The Veliger 46(2):162–168 (April 1, 2003)

THE VELIGER

Distribution of Sister *Littorina* Species, I: Tenacity and the Wave-Exposure Gradient

PAUL A. HOHENLOHE[1]

Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington 98250, USA

Abstract. Data on the ecological differences between the sympatric gastropod species *Littorina scutulata* and *L. plena* are limited, in part because they were long regarded as one species and are difficult to distinguish in the field. I examined their relative distributions along a wave-exposure gradient and tested for differences in adaptations to wave stress by measuring foot length, width, and area across a size range of individuals, and by measuring tenacity on rock and glass substrata in the laboratory. The species were found together at most sites, and both species were found at all levels of wave exposure. Within each species, shell height was strongly related to foot length, width, and area, and to tenacity. Tenacity was directly proportional to foot area, but foot size did not scale isometrically with shell height; larger animals had proportionally smaller feet. Thus the tenacity/drag ratio is also lower for larger snails. The species did not differ from each other in any of these relationships. The distribution results lend support to the hypothesis that *L. plena* is associated with areas of freshwater inflow, such as estuaries and shores near the mouths of rivers, more than its congener.

INTRODUCTION

The gastropod species *Littorina scutulata* Gould, 1849, and *L. plena* Gould, 1849, are broadly sympatric in the northeast Pacific, inhabiting the high intertidal and splash zone of rocky shores. They were long regarded as one variable species (Reid, 1996) and are not easily distinguished in the field. As a result, several studies (e.g., Chow, 1975; Jensen, 1981) may have lumped the two species. Behrens Yamada (1992) found "*Littorina scutulata sensu lato*" to have a wider ecological niche than other *Littorina* species considered, perhaps as a result of combining the two species' niches. Morphological work by Murray (1979, 1982) and molecular work by Mastro et al. (1982) and Kyle & Boulding (2000) have shown them to be distinct species. Nonetheless, data on interspecific differences in distribution and habitat preference are minimal. Reid (1996) studied museum collections of both species and found *L. scutulata* to range from southeast Alaska to southern Baja California, Mexico, and *L. plena* to range from Kodiak Island, Alaska, to northern Baja California. On the basis of these collection sites, Reid (1996:67) found that "*L. scutulata* tends to predominate in the more exposed localities, whereas in very sheltered situations and in salt marshes *L. plena* may occur alone." Rugh (1997) also found *L. scutulata* to predominate on more exposed shores but noted high relative abundances of *L. plena* at exposed sites near the mouths of rivers. On the contrary, other studies have found *L. plena* more abundant at exposed sites (Hohenlohe & Boulding, 2001; S. Behrens Yamada, personal communication). Slight differences in exposure preference in species with overlapping ranges may be common in the genus *Littorina* (Mill & Grahame, 1990; Boulding & Van Alstyne, 1993).

One important adaptation of organisms living on hard substrata in the intertidal is resistance to dislodging by wave action (Denny, 1999). Gastropod species adapted to high wave-energy habitats tend to have longer, broader feet and greater tenacity (Miller, 1974). In the genus *Littorina,* tenacity is a factor in microhabitat preferences among sympatric species (Davies & Case, 1997) and in local adaptation and phenotypic plasticity within a single species (Trussell, 1997). Miller (1974) found *L. scutulata* to have one of the highest tenacity/foot area measures among the gastropods studied (466 ± 28 g/cm^2 foot area for a force normal to the substratum on a crawling snail), although her study predated the taxonomic resurrection of *L. plena,* and she may have combined the currently recognized *L. scutulata* and *L. plena* in her analysis. Curiously, she described a bimodal distribution for the species complex along a wave-exposure gradient of sampling sites. Nonetheless, high tenacity measurements for these species are expected, given both their rocky intertidal habitat and their coiled shells that are expected to induce relatively high drag in flow (Denny, 2000).

