snails from eggmasses on the algae. The gene flow between islands is restricted due to lack of dispersal (Tatarenkov & Johannesson 1994), so a variation between islands could be expected (Crisp 1974). The eggs and snails can be rafting on seaweed from one island to another even though the chances for this can be considered limited. The shores are separated by sandy or rocky habitats which could be considered as a reproductive barrier. On account of the restricted gene flow, random genetic drift has a greater influence on divergence in neutral loci. Because of this we could expect more similarity within shores than between.

Size and shape
In PC1 and PC2 regardless of shore I found a difference between the snails living at the sheltered or exposed habitats. In the experiment carried out by Tatarenkov & Johannesson (1998), the snails living at the exposed habitat grow faster and matured at a larger size than those living at the sheltered habitat, this experiment where however unreplicated in time and on a restricted area (one shore).

PC3 which explains 6.8% of the variance, shows a difference between shores. The small difference in the remaining characters not related to habitat may be a result of isolation by distance. The difference between the shores, and even more between islands in PC3 would thus be a result of stochastic processes.

Why is size an advantage in an exposed habitat? One answer could be that predation from crabs is a bigger problem at exposed habitats. If so, snails having the capacity to grow fast have an advantage, as larger snails are less vulnerable to crab attacks (Johannesson 1996). Kitching, Muntch, & Ebling (1966) and Elner & Raffaelli (1980) suggest that snails from exposed shores has a large foot to prevent dislodgement, and snails living under high risk of predation from shore crabs has small apertures and thick shell to avoid crushing.

At the exposed habitat the snails are more associated to rocks and stones, whereas at the sheltered the snails are sitting on the plants. At the exposed habitat the plants are moved by waves and make it difficult for the snails to live on them, at the sheltered habitat there is no such problem. Presumably, it is easier for the crabs to eat snails sitting on the rocks than on the plants, and therefore selection favours large snails in the

exposed habitat. But why are there no large snails at the sheltered habitat?

So far there is no evidence of assortative mating or time separation for the reproduction (Tatarenkov & Johannesson 1998). So why is there a reproductive barrier and what is it that maintains this? To point out the cause of this difference between and within a location a transplantation experiment is necessary.

Ridges and colours

I reduced the observations to presence or absence of micro-ridges because, I doubt that it is possible to be objective in the judgement between regular and irregular, and because of the subjectivity in the judgement it is not testable. The exact structure can be difficult to see if the ridges are small. This needs more investigation and a more objective method. The difference in micro-ridges between snails of different habitats is not so clear at the Swedish coast as Reimchen found in Wales (Reimchen 1981). A minority of the snails at the exposed habitat have micro-ridges, and a minority of the snails living at the sheltered habitats lacks micro-ridges (fig. 2). Reimchen (1981), however, found that Welsh snails on sheltered habitats had more regular micro-ridges at the periostracum, whereas, snails from exposed habitats had an irregular pattern of the micro-ridges. In Wales the exposed shells are mostly dark whereas the sheltered shells are almost always light coloured. Reimchen (1981) describe the exposed habitat as ”rocky shore” and the sheltered habitat as ”soft bottom”. I believe that the habitats are equivalent in Wales and Sweden. In Sweden the exposed habitats have rocks and boulders covered with macroalgae and the bottom of the sheltered habitat is covered with sand. The macroalgae is attached to bigger stones on the bottom. This means that the snails are more or less restricted to the macroalgae at the sheltered habitat. At the exposed habitat the snails are restricted to the rocks because of the high risk of dislodgement from the macroalgea by the waves. The micro-ridges hypothesis seems an interesting and possible way of finding the character diagnostic for the different habitats. The problem is to find a method to quantify it, so it becomes testable.

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