The intertidal front lines: A comparison of the aggressive response in higher intertidal and lower intertidal clones of the anemone *Anthopleura elegantissima*

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Sessile organisms must contend with different challenges depending on their vertical position in the rocky intertidal zone. Those individuals in the lower part of their species' intertidal range typically experience greater predation and competition while their relatives in the higher intertidal are exposed to more frequent, extreme and longer lasting variation in physical factors. *Anthopleura elegantissima* are found from Baja California to Alaska and form intertidal, clonal colonies that make an aggressive response to nearby non-clonemates. We hypothesized that anemones living higher in the intertidal would have less energy to allocate towards aggression than those in the lower intertidal. We sampled clones from both the lower and higher intertidal at Cattle Point, San Juan Island, WA and, in the laboratory, pitted them in battles against individuals from a single clone from a rock ledge adjacent to the Friday Harbor Labs. Post-battle counts of acrorhagi (specialized battle tentacles), as well as time-lapse videos of the battles, were analyzed for metrics of aggression. There were significantly more acrorhagi in animals from the lower intertidal than those in the higher intertidal. No behavioral battle metric showed a significant difference between the higher intertidal and lower intertidal clones; however, in 11 of 13 metrics lower intertidal clones ranked as more aggressive. Based on the results of the individual analyses of metrics, higher intertidal animals seem to have an increased fighting efficiency, behaving as aggressively as lower intertidal animals, but with fewer acrorhagi. The rank analysis suggests that upper intertidal clones are more limited in their aggression.

Introduction

Sessile animals of the rocky intertidal have to be able to respond to wide swings in temperature with the flooding and exposure of the rocks. While those situated in tide pools experience a less dramatic swing in temperature, they have the added challenge of responding to drastic salinity changes caused by evaporation and the freshwater introduced from rainfall (Truchot and Duhamel-Jouve, 1980).

One such intertidal species is the aggregating anemone *Anthopleura elegantissima*, which is an abundant predator of the Pacific intertidal from Baja, Mexico to Alaska (Secord and Augustine, 2000). *A. elegantissima* is a clonal species that has been shown to clearly segregate based on clone (Francis, 1973a). The animals in each clonal aggregation are genetically identical to each other and genetically unique from other aggregations. Each clonal colony begins as a single polyp and can expand to occupy an area of up to 10 m^2 – 100 m^2 (Sebens, 1982). This is accomplished by binary fission, a mode of asexual reproduction. Reproduction by binary fission usually occurs during the winter months in *A. elegantissima* and is a lower risk mode of proliferating a genotype than generating gametes for sexual reproduction (Sebens, 1982). Colonies do undergo a short period of gamete development and spawning during the late Spring (Sebens, 1977).

A. elegantissima displays an aggressive response unique to the family Actiniidae that is used for the sole purpose of defending the clone. The anemones use acrorhagi, specialized battle tentacles, which are densely packed with nematocysts to attack opponents (Francis, 1973b). Acrorhagi present as white-tipped spherules are located at the fosse, the junction of the column and oral disk. They can be quite inconspicuous while the anemone is not engaged in aggression, hidden under the fifth and outer most ring of feeding tentacles but become highly visible in fighting anemones. The aggressive response unfolds in a distinct way common across all aggregations of *A. elegantissima*. The first stage of each response is distinguished by the initiation phase, in which the animal contracts the side of the oral disk closer to the opponent and begins the peristaltic movement that inflates the acrorhagi (Figure 1; Francis, 1973b). The climax of the response is the violent, down-sweeping motion of the anemone as it contracts the side of its column close to the anemone and applies the nematocyst of the acrorhagi to the ectoderm of the opponent in as fast as 90-120 seconds (Figure 2; Francis, 1973b).