The current study addresses two questions: (1) Are *L. scutulata* and *L. plena* distributed differently along the wave-exposure gradient; and (2) Do they exhibit differences in adaptation to wave stress, either in foot size and

[1] Address for correspondence: USDA Forest Service, Corvallis Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, Oregon 97331; phone (541) 750-7403; Fax: (541) 750-7329; e-mail: phohenlohe@fs.fed.us

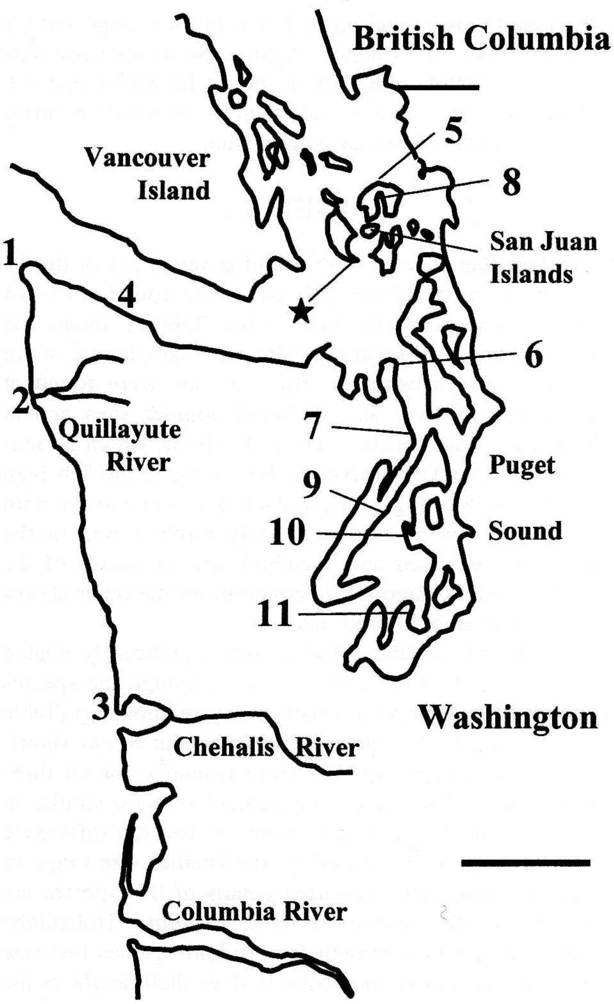

Figure 1. Map of western Washington showing sample locations for exposed (1–3), moderately exposed (4–7), and sheltered (8–11) sites. The star shows the collection site for snails used for foot morphology measurements and tenacity trials. The scale bar is approximately 50 km.

shape or in tenacity, that correlate with their distribution along this gradient?

METHODS

In order to cover a range of habitats from exposed open coast to sheltered cobble beach at a similar latitude, sampling was conducted at 11 sites on the outer coast of Washington state and in Puget Sound (Figure 1). Animals of all sizes were collected from an area of shore and identified by penis morphology (Murray, 1979), a combination of other morphological characters (Rugh, 1997; Hohenlohe & Boulding, 2001), and/or restriction fragment analysis of the cytochrome b gene (Hohenlohe & Boulding, 2001). While this sampling method did not measure density, it is believed to accurately reflect relative proportions of the two species at each site because the two species occupy roughly the same tidal height (Hohenlohe, in press) and microhabitats (Hohenlohe, personal observation). Sampling sites were scored for wave exposure as follows: exposed (facing the open ocean), moderately exposed (facing an inland waterway with 5 or more km of open water), or sheltered (less than 5 km of open water); and for substratum: bedrock, boulders (larger than 0.3 m in diameter and not moved by typical wave action; includes man-made jetties), or cobbles (smaller than 0.3 m in diameter).

To measure tenacity and foot morphology, individuals of both species were collected from the same shore at Cattle Point, San Juan Island, Washington, a moderately exposed, bedrock shore (48°27′N, 122°58′W; see Figure 1) and identified to species using the discrete morphological characters described by Hohenlohe & Boulding (2001), confirmed in males by penis morphology (Murray, 1979; all individuals used were sexually mature). Shell heights were measured with an ocular micrometer on a dissecting microscope. To measure foot length, width, and area, the snails were photographed with a digital camera mounted on a dissecting microscope as they crawled upside down on a glass slide. The images were transferred to a computer and measurements were taken with imaging software. Area measurements used the outline of the foot surface on the substratum, rather than simply the product of length and width. Small metal hooks were fixed with epoxy to the shells of 18 *L. scutulata* and 16 *L. plena* individuals, covering a range of shell sizes in each species. Hooks were oriented perpendicular to and centered over the snails' feet.

