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BULLETIN OF MARINE SCIENCE, 54(1): 17–23, 1994

HOW DO LIMPETS MAINTAIN BARNACLE-FREE SUBMERGED ARTIFICIAL SURFACES?

Uriel N. Safriel, Neta Erez and Tamar Keasar

ABSTRACT

To reveal how limpets maintain barnacle-free patches on man-made substrata, we transplanted intertidal *Patella coerulea* L. (19–27 mm shell length) to experimental glass and steel panels, and compared these panels with control panels, free of limpets. All panels were submerged at 1-m depth in the port of Ashdod, Israel, during the time of peak recruitment of *Balanus amphitrite* (Darwin). Observations through the glass on limpets moving over barnacle-infested glass panels showed that they do not bulldoze young recruits with the front edge of their shell. Stomach analyses did not implicate limpets as barnacle predators. But enclosing the steel panels with a fine net that collected all objects detached from the panel surfaces, revealed that limpets accelerated barnacle detachment and mortality. This presumably is achieved by repeatedly running over the barnacles by the foot of the limpet, thus undermining the barnacles' hold. Barnacles attaining a rostro-carinal diameter >ca. 1.5 mm were not detached by limpets.

Mediterranean limpets *Patella coerulea* (L.) transplanted onto steel panels submerged in port waters, maintained them free of the commonest Mediterranean fouling barnacle *Balanus amphitrite* (Darwin) for several months (Safriel and Erez, 1987; Safriel et al., 1993). Limpets and barnacles broadly overlap in their intertidal vertical distribution (Lewis, 1964; Underwood, 1979), and often interact. For example, Lewis and Bowman (1975) showed that in some circumstances the presence of barnacles promotes the settlement of limpets, presumably by slowing the drying of surfaces to which the post-larvae recruit. Thus, barnacles may facilitate settlement and survival of limpet recruits by decreasing desiccation pressure. On the other hand, on Israeli Mediterranean shores *P. coerulea* is rare in patches with dense cover of the barnacle *Chthamalus stellatus* (Poli), and limpet scars are frequently barnacle-free (Lipkin and Safriel, 1971). A dense barnacle cover may interfere with the limpets' pedal movement, thus impairing the limpets' foraging efficiency, depressing growth and increasing mortality. Limpets, in turn, eliminate barnacle recruits or reduce their survival, thus keeping their home ranges accessible for grazing (Branch, 1981; Underwood, 1979).

Two possible mechanisms may account for the control limpets have over fouling barnacles, and possibly also in non-fouling benthic communities. One possibility is that limpets detach barnacle cyprids and small recruits as they forage. This mechanism was observed by Dayton (1971) as some *Acmaea* species moved over the barnacles, *Balanus glandula* (Darwin), *B. cariosus* (Pallas) and *Chthamalus dalli* (Pilsbry). Denley and Underwood (1979) report similar observations for the limpet *Cellana tramoserica* (Sowerby) and the barnacles *Tetraclitella purpurascens* (Wood) and *Tesseropora rosea* (Krauss). Alternatively, if limpets, indeed, significantly reduce the survivorship of barnacle recruits, then barnacle larvae may select limpet-free sites for their settlement. The ability of barnacles to avoid unfavorable sites for settlement is reviewed by Crisp (1974). Moreover, settlement decisions can be influenced by interspecific chemical cues: larvae of *Balanus amphitrite* avoid settling on two species of live corals as long as the latter secrete low-weight mucopolysaccharide molecules (Standing et al., 1984).

We present results of observations and experiments aimed at revealing the mechanisms by which limpets *Patella coerulea* maintain artificial surfaces sub-

merged in an Israeli port, free of *B. amphitrite*. Understanding this mechanism may help evaluate and improve limpets' performance as biological control agents of marine biofouling (Safriel and Erez, 1987; Safriel et al., 1993).

