Sarah A. Gravem and Steven G. Morgan. 2015
Prey state alters trait-mediated indirect interactions in rocky tidepools
Functional Ecology

Supporting Information

Appendix S1 *Experiments testing the consumptive and nonconsumptive effects of Leptasterias on varying sizes of Tegula in the laboratory*

Predation and size refuge

Methods. We determined the frequency of predation on *Tegula* and other invertebrate prey by examining which prey had been captured by 102 *Leptasterias* throughout the intertidal zone in July 2009 at the Bodega Marine Reserve at Horseshoe Cove in northern California, USA (38° 18' 59.37" N, 123° 4' 16.28" W). For each *Leptasterias*, we recorded the shore level (low, medium, high), seastar oral disc diameter (which can influence potential prey size) and prey maximum diameter (gastropods) or length (mussels and chitons).

We tested for a size refuge of *Tegula* from *Leptasterias* predation by confining 136 pairs of seastars and snails in very small (~125 cm^3) flow-through seawater compartments that minimized the ability of snails to escape in July and August 2011. Snails were collected the days before the experiment in Horseshoe Cove. Because of a local *Leptasterias* mass mortality event in November 2010 (Gravem 2015) *Leptasterias* were collected 17 km north of Horseshoe Cove at Twin Coves (38° 27' 28.83" N, 123° 8' 42.85" W). Snails were grouped into six 3-mm size class increments with 22 - 24 snails each, and half of the snails in each size class were paired with either a large (3 - 5 cm diameter) or medium sized seastar (2 - 3 cm diameter). We recorded the outcome of predator-prey encounters daily for 16 days. We analyzed the main and interactive effects of seastar and snail size on the survival of snails using a nominal logistic regression and associated likelihood ratio tests using R statistical software.

Results. Of the 102 *Leptasterias* observations in the field, only 5 seastars were consuming *Tegula* (4.9 %), and 4 of these snails were small (<12 mm). The other snail was 18.5 mm in diameter, and was being eaten by an extremely large *Leptasterias* (12.6 mm oral disc diameter and at least 50 mm in arm span). Of the 21 seastars eating, at least 6 prey types were identified (Fig. S1a) including *Littorina* spp. (29%), *Lacuna marmorata* (24%), *Tegula* (24%), limpets (10%), *Mytilus californianus* (10%) and chitons (5%).

In the laboratory, vulnerability to predation decreased with snail size (Fig. S1b; logistic regression: $X^2 = 59.5$, $p < 0.001$), and increased with *Leptasterias* size ($X^2 = 6.46$, $p = 0.011$). Small and medium snails were eaten often (76.6% and 40.0% of snails, respectively) and only occasionally survived attacks (14.9% and 15.6%, respectively). Conversely, most large snails were not attacked (63.8% of snails) but when attacked they were rarely eaten and often survived (6.4% and 29.8 % of snails, respectively). Note that our goal was to establish the size refuge rather than to estimate size-dependent consumptive effects or predation rates in the field, and these estimates likely grossly overestimated predation risk in the field where snails can flee.

Size-dependent responses of prey to predator cues

Methods. To determine if less vulnerable large snails responded less strongly to seastars than more vulnerable small snails, we determined the escape responses of snails to both tactile and waterborne chemical cues from *Leptasterias* in February 2013. Snails were collected days before the experiment from Horseshoe Cove. Because of local *Leptasterias* mass mortality events in November 2010 and August 2011 (Gravem 2015; Jurgens *et al.* 2015), *Leptasterias* were collected 104 km north of Horseshoe Cove at Point Arena (38° 54' 47.48" N, 123° 42'

37.83" W). We placed small (6 - 12 mm), medium (12 - 18 mm), and large (18 - 25 mm, within the size refuge) snails in small shallow tanks (33 x 19 cm x 4 cm depth) with each individual located in the center of a “bulls eye” with five 1-cm concentric rings. We exposed 12 - 20 snails of each size class to a tactile cue (direct contact with seastar in clean seawater), waterborne cue (seawater with dissolved chemical cues from seastars bathed for 2 hours at natural tidepool densities of ~0.41 seastars L^{-1}), or no cue (seawater alone). Once the snail emerged from its shell, we recorded the time elapsed at each 1-cm annulus and noted if snails meandered between annuli. We classified a meander as the snail turning >90^{o} within a 1-cm annulus, thereby proving a simple effective measurement of meandering during the observation period. Snails were used only once and within a day of field collection. Seawater was changed and tanks were washed after each trial to eliminate any residual cues.

The average speed (cm s^{-1}) between annnuli was recorded for each snail, excluding annuli where snails meandered since the distance inherently increased. Because speed increases with size, we calculated “size-corrected relative speed” for each snail to determine whether the snail moved faster or slower than average for its size for each cue type (mean speed of an individual / mean speed of snails in the individual’s size class for all cue types). Meandering frequency was calculated as the percent of 1-cm annuli in which meandering occurred. We analyzed the effects of seastar cue (no cue, waterborne or tactile) and snail size (small, medium or large) and their interactions on size-corrected relative speed and meandering frequency using General Linear Models (GLM) in JMP software (SAS Institute Inc., Version 9, 2010).

Results. Despite low risk of predation, large snails reacted strongly to seastar cues, moving the fastest when exposed to imminently threatening tactile cues, less when exposed to prospectively threatening waterborne cues, and slowest when no cues were present (Fig. S2a; Seastar cue x Snail size: $F_{4,138} = 5.53$, $p < 0.001$). Medium snails also responded most strongly to tactile cues relative to controls (Fig. S2a; Tukey: $p = 0.003$). Surprisingly, neither tactile nor waterborne cues increased the speed of small snails relative to controls, despite their high vulnerability to predation (Fig. S2a; Tukey: $p > 0.987$ for all comparisons). However, small snails meandered more often than large snails regardless of the cue (Fig. S2b; snail size: $F_{2,138} = 4.21$, $p = 0.017$). Overall, snails meandered most when exposed to nondirectional waterborne cues but went straightest when exposed to unidirectional tactile cues (Fig. S2b; seastar cue: $F_{2,138} = 3.16$, $p = 0.046$). However, though this was not the case for small snails, which tended to meander frequently regardless of cue type (seastar cue x snail treatment: $F_{4,138} = 2.14$, $p = 0.079$).

Literature Cited

Gravem, S.A. (2015) Linking antipredator behavior of prey to intertidal zonation and community structure in rocky tidepools. PhD dissertation PhD, University of California, Davis, CA.

Jurgens, L.J., Rogers-Bennett, L., Raimondi, P.T., Schiebelhut, L.M., Dawson, M.N., Grosberg, R.K. & Gaylord, B. (2015) Patterns of mass mortality among rocky shore invertebrates across 100 km of Northeastern Pacific Coastline. *Plos One,* **10,** e0126280.