Tenacity was measured on each individual with the apparatus shown in Figure 2. Snails were placed on the substratum in filtered seawater at ambient seawater temperature (14 to 15°C) and the spring scale attached to their hook. When the animal began to crawl normally, tensional force normal to the surface of the substratum was smoothly increased using a micromanipulator until the animal released from the substratum. Tenacity, equivalent to the maximum tensional force just prior to release, was measured in grams to the nearest 0.5 g to allow comparison to previous results (e.g., Miller, 1974; 1 g is equivalent to about 0.0098 N of force). Since none of the snails exceeded 0.25 g in mass, each snail's weight fell within the rounding error of the measurements and was not subtracted from the measurements. Each individual was tested in this way 10 times on a glass substratum and 10 times on smooth basalt rock. The order of individuals tested was randomized, and the substratum was cleaned between individuals. Because of the variability of measurements for each individual (also found by Miller, 1974, and Davies & Case, 1997), including several very low values, the maximum tenacity for each set of 10 trials was used in all further analyses.

Regression analysis, using the model $Y = aX^bE$, which

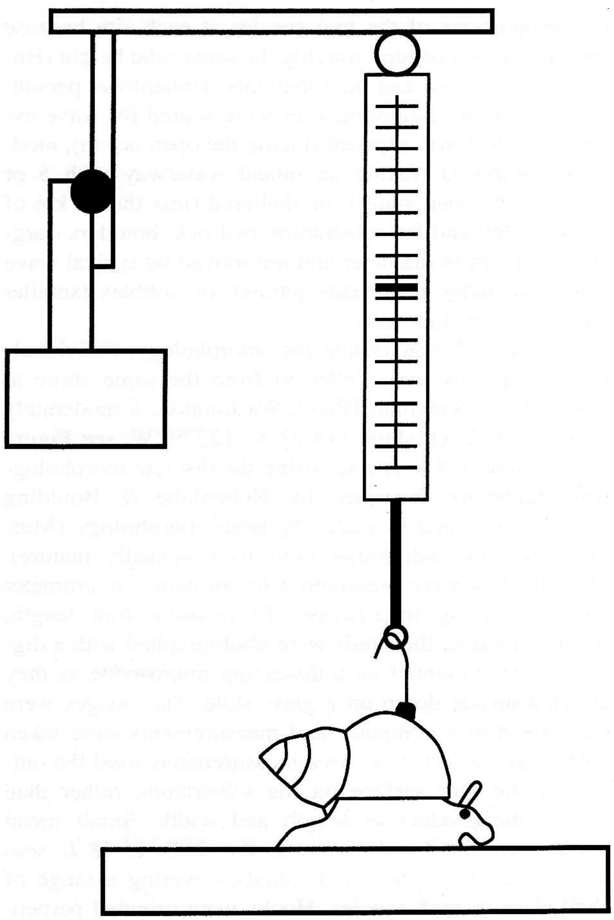

Figure 2. Diagram of apparatus used to measure snails' tenacity to rock or glass substratum. Force was gradually increased using the micromanipulator, and the maximum force required to pull the snail off the substratum was recorded in grams.

is equivalent to simple linear regression on the logarithm-transformed data, was used to determine the form of the relationships among the measured variables within each species (Zar, 1996). The five relationships tested were: foot length, width, and area versus shell height; tenacity versus foot area; and tenacity versus shell height. These results were then used to test two null hypotheses: that the species do not differ from each other in the form of any relationship, and that the form of each relationship does not differ from the predicted form within each species. In the first case, a Student's t-test was used on the exponent b, and then, if the species did not differ with respect to b, on the coefficient a (Zar, 1996). For the second hypothesis, isometric growth was expected to produce a linear relationship ($b = 1$) for foot length and width versus shell height, and a quadratic relationship ($b = 2$) for foot area versus shell height. A linear relationship was also expected for tenacity versus foot area based on previous work (Miller, 1974; Etter, 1988; Trussell, 1997), and thus a quadratic relationship was expected for tenacity versus shell height. Again a Student's t-test was used (Zar, 1996), although it should be noted that the calculations of variance and degrees of freedom differ between the two types of comparison.