Materials and Methods

Observations and experiments were carried out at the Port of Ashdod (31°50′N, 34°39′E), Mediterranean Sea, Israel during August 1984, when recruitment of *B. amphitrite* was at its peak. Glass panels of 11.5 × 5.0 × 0.2 cm in size and shipping steel panels (painted with anti-corrosive paint) of 20 × 20 × 0.2 cm were hung vertically from a pier at 1-m depth, when housed in an apparatus allowing repeated lift-up for inspection and manipulation (Safriel and Erez, 1987). These smooth, vertical surfaces were chosen to simulate submerged marine constructions or hulls of boats. Barnacles recruited to these panels immediately following their submergence. Limpets of 19–27 mm shell length were then collected from intertidal boulders at the edges of the port, and transplanted onto the panels. The limpets' foraging movements were observed within aquaria, either on the pier under shade, or in the laboratory. Limpets' behavior was inspected and barnacles' survivorship was determined with the aid of a long-arm Nikon binocular stereoscope. Data were recorded on coding sheets and analyzed using SPSS (Nie et al., 1975, 1981) on a CDC-6400 Cyber computer, and SAS-PC version 6.03 (SAS Institute, 1988).

Results and Discussion

Does the Limpets' Pedal Mucus Prevent Barnacles' Settlement?—We attached 10 limpets to each of 7 glass plates submerged in an aquarium on the pier, and at the same time set up an adjacent aquarium with identical plates but with no limpets. The limpets moved over the panel surfaces, thus covering them with their pedal slime. After 4 h, the limpets were removed and all 14 experimental panels were submerged in the sea at 1-m depth for 17 h. Then all panels were lifted up for counts of recruiting barnacles. The mean numbers (±SD) of barnacles on mucus-covered and on control plates was 5.7 ± 3.3 and 4.3 ± 3.0, respectively. This difference was not significant (one-tailed Mann-Whitney *U*-test). Thus, we were unable to show that barnacle settlement (at mean rate of ca. 0.005 recruits·$cm^2 \cdot h^{-1}$) was affected by the slimy trail produced along the trajectory of the moving limpet.

Do Limpets Bulldoze Young Barnacle Recruits with the Edges of Their Shell?—We submerged 12 glass panels in the sea, and lifted them up by the end of the third day after submergence, when the settled barnacles reached rostro-carinal diameters of 0.30 ± 0.10 mm (±SD). We then attached one limpet to each panel, and submerged the panel in a sea-water filled aquarium, with the attached limpet upside down. It was thus possible to observe the effect of the limpet's movements on the barnacles through the glass which carried them. Six of the plates were observed in a shaded location in the port, and the six remaining plates were observed in the laboratory. Observations lasted 6–30 h. During these observations we detected several occasions where barnacles became "trapped" between the front edge of the limpet's shell and its foot, where they continued their cirral activity. The height of the barnacle's shell was about 0.3 mm, and the edges of the moving limpet shell were lifted about 1–2 mm above the panel's surface. Thus, limpets (*P. caerulea*) did not knock down new recruits with the edges of their shell, unlike Dayton's (1971) observations on *Acmaea*.

Do Limpets' Pedal Movements Kill Metamorphosed Barnacles?—In the previously described experiment we noticed that many barnacles, which had just been run-over by a limpet, remained intact and active. Thus, repeated running-over by the foot may be required to fatally damage barnacle recruits. For exploring this possible mechanism, we attached 15 limpets to one member of each pair of panels

("limpet panel"), at day 3, 5, 7, 9, 11 and 13 after submergence (and onset of barnacle settlement). The other member of each pair served as a control. Immediately after the limpets attached themselves to the panels, both members of each pair were wrapped in a plankton net bag (130-μm mesh size), allowing for plankton penetration but preventing loss of detached barnacle recruits or their parts. After 24 h both limpet and control panels were lifted up, and the contents of each plankton net was carefully collected for inspection and counts. Then the clean net bag was restored in its previous position and the panels submerged for another 24 h, followed by an identical, final inspection. Thus, limpets were given the opportunity to affect populations of variable size-structure, ranging from 3 to 13 days of development, and it was possible to assess the effect of limpets, independent of non-limpet mortality causes.

