Local adaptation of a marine snail to divergent selection pressures

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ABSTRACT

Speciation and evolutionary divergence in close geographical context is the focus of numerous scientific debates. How can species diverge in the face of gene flow that homogenizes allelic composition differences and in this way oppose divergence of phenotypes? In this project, I used the rough periwinkle Littorina saxatilis, as a model species to test how phenotypic divergence evolves under strong differential selection applied over short distances. In western Sweden, the shore alternates from wave exposed cliffs to sheltered boulder beaches. Snails in these two micro-habitats exhibit different morphs; wave exposed environments selects for individuals of small size and thin shell that offers minimal exposure to hydrodynamic forces. In contrast, boulder beaches, that hosts much higher densities of crabs, favours and selects for individuals that grows large, with a thick shell and a cautious behaviour. In contact zones of these two micro-environments hybrid populations are present that contain snails of intermediate phenotypes.

In this study, two projects have been realised in parallel. Firstly, I exploited data of a large sampling of snails along a contact zone and adjacent micro-environments. 625 pictures were used to analyse the variation of foot area and aperture area across the contact zone. The analysis showed a clear independent effect of the environment on the foot area of the sea snails. Secondly, I tested the response of adult snails that had been exercise in a miniature flow tank to an increasing physical stress of strong current exposure using a flush-through flume device. I did not find a significant difference in the attachment strength of snails from the treated group and the control group, however I noticed a possible tendency of a change in treated snails behaviour, as they seemed to have a quicker activity to get a grip of the substratum after threat (p-value 0.079). This research opens new perspectives and methods to understand how selection acts on such a complex trait as snail attachment strength.
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I. INTRODUCTION

Emergence of positively selected variation and its propagation in wild populations is responsible for diversification of species, allowing life forms to colonize diverse ecological niches [1]. When evolutionary divergence happens in a scenario of geographical proximity (parapatry or sympatry), species diverge despite a gene flow that homogenizes the allelic composition of the divergent populations and so opposes the formation of reproductive isolation [2].

The rough periwinkle *Littorina saxatilis* is an ideal model for studies of evolutionary divergence without geographical isolation [3,4]. This species is widely distributed across the North-Atlantic coast and has diversified in several ecotypes present across most of the species area of distribution [5]. Most intertidal environments are divided into a mosaic of micro-habitats with different physical and biotic characteristics, for example, more or less exposed wave action, cliff or boulder substrata. *Littorina saxatilis* populations appear to have repeatedly and independently evolved into ecotypes that are locally adapted to specific niches [6]. For example, on the west coast of Sweden it is easy to separate two different ecotypes of snails, a crab ecotype and a wave ecotype, and these were the focus of the present study. The “exposed” microhabitat is a portion of the shore that is affected by strong hydrodynamics forces. The bare rock is smooth and often with a steep slope, it offers a surface that can be violently swept by waves. Periwinkles that colonises this zone are of the ecotype E (for "Exposed"), also called “wave” ecotype. This ecotype is distinguishable by its small size (approximately 5 mm length for the adult) and its very thin shell, that is easily crushed by the pressures between fingers. The size reduction and shell thickness reduction allows to minimize the surface offered to waves and reduce the drag force exerted on the snail, thereby lowering the risk of sweeping away from the substratum. The wave ecotype has a large aperture in its shell in relation to the whole small size of the snail, likely to allow for a better grip in this highly wave exposed habitat [7, 8].

-The “sheltered” microhabitat is made of boulder that breaks wave strength. This habitat slope is gentle and wave strength is reduced by the boulders. Less wave energy allows for increased development of macroalgae like *Fucus*. Predation on the periwinkle exerted by high densities of crabs (mainly the intertidal green crab *Carcinus maenas*) becomes one of the major selection forces in this environment. Here adult snails have an increased growth rate and a larger adult size, shell is thickened and requires hammer strength to break. Aperture is proportionally smaller than the one of the exposed ecotype. It has been documented that an increase in size, shell thickness and reduced aperture are advantageous to resist crab attacks [8].

Besides the morphological differences announced above, the behaviour of the “crab” and “wave” ecotypes also differ. The snails adapted to crab predation have a “scared” behaviour, and they retract in the shell under threat and emerge out after a prolonged period of time. Whereas wave adapted snails are significantly bolder, emerging out of the shell much quicker after disturbance [9].