RESULTS

Both *Littorina* species were found at seven out of the 11 sites sampled, though only *L. plena* was found at two of the three exposed outer coast sites. Table 1 shows the proportion of *L. plena* at each site, total sample size, wave exposure, and substratum. Both species were found at moderately exposed and sheltered boulder sites and at sheltered cobble beaches. The only site at which *L. scutulata* was found exclusively is Eastsound in the San Juan Islands (site 8 in Figure 1), a result that is consistent with past work. *Littorina plena* is only rarely found in the moderately exposed and sheltered interior shores of the San Juan archipelago but is common on the outer shores (Hohenlohe, unpublished data).

Foot length, width, and area were significantly related to shell height within each species, although the species did not differ from each other in any relationship (Table 2). However, in *L. scutulata,* the exponent b was significantly lower than expected from isometry for all three relationships. The regression parameters were similar in *L. plena,* but the statistical power to test the difference from isometry was reduced by the smaller size range of *L. plena* individuals measured (snails of this species are typically smaller than those of *L. scutulata;* Hohenlohe & Boulding, 2001). Nonetheless, in both species foot size apparently increases more slowly than shell height as the animals grow (Figure 3).

Tenacity on the rock substratum was significantly related to both shell height and foot area within each species, though again the species did not differ from each other in either relationship (Table 2). The relationship between tenacity and foot area did not differ significantly from linear (Figure 4). Given the relationship between shell height and foot area, tenacity thus increases slower than the square of shell height as the animals grow. Again this deviation from isometry was significant only in *L. scutulata,* perhaps because of the smaller size range of *L. plena* individuals measured.

Snails had greater tenacity on the rock substratum than on glass (paired t-test; $P = 0.034$), although in each species the maximum value was recorded on glass. On rock substratum, *L. scutulata* had an average tenacity/foot area of 174.4 ± 12.4 g/cm^2 (mean $\pm$ SE) and a maximum of 273.0 g/cm^2, while *L. plena* averaged 180.2 ± 11.9 g/cm^2 with a maximum of 258.9 g/cm^2. On glass, *L. scutulata* averaged 172.5 ± 14.2 g/cm^2 with a maximum of 324.5 g/cm^2, while *L. plena* averaged 164.3 ± 15.3 g/cm^2 with a maximum of 330.8 g/cm^2.

Table 1

Proportion of *Littorina plena* in samples of *L. scutulata* and *L. plena* collected at 11 sites in western Washington (see Figure 1). Proportions in bold highlight sites where only one species was found.

Exposure site	Latitude	Longitude	Substratum	N	Proportion *L. plena*
Exposed					
1. Tatoosh Island	48°24′N	124°44′W	bedrock	133	0.89
2. Rialto Beach	47°55′N	124°38′W	bedrock	15	**1.00**
3. Pt. Brown	46°56′N	124°11′W	boulders	67	**1.00**
Moderately exposed					
4. Sekiu	48°16′N	124°18′W	boulders	26	0.69
5. Orcas Island	48°43′N	122°54′W	bedrock	26	0.31
6. Fort Worden	48°9′N	122°46′W	boulders	65	0.35
7. Termination Pt.	47°52′N	122°38′W	boulders	13	**1.00**
Sheltered					
8. Eastsound	48°41′N	122°54′W	bedrock	38	**0.00**
9. Seabeck	47°39′N	122°47′W	cobbles	23	0.87
10. Sinclair Inlet	47°32′N	122°41′W	boulders	35	0.71
11. Kopachuck Park	47°19′N	122°41′W	cobbles	70	0.67

Table 2

Relationships among size, foot morphology, and tenacity on rock substratum. Shown are regression parameters for the equation $Y = aX^bE$ within each species, tests for isometry of each relationship within species (see text for details), and tests for differences between the species for each parameter. Isometry and interspecific differences were tested with a Student's t-test (Zar, 1996).

Variables	Within species				Isometry	
species (n)	a	b	r	p	b_{exp}	p
Foot length (Y) vs. shell height (X)						
L. scutulata (18)	0.966	0.708	0.863	< 0.001	1	0.013
L. plena (16)	0.995	0.719	0.764	< 0.001	1	0.107
Between species	p > 0.2	p > 0.5				
Foot width (Y) vs. shell height (X)						
L. scutulata (18)	0.502	0.826	0.982	< 0.001	1	< 0.001
L. plena (16)	0.523	0.800	0.804	< 0.001	1	> 0.2
Between species	p > 0.5	p > 0.5				
Foot area (Y) vs. shell height (X)						
L. scutulata (18)	0.443	1.502	0.957	< 0.001	2	< 0.001
L. plena (16)	0.453	1.528	0.871	< 0.001	2	0.062
Between species	p > 0.2	p > 0.5				
Tenacity (Y) vs. foot area (X)						
L. scutulata (18)	1.652	0.934	0.895	< 0.001	1	> 0.5
L. plena (16)	1.582	1.049	0.881	< 0.001	1	> 0.5
Between species	p > 0.5	p > 0.5				
Tenacity (Y) vs. shell height (X)						
L. scutulata (18)	2.627	1.511	0.922	< 0.001	2	0.007
L. plena (16)	2.053	1.764	0.845	< 0.001	2	> 0.2
Between species	p > 0.2	p > 0.5				