The speed and range of motion required of an anemone during the aggressive response incurs some energetic cost. Anemones must spend a considerable amount of energy generating and maintaining acrorhagi and repairing ectoderm that is damaged during battle. These costs are so significant that clones have been shown to have a high level of social organization, with clone members on the edges of the clones specialized as non-reproducing, acrorhagi-laden warriors, and those in the middle being more specialized for gamete production (Francis, 1976). However, anemones occurring in the higher intertidal and lower intertidal must contend with a differing set of challenges. For example, in the Pacific Northwest, anemones in the higher intertidal can endure up to five more hours of low tide exposure daily during spring tides than those in the lower intertidal. During this time of exposure in the summer, polyps can be subjected to a prolonged increase in temperature as well as the damaging effects of UV radiation and desiccation. In the winter, a prolonged period of cold temperatures, harsh winds, and rainfall pose severe regulatory challenges.

A. elegantissima also play host to the algal endosymbionts zooxanthellae and zoochlorellae that they rely on for supplemental nutrition to the invertebrates they catch falling off the rocks (Verde and McCloskey, 2002; Bergschneider and Muller-Parker, 2008). Producing UV protection, heat shock proteins, and proteins to break down accumulating intercellular oxygen may also be especially energetically expensive for anemones in the high tide region (Richier, et al., 2008).

By contrast, anemones lower in the intertidal have to contend with desiccation and effects of temperature and UV far less. Instead, animals of the lower intertidal are in danger to predation by *Aeolidia papillosa* and *Dermasterias imbricata* (Francis, 1973b). Also, anemones in the lower intertidal, with a prolonged period of submersion, have more time to wander around the substrate, increasing their potential for bumping into anemones in another clone (nonclonemates). This likely requires a large allocation of energy to tissue repair and maintenance of protective nematocysts within acrorhagi as they defend their territory. Further, anemones in the lower intertidal tend to aggregate more loosely than those in the higher intertidal (personal observation). Those in the higher intertidal tend to have tighter aggregations that serve to decrease the overall surface area to volume ratio and thus diminish desiccation (L. Francis, personal communication, 2013). The presence of wet, cool neighbors also provides a temperature buffer (Merz, et al., unpublished data).

If the costs associated with physiological regulation are large for anemones from the high intertidal, then we anticipate that they will engage in the aggressive response less often than those in the lower intertidal. We also expect those higher intertidal anemones that do engage in aggression to display a less robust response than those in the lower intertidal.

Materials and Methods

Sample Collection

Polyps of *A. elegantissima* were collected from Cattle Point, San Juan Island, WA (48.450688,-122.966887), a rocky inner coastline on the Strait of Juan de Fuca. *A. elegantissima* live roughly between +0.0 m and +1.2 m MLLW in the intertidal (Kozloff, 1983). Twenty separate clones from the lower intertidal and twenty clones from the higher intertidal were carefully removed from substrate by hand. Lower intertidal clones were selected from just above the uppermost extent of the brown algae *Saccharina sessilis* (~0.0 m) and higher intertidal clones were selected from the uppermost reach of the barnacle range (~1.2 m). At both respective levels of the intertidal, sampling occurred at every twenty meters, unless it was clear that unrelated clones were settled fewer than twenty meters from each other. Polyps at the edge of clones were specifically selected to maintain consistency and to ensure that animals from the midclone that are more specialized for reproduction rather than aggression were not tested.

Four to six polyps from each clone sampled were immediately transported to Friday Harbor Laboratories. Each clone was placed into its own plastic container, the bottom of which was covered with light-colored bivalve shells or rocks on which they settled. The containers were placed in a sea table with flowing water and covered with mesh to prevent dispersal of the polyps. The few polyps that did escape from their containers were quarantined and not used in experiments.

Polyps of *A. elegantissima* from a single clone on the rock ledge adjacent to the Friday Harbor Laboratories (FHL clone) were collected as needed and housed in the laboratory in the same way as those from Cattle Point. These polyps were used as the common opponent against which the forty Cattle Point clones were tested.