The fitness advantage provided by specific ecotype alleles to their microenvironment has been tested in previous studies showing that individuals from the sheltered habitat placed in an exposed environment suffer reduced growth rate and increased mortality [10]. The contact zone of the divergent ecotypes is populated by hybrids with mixed allelic compositions and intermediate phenotypes. The existence of such a zone indicates an ongoing gene flow between the diverging populations, however, gene flow is restricted over the hybrid zone [11]. *Littorina saxatilis* has limited dispersal abilities. Indeed, this littorinid is a direct developer meaning it's early stages of development do not include a pelagic larval stage. This restrains greatly the dispersion of the offspring to the distance that the individuals crawl in a lifetime. Weak dispersal abilities tend promote enhanced genetic local adaptation [12] and *Littorina saxatilis* model supports this theory. Population divergence under the selection made by wave action and crab predation is strongly reinforced by the size assortative mating system of *Littorina saxatilis* [13].
The phenotypic variation across the contact zone is a result of the differential selection pressures affecting the microhabitats. Aperture area, for example, is under opposing selection pressures. Crab predation pushes the local populations of *Littorina saxatilis* to evolve towards a narrow aperture that provides increased defence against some of the crabs attacking techniques. Indeed, crabs have distinct strategies to attack their prey, depending on the species of crab and its type of chelae, and a species like *Carcinus maenas* have at least 5 different strategies to attack and eat periwinkles [7]. Aperture area plays an important role in lowering the risks against the crab pulling the snail tissue out of its shell. Foot area is somewhat related to aperture area but is expected to play an important role in the adhesion force to the substratum, as suggested by the morphology of wave adapted *L. saxatilis* and *Nucella sp.* [14]. Both aperture and foot size of wave ecotype snails are larger than in the crab ecotype, in relation to their respective size [12], yet it is not known if the area of the foot is entirely explained by the aperture area of the snail, and how strongly correlated these two traits are.

The phenotype of an organism is the result of its genotype and the environmental conditions it faced. Geographically close but contrasting micro-habitats generate steep selection gradients (e.g. in crab density and wave energy) and it seems reasonable that the species could have developed adaptive plasticity to further strengthen the fitness of the snails. In an experiment, Hollander & Al. exposed juvenile periwinkles to crab predation cues or to exposure from turbid water, and they found that the genotype does not entirely fix the snail to one phenotype but allows the snails to develop towards better adapted phenotypes in a specific environment [15]. The plasticity revealed in this study made up for a new question, to understand if *L. saxatilis* is plastic enough to cope with temporal environmental variation. Quick acclimation to environmental change has been reported under temperature variation in periwinkles [16], however it is unknown whether acclimation can happen regarding strong habitat-specific selective forces such as the energy of hydrodynamic forces.

**2. OBJECTIVES**

This project includes two different topics:
1) Investigating the link between aperture area and foot area across the contact zone and understanding if aperture in the shell is a variable that entirely explains the foot area variation of the "crab" and "wave" ecotypes.
2) Testing whether adult snails exercised in a water flow show different acclimation to the current exposures than snails stored in still water.

These questions were treated in two different approaches. 1) I exploited the big amount of morphometric data extracted from a sampling across a complete contact zone between diverging ecotypes. 2) I set up a pilot study to investigate for the possible development of the grip strength of sheltered adult ecotype trained daily to resist a water flow, while developing with the support of several colleagues a new method to estimate the grip strength in a flume.
3. MATERIAL AND METHODS

Project 1: Linking foot area, aperture area and environment

A- Sampling (2013) and morphometry

During summer 2013, R.K. Butlin, A. Westram, K. Johannesson and colleagues performed a large sampling over five contact zones of the “wave” and “crab” ecotypes. Data from one of the sampled zones, approximately 360 m long south shore of the island of Ramsö (58.824385, 11.062199) was used in the present study. 625 individuals were collected on this intertidal zone. The precise coordinates of each snail were recorded with a “total station”. A Bézier curve was fitted to all the snail positions. Frequent point estimate of environmental parameters allowed for a description of the proximal environment in a radius of 4 metres around each snail. Several density gradients were established assigning a value ranging from -1 to 1 indicated the composition of the proximal environment around each snail based on presence or absence of boulders, Fucus, and barnacles.

For each snail, pictures showing the aperture of the shell were chosen for the present study. These pictures were photographed under magnification by putting the aperture plan of a snail in the horizontal plan parallel to the ground and photographing all 625 individuals.