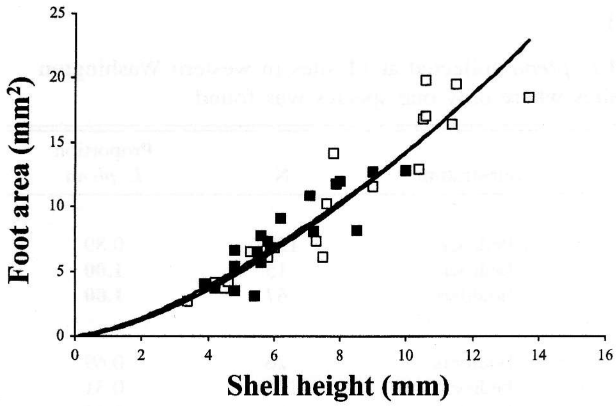

Figure 3. Foot area correlates with shell height in *Littorina scutulata* (open squares) and *L. plena* (dark squares), but the species do not differ statistically (see Table 2).

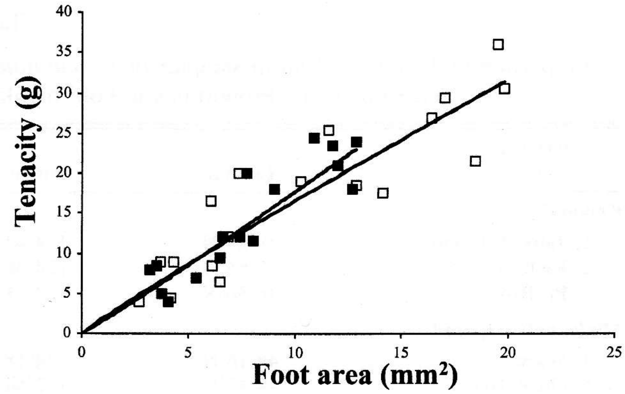

Figure 4. Tenacity, measured as the maximum force required to pull a snail off a flat rock substratum. Tenacity correlates with foot area in *Littorina scutulata* (open squares) and *L. plena* (dark squares), but the species do not differ statistically from each other (see Table 2).

DISCUSSION

The gastropod species *Littorina scutulata* and *L. plena* overlap broadly along the wave-exposure gradient. Contrary to the conclusions of Reid (1996), both species were found at all three exposure levels from sheltered cobble beaches to exposed bedrock shores. In fact, *L. plena* appeared to be the predominant species at the most exposed sites, whereas *L. scutulata* reached its highest proportions at the moderately exposed sites and at one sheltered site. However, if wave exposure plays a role in defining these species' relative habitats, one might expect differences in adaptations to resisting wave forces. No such differences were found in any of the foot morphology and tenacity measurements taken here; the species were indistinguishable in these respects.

Several forces act on animals in intertidal habitats as a result of wave action. Accelerational force depends on the volume of water displaced by the organism, and in these *Littorina* species (typically less than about 1.5 cm in shell height) it would be small compared to lift and drag (Denny, 2000). Lift may be the critical force affecting many limpets in the intertidal and is proportional to the projected area of the shell; however, as the height/radius ratio increases, drag forces become more important (Denny, 2000). For these *Littorina* species, the height/radius ratio far exceeds 1.0, although the asymmetrical shape of *Littorina* shells may still produce important lift forces (Denny, 2000). Two types of drag forces may play a role on wave-swept organisms: skin-friction drag is proportional to the surface area of the animal, but is expected to be of minor importance because of the high water velocities in breaking waves (Denny, 1995). Pressure drag depends on the orientation of asymmetrically shaped organisms but is also proportional to the frontal area of the organism (the area projected onto a plane perpendicular to the direction of water motion), as measured in two snail species by Denny (1995). Drag and lift forces may either counteract or augment each other, depending on the orientation of the shell (Denny, 2000).