Battles

Anemones were allowed at least 24 h to settle on the substrate in their containers. To pit a battle, a size-matched anemone from the FHL clone was placed, on its shell or rock fragment, into a container in a sea table with flowing water. Then, a polyp from one of the Cattle Point clones was transferred quickly, attached to its shell or rock fragment, from its housing container into the container with the FHL clone member. Occasionally, a polyp from either the FHL or Cattle Point clone came detached from its substrate upon transfer; the battle was still conducted, but note was taken during video playback of when the polyp had re-adhered to the substrate. Each FHL polyp was used only once. Because

A. elegantissima reproduces asexually, each individual from the FHL clone is genetically identical. Polyps pitted against each other had been in the laboratory for nine days or fewer.

Battles were filmed two at a time, in side-by-side containers from overhead by a camcorder suspended over the sea table. Battles were filmed for 12 h in time lapse at a rate of 1 frame taken every second, beginning between 9:00 am and 10:30 am, to control for any diurnal pattern or rhythm of aggression. After filming, polyps were moved to isolated containers in a sea table to recover.

Disk Measurement and Acrorhagi Count

Within 12 h of the conclusion of each battle, anemones were transferred into a glass dish filled with seawater. Measurements of the diameter of the oral disk and pedal disk were taken using calipers. Anemones were then anesthetized by adding about 25 mL of 10% $MgCl_2$ in reverse osmosis water. More $MgCl_2$ was used as needed. After an hour, anemones were sufficiently anesthetized in order to be able to fold back their feeding tentacles and view the acrorhagi without the polyp contracting. Anemones in the dish were then placed under a light microscope, and I counted the number of acrorhagi present. Acrorhagi appear as whitish beads at the base of the last ring of tentacles where it meets the column. Some acrorhagi were less defined, and presented as a more faint white tipped bead; these were counted and included in acrorhagi count, too. I also counted the number of acrorhagi present in 3-5? of the clonemates of each anemone that battled.

Video Analysis

Video footage of the battles were played back at 30 frames per second, equaling a condensed footage of 24 minutes for 12 hours of battling. Videos were analyzed for indicators and metrics of aggressiveness (Table 1). After measurements and battles were complete, animals were returned to the field.

Statistical Analysis

Because the data were not normally distributed, a Wilcoxon Rank Sum test was performed on each of the battle metrics to test for significant difference in response between higher and lower intertidal animals. Each metric was then ranked according to whether the lower intertidal polyps showed the more aggressive response and based on these rankings a chi-square analysis was performed. We assumed each battle metric to be independent from each other.

Results

There was no significant difference in the size of pedal disk in test animals from the higher and lower intertidal (t=0.1144, d.f.=1, p=0.74). The minimum oral disk diameter measured was 8.1 mm, while the maximum was 29.5 mm. The minimum pedal disk size was 17.0 mm with the maximum at 45.3 mm. Battling animals from the higher intertidal had only about half the number of acrorhagi as those from the lower intertidal (t=6.6834, d.f=1, $p<0.01$; Figure 3). This same pattern held true for non-battling clonemates (t=11.1199, d.f.=1, p=0.002; Figure 4). Statistical analyses on each of the aggression metrics showed no significant difference in any of the other metrics of aggression between those animals from the higher intertidal and those from the lower intertidal (Table 2). However, if for each of these metrics we use the mean and median values from each population to establish a rank order “winner”, a distinct pattern emerges. If there were no difference in the aggressiveness of anemones from high and low intertidal, then we would expect that the rankings of approximately half of the measured metrics of aggression would be “won” by both populations of anemones. However, in 10 of 13 metrics the animals from the lower intertidal score as more aggressive. In two metrics (time to disengage and distance towards time) the higher animals have a more aggressive rank and for one metric (distance away) there is a tie. A chi-square analysis of this pattern of aggression metrics indicated that, on average, lower intertidal animals displayed more aggressive behavior ($\chi 2=4.923$, d.f.=1, $p<0.05$; Table 3).