Pictures of the extended foot have also been taken by putting the snail on a wet glass surface and taking photographs of the foot from underneath. I used the maximum foot area measured (among several replicate measurements) for each individual to have a closer estimation of the real value of the maximum spread of the extended foot area. I also measured width of the shell, length, area of the shell and area of the outer aperture using the software Image J, relying on a standard scale placed on each pictures. Shell area was measured by following the 2D shape of the shell on the pictures. Outer aperture was measured following the line that separated the visible part of the shell in contact with the foot with the outer part.

B- Statistical analyses of foot and aperture areas

To investigate whether aperture area was a good predictor of the foot area, I analysed the morphometric data collected with Image J. Juvenile and parasitized snails were not included in the analysis. A simple foot index based on the ratio of Foot area/Shell area was calculated and plotted along the Bézier path (that represents the average distribution of snails along the shore) to display the variation in relative foot area.

I used a linear model to analyse the variation in foot area for given aperture values, and achieved homogeneous variances by log transformed all area estimates. Fisher tests on log transformed foot data and aperture in both rocky and boulder environments did not evidence heteroscedacity. The residuals appeared to be normally distributed around the regression (Shapiro test p value of 0.65), and did not reject assumptions of linearity. All statistics were run using the statistical software R.

Project 2: Exposing adult sheltered snails to different current forces

A - Sampling and treatment

One hundred snails were sampled along 30 m on the sheltered shore of Saltö island (58.875063, 11.117008) and kept in a fridge overnight. Snail sizes were measured and 36 were selected for their homogeneous size of 1 cm ± 1 mm.
The goal of the treatment was to test if under daily exposure of current, the snails would acclimate and become more resistant to wave exposure. The treatment was realized by putting snails in a cylindrically shaped container and creating a current by the use of a magnetic stirrer. The device did not allow the snails to occupy spots where the current did not apply horizontal pressure on the shell, applying the drag force on the snails from a horizontal vector. The 36 individuals were separated in 3 distinct groups of 12 each: a "Strong current" group, a "Moderate current" group, and a “No current” or control group. The current exposure period lasted 1h30 per day for each treated group and for a total duration of 20 days. To avoid snails regrouping and deflecting too much the current, the boxes contained 6 snails (half a group) per training set. Outside the treatment and measuring periods, the snails were kept in separate aquaria in the laboratory. Water was renewed every day, food was distributed in excess between the groups and consisted of algal biofilms and green algae (*Ulva* sp).

**B - Testing behaviour**

After the treatment period, I also tested behavioural differences between groups. The snails were placed upside down in water filled Petri dishes, this manipulation scared the snails that retracts in the shell for an amount of time before coming out, then scanning the environment and attempting to reverse the shell to get grip of the substratum. The number of active individuals was counted at given times (30, 60, 120, 240, 420, 600 and 900 sec) after the beginning of the experiment. I considered an individual as active if it scanned the environment by moving the antennas out of the shell and/or to actively moving the foot to reverse. The results of this experiment were treated by a generalized linear model following a binomial distribution to look into the effect of the variables on the probability of a snail to turn active.

**C - Testing adhesion**

The adhesion capabilities of the individuals after the training period was estimated by using a high-speed flume that attempts to dislodge snails by delivering a strong water flow over a short period of time (up to 20 litres a second in a 10 cm diameter pipe). The intensity of the water flow can be controlled by increasing or reducing the degree of aperture of the valve placed in the end of the tube (see illustration 1).

Periwinkles were tagged individually and placed in the flume. To discriminate the abilities of snails to keep hold of the substratum, a routine of flushes was performed 3 times for every group. Each set consists of 3 gentle flushes to ensure periwinkles held grip and could anticipate the current direction. These 3 gentle flushes were followed by 5 flushes of a given flow strength (angle 75° of valve) (1 minute interval between flushes). If snails had succeeded holding grip, 5 more flushes were performed with maximum current strength (90°). At each flush, the individuals that failed to maintain the grip were recorded. Finally, every individual was assigned a score based on the sum of flows successfully held (ranges from 0 to 30).

The data provided by this method was treated with a Kruskal-Wallis test using R.
4. RESULTS

4.1 Project 1: Linking foot, aperture area and environment

Figure A: «Foot area/Shell area» ratio plotted along the Bézier path (that represents the distribution of snails along the shore), to display the variation in relative foot area. Individuals are coloured according to a color palette that graduates in relation to their proximal environment characteristics (in a radius of 4 meters). The colours changes from blue (Rock estimator = 1) for the exposed habitat, to red for the sheltered, boulder habitat. (Rock estimator = -1). The ratio seem to vary accordingly to the substrata were snails were sampled.