All nets contained empty shells, free dead animals, dead animals intact within their detached shells, and a mixture of individual, separated shell- and opercular-plates. The number of intact barnacles and free dead barnacles detached from limpet panels was 2.8 and 1.5 greater than that detached from control panels from populations aged 3–5 and 7–9 days, respectively (Table 1). In all the above-mentioned categories limpets detached significantly more items than detached from other, unknown causes.

In populations aged 11–13 days, there were no significant differences between limpet and control panels (Table 1), suggesting that barnacles attaining a certain size range become immune to limpet-induced mortality.

Do Limpets Consume Barnacle Recruits?—Twenty-six limpets, which had been used in the experiment described in the previous section, were removed at the end of the experiment, sacrificed, and preserved in 5% formalin solution. They were dissected in the laboratory and their stomach contents examined under $\times 100$–$\times 400$ magnification. These reflected the limpets' diet during the previous 48 h, when they had foraged on the panels. The stomach contents were found to include diatoms' shells, fragments of multicellular algae and sand grains, but no skeletal, cuticular or other parts of barnacles. This finding supports numerous other studies (reviewed by Branch, 1981), which show that most limpets are generalist herbivores. Thus, in our experiments the removal of barnacles was not performed by radular scraping and subsequent ingestion.

Is Limpet-induced Mortality of Barnacles Size-dependent?—A grid of 36 1 × 1 cm plots was marked on each of the steel panels used for the experiment just described, and the position of each barnacle and its base diameter were mapped on the day of limpet attachment. The panels were inspected after 1, 2 and 3 days. At each inspection, every panel was compared with the map, and the state of each marked barnacle (disappeared, died, or remained intact) was recorded. For calculation of size-dependent mortality, each barnacle was then allocated to one of seven groups according to its size (<0.5 mm, 0.5–0.7 mm, 0.8–1.0 mm, 1.1–1.3 mm, 1.4–1.8 mm, 1.9–2.1 mm, >2.1 mm). The effects of the presence of the limpets and of barnacle size on the barnacle removal proportion in each size group were tested by 2-way ANOVA (Table 2). Barnacle size significantly affected their survival from the first day of observation and on, while the effect of limpets became significant only on day 3. A significant interaction between the independent variables was also attained on day 3, indicating that more small barnacles were removed on limpet panels than on control panels.

The same trend is reflected in the regression of detachment proportion on size (Fig. 1). Extrapolation of this regression predicts that immediately after settlement 10% and 66% of barnacles are detached, on non-limpet and limpet panels, re-

Table 1. Numbers of detached barnacles at 3–13 days since recruitment

Age (days)	Number of panels	Treatment	Whole barnacles Mean ± SD	Whole barnacles P	Shell-less barnacles Mean ± SD	Shell-less barnacles P	Empty barnacle shells Mean ± SD	Empty barnacle shells P	Plates and operculi Mean ± SD	Plates and operculi P
3–5	4	Limpets	22.5 ± 7.7	0.009	30.3 ± 6.1	0.01	18.5 ± 15.2	0.037	19.5 ± 5.0	0.01
	4	No limpets	0.8 ± 1.5		18.0 ± 4.2		15.8 ± 4.6		2.5 ± 1.7	
7–9	4	Limpets	128.0 ± 72.4	0.041	417.8 ± 292.0	0.074	127.8 ± 49.7	0.041	14.8 ± 9.1	0.04
	4	No limpets	39.0 ± 55.4		316.0 ± 312.9		53.3 ± 37.6		6.8 ± 7.9	
11–13	2	Limpets	72.0 ± 45.3	0.219	288.0 ± 11.31	0.219	321.0 ± 182.4	0.219	36.0 ± 28.3	0.06
	2	No limpets	61.0 ± 52.3		190.0 ± 14.42		190.0 ± 14.4		4.0 ± 5.7	

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Table 2. Significance of effects of barnacle sizes and presence of limpets on the proportion removed of each size group (2-way ANOVA). Removal proportions were arcsine-transformed and weighted by the number of individuals in the size group.