Discussion

While there was no significant difference between the behaviors of higher and lower intertidal clones when each metric was considered separately, the pooling of the metrics into a ranked chi-square analysis that indicates an overall more aggressive response in the lower intertidal animals raises a double-edged question. Paired with the result that animals in the higher intertidal are working with significantly fewer acrorhagi than those in the lower intertidal, we are left with two sets of questions. The analysis of the aggression separately seems to indicate that higher intertidal animals may somehow be more efficient in their battling than lower intertidal animals. They are just as aggressive and successful as those from the lower intertidal, but with less “battle” equipment. On the other hand, if we consider the more holistic analysis of the aggression metrics, the lower intertidal animals’ relatively higher aggressiveness raises the question of what makes individuals viable in the higher intertidal zone. Assuming that larvae settle at random through the intertidal, are most larva be able to successfully modify their behavior to allocate energy properly according to the physical demands of the high intertidal or will those genetically predisposed to strong aggression or an inappropriate allocation of energy be unsuccessful?

We plan to continue developing metrics for analyzing aggression. As examples, we believe looking at the anemone's speed towards or away from its opponent, the orientation of an anemone's tentacles towards or away from a nonclonemate, or the amount of time spent with tentacles retracted could be indicators of aggression that may give further confirmation of . Additionally, although only the first 12 hours of footage of each battle was analyzed for this study, we look forward to expanding our analysis to 24 hours of battle footage. This may also allow us to get a glimpse into post-battle behavior in some instances. Lastly, we plan to compare acrorhagi size between higher and lower intertidal individuals to glean further insight on the effects of the energetic allocation differences that likely exist.

The potentially diminishing capacity for aggression as a result of the abiotic stresses of the higher intertidal could bear stark implications for the viability of *A. elegantissima* as a species in the intertidal region. Helmuth, et al. (2002) shows that at Friday Harbor, in particular, intertidal animals have a disproportionate amount of midday sun exposure during the summer months than those at other sites along the West Coast. As one of the sole predators and defenders of the higher intertidal, it will be important to predict the success of *A. elegantissima* as the water and air continue to warm.

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Figures

Aggression Metric	Definition
Time to initiation	The time it takes the anemone to enter the initiation (first) phase of the aggressive response.
Percent of battles with matches	The percentage of matches in which the anemone engaged in a battle at least once.
Inflation lag	The amount of time between the point at which the anemone's acrorhagi were inflated and the point at which the anemone had entered the initiation phase of the aggressive response.
Time to 1st attack	The time it took for the anemone to make its first attack.
Disengage Time	The time at which an anemone engaged in battle visibly withdrew from the battle, either by deflating their acrorhagi, moving away, or leaning away from the opponent.
Distance towards	The net distance an anemone moved towards its opponent over the course of a match.
Distance towards time	The time at which an anemone began movement towards the opponent during a match.
Distance away	The net distance an anemone moved away from its opponent over the course of a match.
Distance away time	The time at which an anemone began movement away from the opponent during a match.
Number of acrorhagi	The number of acrorhagi present in the test animal post-battle.
Number of attacks	The total number of attacks mounted by an anemone over the course of a match.
Attack success rate	The rate at which an anemone was successfully landing attacks on the opponent, as evidenced by acrorhagi being applied to the ectoderm of the opponent.
Total tentacle contact	The number of distinct instances of tentacle contact between the two anemones. At least ten seconds had to be present between instances of tentacle contact to consider them distinct interactions.

Table 1. A description of the aggression metrics used to analyze differences in behavior between higher and lower intertidal animals. All metrics of time were corrected to the time of initial tentacle contact between the test animal and its opponent. Disengage time was corrected to the time of the first attack.