Figure B: Plot of foot area against aperture area. As for Figure A it distinguishes habitat by colour.

Snails collected in the boulder environment seem to have a smaller foot for a given aperture area.
Figure C:
Plot of the log transformation of the values of the foot area and aperture area.

The distribution of log(Foot area) against log(aperture area) seems linear and the variance constant, allowing to use an analysis of variance on a linear model to test the effect of the rock estimator on the foot area.

Table 1: Results of model analysing how foot area relates to aperture size and micro environment (cliff or boulders).

The adjusted R-squared of the linear model is 0.8856

<table>
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<tr>
<th>Response</th>
<th>Df</th>
<th>Sum Square</th>
<th>Mean Square</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(Aperture)</td>
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<td>127.777</td>
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<td>&lt;2e-16</td>
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<tr>
<td>Rock</td>
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<td>4.636</td>
<td>4.636</td>
<td>112.0731</td>
<td>&lt;2e-16</td>
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<tr>
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<td>0.002</td>
<td>0.002</td>
<td>0.0535</td>
<td>0.8171</td>
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<tr>
<td>Residuals</td>
<td>411</td>
<td>17.003</td>
<td>0.041</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The analysis confirms that both aperture and rock are highly significant variables explaining the foot area (p-values >2e-16). The interaction of log(aperture) and the Rock estimator is not significant (p-value 0.82)
4.2 Project 2: Exposing adult sheltered snails to different current forces.

A – Testing behaviour

Figure D : Evolution of the number of snails in the behavioural experiment.

The number of active snails was recorded for each group at time intervals. The average variation over the duration of the experiment is plotted. Red bars represent standard deviation of the 3 replicates.

The curves follow a trend that seem to match the intensity of the treatment. Treated groups had a higher number of individuals earlier than the control group. The number of active individuals stabilizes at a number of approximately 5 individuals out of 12 for all 3 groups.

Table 2 : Results of the analysis of deviance on the binomial linear model (Chi square test)

|       | Df | Deviance | Resid. Df | Resid. Dev | Pr(>|Chi|) |
|-------|----|----------|-----------|------------|----------|
| NULL  | 20 |          |           | 18.7679    |          |
| group | 2  | 5.0546   | 18        | 13.7133    | 0.07987  |
| time  | 1  | 3.2249   | 17        | 10.4883    | 0.07252  |
| group time | 2 | 3.6299 | 15 | 6.8585 | 0.16285 |

The analysis shows us a p-value of 0.08 for the variable « group ». This is not significant effect at the standard 5% alpha risk. However, this may be due to a lack of power in the analyses, as time is not significant either at the 5 % alpha risk.
B – Testing adhesion

Figure F: Number of fixation successes of the individuals according to their groups. The box plot does not show any trend in the fixation abilities of the groups and displays the large variation intra-group (even revealing an individual that successfully held 28 out of 30 flushes in the control group).

The results of the fixation success was tested with a Kruskal-Wallis rank sum test that calculated a p-value of 0.5897 which confirms that there was no significant difference between the three treatments.
5. DISCUSSION

5.1 Project 1 : Linking foot area, aperture area and environment

The snails living in the boulder environment (red color dots on Figure A) have a smaller foot in respect to their size in comparison to the snails living in the wave environment, as initially expected. The crab « foot/aperture ratio » is less variable around the mean of the ecotype than the corresponding variance for the wave ecotype. Indeed, shell proportions varies more than foot proportions along the sampled zone therefore the « foot/shell ratio » is more restrained for the crab ecotype. Distances as short as 30 meters (e.g. from position 170 to 200 on the path) seem sufficient to switch from one ecotype to the other.

The individuals projected on the positions around the interval 110 – 130 on the Bézier path appear to have a smaller foot index than the other individuals of the cliff habitat. The plots of other phenotypic traits such as length and aperture size along the path suggest that the snails at this position have a crab adapted phenotype, but the rock estimator does not indicate a boulder topography. In the field however, it corresponds to a large crevice, the estimation of Fucus density at this spot are almost as high as in the boulder region. The phenotypic study of the snails around that spot suggest that crab densities are high enough in this precise location to select for the crab adaptations of Littorina saxatilis. To confirm this hypothesis, this location is particularly interesting as it seems to be under high selection pressures because waves acts directly on a rock substratum but Fucus and presumably crabs have colonised the gully. Combination of the selective pressures possibly lowers the density of snails in this microhabitat. An interesting study could further map the energy of waves as well as the crab density on the zone and verify that the observed snails morphometric variations in this gully are correlated to the presence of crabs, and if this short portion of crab environment reduces has lower density of snails.