Nonetheless, the forces imposed on wave-swept snails are expected to be roughly proportional to the square of shell height. Here, tenacity increased slower than the square of shell height in both species. The ratio of tenacity to drag and lift forces thus decreases as the animals grow, suggesting the possibility of an upper size limit set by drag and lift forces (Denny, 1999). This could potentially favor *L. plena,* which tends to be smaller (Murray, 1982; Chow, 1987; Hohenlohe & Boulding, 2001), and explain the predominance of *L. plena* on the exposed shores sampled here. However, intertidal animals in nature do not typically approach the theoretical size limits (Denny, 1999). These *Littorina* species seem to be no exception, since they fall well within the ranges of foot area/shell height and tenacity/foot area measured in many other, mostly larger, gastropod species (Miller, 1974; Etter, 1988).

Other related factors may provide advantages to smaller size, including the ability to stay within the fluid boundary layer where drag forces are reduced, and the ability to fit into crevices in the substratum as shelter from wave action and predation. The measurements of tenacity in *L. obtusata* by Trussell (1997) suggested that individuals may also limit their growth or alter their behavior or microhabitat in response to wave exposure. None of these factors, however, appears to exclude either *L. scutulata* or *L. plena* from the most exposed shores, and the wave-exposure gradient does not separate these species ecologically.

What other ecological dimension could be important? The two exposed sites where *L. plena* was found exclusively (sites 2 and 3; see Figure 1) lie less than a kilometer north of the mouths of the Quillayute River and the Chehalis River estuary, respectively. In addition, the most protected sites in Puget Sound, which are heavily influenced by freshwater run-off from the adjacent mainland, had relatively high abundances of *L. plena.* With the exception of the inner waters of the San Juan Islands (site 8), where *L. plena* is generally not found, the sites with the highest relative abundance of *L. scutulata* (sites 5 and 6) were moderately exposed sites that are not near major sources of fresh water and that receive relatively well-mixed ocean water because of strong tidal action in the area. These results support Rugh's (1997) hypothesis that *L. plena* may be well adapted to lowered salinities. However, the abundance of *L. plena* at Tatoosh Island (site 1), an exposed shore far from any major freshwater source, demonstrates that this species does not require freshwater inflow to persist. Both species, because of their habitat on rocks and in tidepools of the high intertidal, combined with the relatively wet climate of the northeast Pacific, are exposed to periodically reduced salinity directly from rainfall. The association of *L. plena* with freshwater inflow, then, may depend not so much on salinity itself but rather on nutrient composition, algal flora, or other components of river and estuarine environments.

Miller (1974) reviewed several mechanisms that are likely to play a role in gastropod attachment: suction, simple adhesion (by a thin film of liquid), adhesion with sticky secretions from the foot surface, and muscular action. All four of these mechanisms are consistent with the linear relationship shown here between tenacity and foot area: suction force depends on pressure and area, adhesion should be proportional to the surface area in contact, and force produced by muscular contraction is proportional to cross-sectional area. Similarly, both Etter (1988) and Trussell (1997) also found linear relationships between tenacity and foot area in intertidal gastropods. Snails tested here had significantly higher tenacity on the rock substratum than on glass, although the maximum tenacity in each species was recorded on the glass substratum, which is more consistent with previous work. Miller (1972) tested *Nucella* (previously *Thais*) *emarginata* on smooth plexiglass and rock substrata and recorded higher tenacity on plexiglass for a force normal to the substratum. Davies & Case (1997) found a significant tenacity improvement for *L. littorea* and *L. obtusata* on a polished rock substratum compared to a rougher substratum, and concluded that muscular action was not the limiting factor in attachment. However, Miller (1974) found that oxygen is required for attachment, suggesting that tenacity is an active process involving more than simple adhesion. The strength of pedal secretions may depend not only on the rugosity but also the molecular nature of the substratum. Davies & Case (1997) used the same material polished to varying degrees, thus eliminating molecular differences and suggesting that other forces also play a role. On polished rock, and particularly on a glass or plexiglass substratum, suction and adhesion by a thin film of liquid may play a more important role than it does in nature. The results presented here cannot rule out any of the mechanisms of snail tenacity proposed by Miller (1974).