Aggression Metric	t-value	p-value
Time to initiation	0.1524	0.6952
Inflation lag	0.0149	0.9029
Time to 1st attack	0.3580	0.5496
Disengage Time	0.0030	0.9566
Distance towards	0.00	1.00
Distance towards time	0.15	0.6985
Distance away	0.0596	0.8071
Distance away time	0.1974	0.6569
Number of attacks	0.0239	0.8772
Attack success rate	1.7041	0.1917

Table 2. Statistical analysis of the above aggression metrics all yielded no significant difference in the behavior between higher and lower intertidal clones.

Aggression Metric	More Aggressive Response	Higher Intertidal Mean	Lower Intertidal Mean	Test Group Displaying More Aggressive Response
Time to initiation	faster	665 sec	428 sec	Low
Percent of matches with battles	higher	60%	75%	Low
Inflation lag	shorter	39 sec	36 sec	Low
Time to first attack	faster	236 sec	213 sec	Low
Disengage time	longer	340 sec	269 sec	High
Distance towards	greater	15 mm	17 mm	Low
Distance towards time	faster	381 sec	484 sec	High
Distance away	less	26 mm	26 mm	Tie
Distance away time	longer	187 sec	265 sec	Low
Number of attacks	more	5	7	Low
Attack Success Rate	higher	.24	.40	Low
Total tentacle contact	higher	9	7	Low
Number of Acrorhagi	more	16	32	Low

Table 3. On average, lower intertidal animals displayed the more aggressive behavior, as indicated by the rankings assigned. Times (in seconds) have not been adjusted from time-lapse speed to real time.

Figure 1. The left anemone is in the initiation (first) phase of the aggressive response. It has contracted the side of the oral disk closer to the opponent and begun the peristaltic motion needed to inflate the acrorhagi, indicated by the bulging column

Figure 2. In the anemone at right, inflated acrorhagi can be seen as white tipped, broader tentacles, below the stringier feeding tentacles, that will be used to apply nematocysts to the ectoderm of the opponent to the left.