The wave ecotype clearly have have a higher foot size for a given aperture area. A possible bias taking part in our measure of the foot is the different behaviour of the ecotypes, and although maximum measures of the foot area were used to get as close as possible to the complete expansion of the foot values, the area may have been underestimated for some individuals. Perhaps this effect is more pronounced for the crab ecotype as its more cautious behaviour makes it more reluctant to extend the foot and crawl at the moment of the picture. Nonetheless, the plot reveals a clear linear relationship between the foot area and the aperture area for the smaller snails from the rocky habitat and this linear relationship is less tight for higher values of aperture. The results observed on the « Figure , we can suppose that many of the large rocky-habitat snails that we observe on Figure B have been sampled in the crevice (position 110-130) and are in fact facing crab predation. Therefore the phenotype for the larger rocky snails on this plot is most likely an intermediate ecotype that does cope with crab predation and increases the variance of the larger wave ecotype on the plot.

The variance in both foot size and aperture size increasing with the size disappears by log transforming the data (Figure C), revealing the different spread of the foot/aperture ratio for the two ecotypes. Under log transformation, the foot/aperture ratio between rock positions and boulder positions snails appear to be distributed around two distinct parallel lines. The anova realized on the linear model revealed the highly significant effect of the rock estimator (p-value <2e-16), meaning that the aperture area is not a complete predictor of foot are, as foot area is also correlated to the topography where the snail was collected. For the most part wave adapted periwinkles display a foot area that follows strictly the aperture area, suggesting that the foot growth could be limited for this individuals by the available area of aperture. Crab ecotype do not follow that strict linear link, but instead have “spare” space in the aperture due to the smaller foot. As stated in the introduction, it is known that aperture area tends to maximise a trade-off between defence against crab predation and providing space to deploy larger foot mass and increase fixation. The impact of crab action on the foot size is not well known as its
development is believed to be an adaptation to dislodgment [8, 17]. My results suggest that foot size reduction in crab ecotype could also be adaptive: perhaps (1) a smaller foot increases the ability to retract deeper in the shell and counter pulling attacks of crabs, (2) smaller foot possibly allows for a quicker retraction and offers less tissue to attack when outside deployed, (3) from an outside perspective, crabs may attack preferentially snails with a bigger foot for increased reward. We can also hypothesize that the foot reduction in crab ecotype does not have a direct effect on the fitness of the ecotype but instead follows the reduction of the inner aperture, a reduced entrance that is visible on some crab adapted snails. Plasticity in response to environmental stress during growth may play a part in the maximum development of the foot [15], this could be tested comparing the morphology of laboratory raised “wave” ecotype with wild populations.

In every scenario, the genetic background that drives development of the snail features alleles that control foot development and others that control shell formation (such as aperture). In some cases, this alleles may be mismatching with each others, and create unbalanced phenotypes over the hybrid zone. Even considering they are best adapted to the ecotone than “crab” and “waves” ecotypes, hybrid populations are likely to show greater variability and lowered fitness than the these, leading to disruptive selection applied at the contact zones, further dividing the populations.