Source of variation	DF	Time after application of limpets		
		1 day	2 days	3 days
Barnacle sizes	6	0.0001	0.0001	0.0001
Limpet presence	1	0.072	0.208	0.0001
Interaction	6	0.097	0.683	0.030
Total explained	13	0.0001	0.0001	0.0001
r^2		0.690	0.631	0.902

spectively. Both limpets and non-limpet detaching agents cease to affect barnacles when they reach size group 5–6 (1.4–2.1 mm diameter). *B. amphitrite* reach this size range, on the average, after 14.9–23.3 days of submergence [linear regression of size on time in water, $r^2 = 0.397$, regression coefficient = 0.083 ± 0.004 (±SE), $P < 0.001$]. Similarly, *Cellana tramoserica* can not detach *Tetraclitella purpurascens* and *Tesseropora rosea* larger than 3–4 mm in base diameter (Denley and Underwood, 1979). On the other hand, *Semibalanus balanoides* (L.) 10 days and older are liable to crushing by limpets, while smaller recruits are not damaged when limpets move over them (M. A. Kendall, pers. comm.). Finally, the intercept and the negative regression coefficient of removal rate by limpets on barnacle size, increased with number of days of limpets' activity, suggesting a cumulative effect of the limpets' movement.

Our experiments and observations suggest that *P. coerulea* limpets approximately 20 mm in diameter do not maintain artificial surfaces submerged in a port free of *B. amphitrite,* 1) by directly discouraging them from settling, 2) by rasping and thus preying upon new recruits, or 3) by knocking them over by the shell edges, but rather by repeatedly running over them with the foot, presumably during their grazing excursions. It seems that by running over a newly established, or even slightly older barnacle which is run-over repeatedly, the barnacle's hold on the substratum, as well as its general environmental resistance, are somehow weakened. Our experiments suggest that limpets may act mainly as amplifiers, rather than direct inducers, of barnacle mortality. Yet, this grants them a sufficient edge in their struggle with barnacles for space monopolization, provided they maintain ample movement. If their movements are insufficient, barnacles within their home ranges can incidentally and temporarily evade the effect of limpets, which may allow them to grow and reach a "safe" size. Movement of *P. coerulea* increases when intraspecific competition for a rich food supply is high; but also when food is scarce, yet its future acquisition if it later becomes abundant, is threatened by the presence of other limpets (Keasar and Safriel, 1994). We suggest that a similar increase in limpet movement can occur during times of heavy interspecific competition for space, i.e., during periods of heavy barnacle recruitment. Limpets may then take off from their "home scars" for "patrolling" expeditions of their home ranges, even when their nutritional condition does not require further foraging.

Settling barnacles exhibit a species-specific aggregative behavior (Crisp, 1990). Surfaces which are kept clean of barnacles by limpets may thus be less attractive for the settlement of new cyprids. This may reduce the need for limpets to perform movement aimed at removal of barnacles.