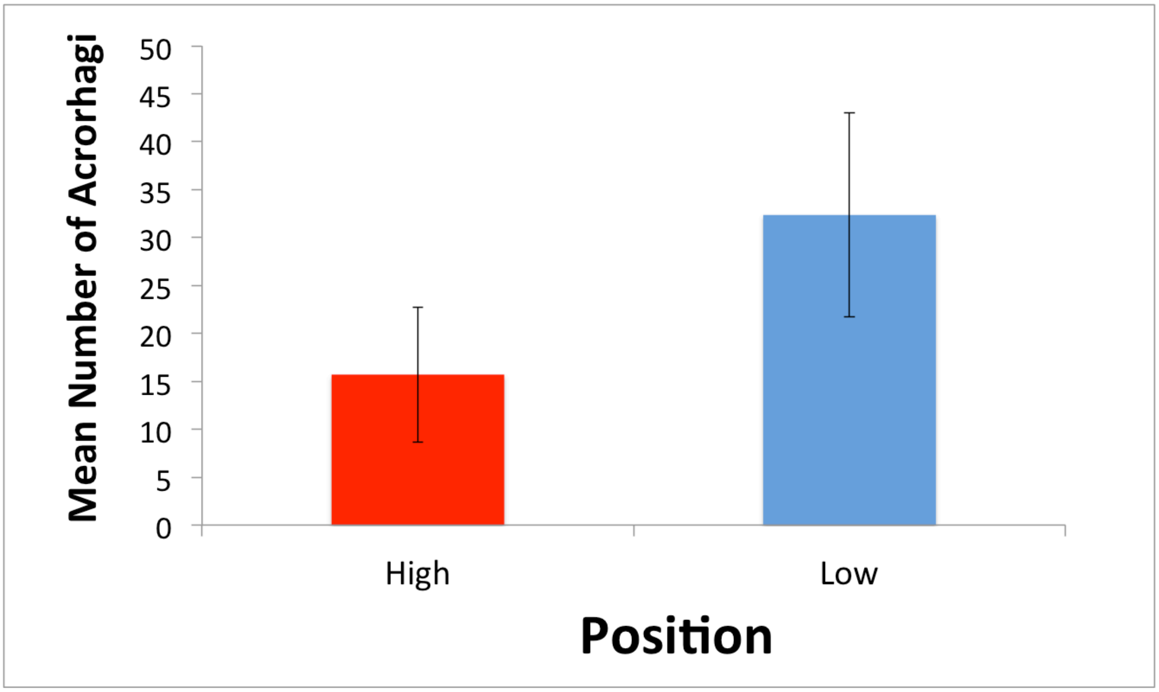

Figure 3. Anemones from the higher intertidal had significantly fewer acrorhagi than those from the lower intertidal when analyzed post-battle (t=6.6834, d.f=1, p<0.01).

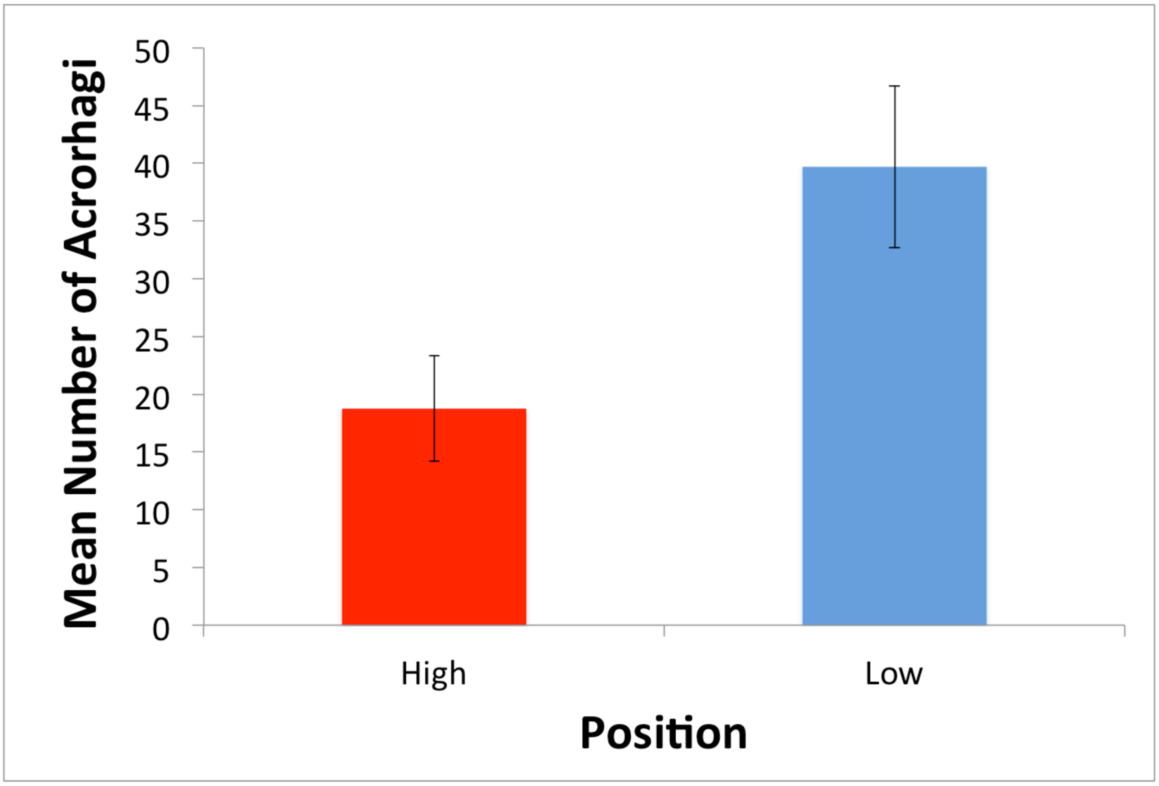

Figure 4. Of non-battling anemones from the same clone as those battled in lab, those from the higher intertidal had significantly fewer acrorhagi than those from the lower intertidal (t=11.1199, d.f.=1, p=0.002).