5.2 Discussing project 2: Exposing adult sheltered snails to different current forces.

The experiment looking into acclimation mechanisms to strong flow force arose several challenges, and the first was to create a sufficient and homogeneous current to trigger a plastic response from the snails was successfully solved by the use of magnetic stirrers which appeared to be a good option to create a homogeneous circular current that pressured the snail to maintain grip. However if too many snails are placed in a current box, they will tend to clump up and use the group as a block to reduce the flow pressure. The behaviour of the snails in the current boxes showed interesting patterns, as I believe they migrate down when disturbed either by the current or the vibrations of the box. The conditions in this experiment are far from natural environment but this observation leads to think that an increase in hydrodynamics forces increases could trigger a quick behavioural reaction of shelter seeking. This behaviour poses new questions, how does littorinid proceed to seek shelter against dislodgement in case of increasing waves? Crawling out of the water, in crevices or to sheltered positions behind rocks could be plausible reactions, in any of these cases, it results in a reduction or interruption in time allocated to grazing. Previous studies exposing periwinkles to predatory cues has shown significant reduction in grazing time of snails leading to lowered growth and fitness of these individuals [18]. I suspect this shelter seeking behaviour that protects against strong wave action to induce the same effect: the stimuli that provokes this behaviour in “crab” ecotype is strong in exposed shores, that may very well contributes to isolate the ecotypes by microhabitat by not only reducing the distance that snail crawls under high wave action [19] but also lowering the fitness of migrating individuals to the exposed shore. The behavioural test provided interesting results that appear to match the intensity of the treatments (Figure D). The groups that faced strong currents and the medium current actively tried to get a grip of the substratum faster than the control group. It is interesting that in each group, approximately half of the snails reached an “active” state after 15 minutes, evidencing strong differences between individual behaviour. Assuming that a big part of the behaviour is genetically driven, it is a possibility that the snails that exhibit “extreme” behavioural response: very quick activity, or no activity over 15 minutes have genetic dispositions that favour their respective behaviour. The different sensibility to stimulus that is the strong current of the treatment can explains the pattern we observe. However these results are to be taken with caution as the low number of snails per group overweights the importance of very active individuals as well as very passive ones, and these were present in every group. Thus, the random distribution in the groups of these individuals are a concern in this study. The analysis of deviance on the generalized
A binomial linear model calculates a p-value of 0.08 (Table 2) for the effect of the group, meaning that under null hypothesis, we could observe this pattern or more extreme ones for almost 8% of similar experiments, such value is not low enough to assess with certainty that the treatment induced a separation in the snails’ behaviour according to their group. It strongly encourages the further research in behavioural response to current exposure.

A second challenge was to precisely measure individual adhesion, and numerous trials of pulling a loop glued to the snail's shell with a precise dynamometer were judged to be not consistent enough and far from the natural conditions. Developing and building the high speed flume described in “C - Testing adhesion - Material and Methods” provided a technique much closer to the natural system. The device discriminated repeatedly some individuals with high adhesion efficiency and others with consistent poor adhesion. For example, the outlier observable in figure F of the control group represents the average score of an individual that has succeeded maintaining its grip for almost every set to the completion (28 out of 30 maximum number flushing). The results are convincing in saying adhesion of the treated individuals did not increase (p-value = 0.59 with the Kruskal-Wallis test). It is difficult to assess whether the intensity of the treatment, both in current strength and duration per day, would have been enough to induce acclimation of these adult sheltered individuals in the case of such plasticity existing.

Maintaining adhesion in face of water flow is a crucial and complex trait in which shell shape and size, as well as foot characteristics play a role. It is fair to say that shell shape and size are components that are fixed for adult snails, if adhesion was to increase by acclimation it would probably be by an augmentation in the foot mass. Synthesis of muscular tissue is likely to take a prolonged time and require additional energy intake to cover energy spent by both effort and foot growth. In this experiment, snails were directly exposed to the flow of the treatment and had limited options to shelter and reduce the flow pressure. In natural conditions staying fixed to the substratum under high energy waves would probably not be possible without the appropriate behaviour, a key and more adjustable component than foot characteristics to minimize energy expenses.

6. CONCLUSIONS

The variation in foot area in the hybrid zone was not entirely predicted by the variation in aperture area, but showed, in addition, a strong correlation with the microhabitat. This result suggests that foot and aperture are not equally affected by the environmental factors and selection forces ensuing. While wave ecotype have closely related aperture and foot suggesting that aperture might act as a limiting factor for foot growth, a relatively smaller foot in crab dense environment could be an exclusive adaption to the “crab” environment. The morphometric data collected in this study provides data to fit cline models and apprehend how selection shapes these traits over a hybrid zone. By mapping both environmental pressures and individuals characteristics, the study of Littorina saxatilis ecotypes and hybrid zones can provide fundamental knowledge to understand divergence and speciation processes in sympathy unveiling how natural selection is sufficient to structure populations without geographic isolation.

The acclimation pilot study revealed major difficulties in measuring adhesion of snails to the substratum. However, an innovative solution was presented with the development of a high-speed flume. Coupled with particle image velocimetry, it will allow for precise measuring of adhesion abilities and investigate topics such as hydrodynamics characteristics of snail shell. While in this experiment, the training did not appear to have any significant effect on the snail adhesion skills, however it seems to affect their behaviour. These results are preliminary and deserve further experiments using a higher number of individuals. It may be worthwhile measuring the behaviour and adhesion efficiency after a period of treatment in an exposed natural environment.
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8. REFERENCES