Acknowledgments. This work was supported by a National Science Foundation graduate fellowship and a National Science Foundation training grant in mathematical biology. Thanks to A. Kohn for assistance with this project and to A. Kohn, R. Strathmann, G. Odell, B. Roth, and two anonymous reviewers for helpful comments on the manuscript.

LITERATURE CITED

Behrens Yamada, S. 1992. Niche relationships in Northeastern Pacific littorines. Pp. 281–291 in J. Grahame, P. J. Mill & D. G. Reid (eds.), Proceedings of the Third International Symposium on Littorinid Biology. Malacological Society of London.

Boulding, E. G. & K. L. Van Alstyne. 1993. Mechanisms of differential survival and growth of two species of *Littorina* on wave-exposed and on protected shores. Journal of Experimental Marine Biology and Ecology 169:139–166.

Chow, V. 1975. The importance of size in the intertidal distribution of *Littorina scutulata* (Gastropoda: Prosobranchia). The Veliger 18:69–78.

Chow, V. 1987. Morphological classification of sibling species of *Littorina* (Gastropoda: Prosobranchia): discretionary use of discriminant analysis. The Veliger 29:359–366.

Davies, M. S. & C. M. Case. 1997. Tenacity of attachment in two species of Littorinid, *Littorina littorea* (L.) and *Littorina obtusata* (L.). Journal of Molluscan Studies 63:235–244.

Denny, M. W. 1995. Predicting physical disturbance: mechanistic approaches to the study of survivorship on wave-swept shores. Ecological Monographs 65:371–418.

Denny, M. W. 1999. Are there mechanical limits to size in wave-swept organisms? The Journal of Experimental Biology 202: 3463–3467.

Denny, M. W. 2000. Limits to optimization: fluid dynamics, adhesive strength and the evolution of shell shape in limpet shells. The Journal of Experimental Biology 203:2603–2622.

Etter, R. J. 1988. Asymmetric developmental plasticity in an intertidal snail. Evolution 42:322–334.

Hohenlohe, P. A. In press. Distribution of sister *Littorina* species, II: geographic and tidal-height patterns do not support sympatric speciation. The Veliger.

Hohenlohe, P. A. & E. G. Boulding. 2001. A molecular assay identifies morphological characters useful for distinguishing the sibling species *Littorina scutulata* and *L. plena.* Journal of Shellfish Research 20:453–457.

Jensen, J. T. 1981. Distribution, activity, and food habits of juvenile *Tegula funebralis* and *Littorina scutulata* (Gastropoda: Prosobranchia) as they relate to resource partitioning. The Veliger 23:333–338.

Kyle, C. J. & E. G. Boulding. 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Marine Biology 137: 835–845.

Mastro, E., V. Chow & D. Hedgecock. 1982. *Littorina scutulata* and *Littorina plena:* sibling species status of two prosobranch gastropod species confirmed by electrophoresis. The Veliger 24:239–246.

Mill, P. J. & J. Grahame. 1990. Distribution of the species of rough periwinkle (*Littorina*) in Great Britain. Hydrobiologia 193:21–27.

MILLER, S. L. 1972. Adaptive design of locomotion and foot form in prosobranch gastropods. Ph.D. dissertation. Department of Zoology, University of Washington: Seattle. 183 pp.

MILLER, S. L. 1974. Adaptive design of locomotion and foot form in prosobranch gastropods. Journal of Experimental Marine Biology and Ecology 14:99–156.

MURRAY, T. 1979. Evidence for an additional *Littorina* species and a summary of the reproductive biology of *Littorina* from California. The Veliger 21:469–474.

MURRAY, T. 1982. Morphological characterization of the *Littorina scutulata* species complex. The Veliger 24:233–238.

REID, D. G. 1996. Systematics and Evolution of *Littorina.* The Ray Society: London. 463 pp.

RUGH, N. S. 1997. Differences in shell morphology between the sibling species *Littorina scutulata* and *Littorina plena* (Gastropoda: Prosobranchia). The Veliger 40:350–357.

TRUSSELL, G. C. 1997. Phenotypic plasticity in the foot size of an intertidal snail. Ecology 78:1033–1048.

ZAR, J. H., 1996. Biostatistical Analysis, 3rd ed. Prentice-Hall: Upper Sadle River, New Jersey. 622 pp.