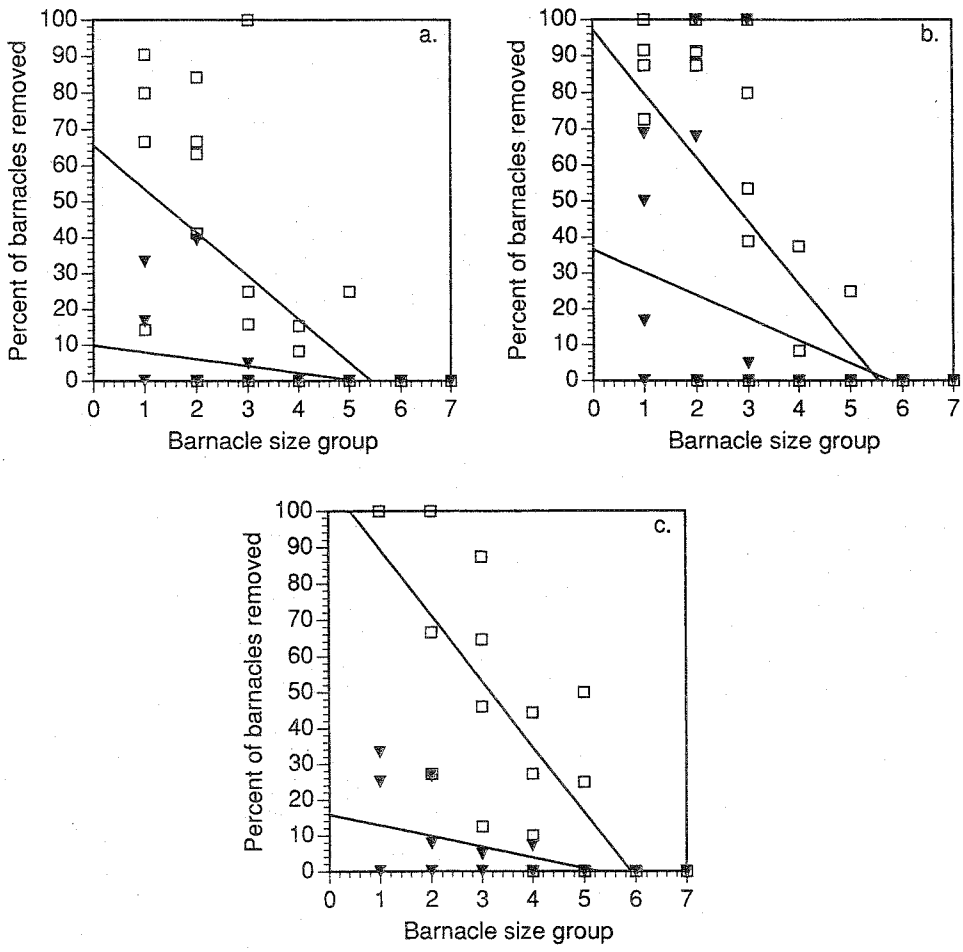

Figure 1. Detachment proportion of barnacles, as a function of their size, described by linear regression. Size groups 1–7 correspond to rostro-carinal diameters of <0.5 mm, 0.5–0.7 mm, 0.8–1.0 mm, 1.1–1.3 mm, 1.4–1.8 mm, 1.9–2.1 mm and >2.1 mm. Squares—limpet panels, triangles—non-limpet panels. a—One day after limpet application. Regression equations are $Y = -12.10X + 65.66$, $r^2 = 0.36$, $P = 0.001$ for limpet panels, $Y = -1.93X + 9.93$, $r^2 = 0.12$, $P = 0.094$ for non-limpet panels. b—Two days after limpet application. Regression equations are $Y = -17.56X + 97.34$, $r^2 = 0.49$, $P = 0.001$ for limpet panels, $Y = -6.35X + 36.63$, $r^2 = 0.13$, $P = 0.077$ for non-limpet panels. c—Three days after limpet application. Regression equations are $Y = -18.29X + 108.10$, $r^2 = 0.63$, $P = 0.001$ for limpet panels, $Y = -3.01X + 15.91$, $r^2 = 0.30$, $P = 0.01$ for non-limpet panels.

Safriel and Erez (1987) and Safriel et al. (1993) showed that limpets control marine biofouling on artificial surfaces submerged in a port. The results reported in this paper point at the mechanism by which this feat is accomplished.

Acknowledgments

This research was supported by Resources For The Future, Inc., by the Robert Szold Institute for Applied Science and the U.S.-Israel Binational Science Foundation. We would like to thank S. Pisanti, D. Litvin and J. Shalmoni for their help in the various stages of this research.

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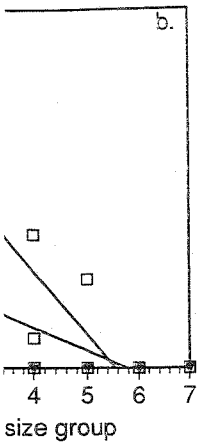

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Date Accepted: December 1, 1992.

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