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Sophia Walton
Bowdoin College

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Mutual benefits of inducible defenses to crab predators in the blue mussel *Mytilus edulis* in a
multi-predator environment

An Honors Project for the Department of Biology

Sophia Walton

Bowdoin College, 2021

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ABSTRACT

The blue mussel *Mytilus edulis* alters its phenotype in species-specific ways in response to either green crab (*Carcinus maenus*) or sea star (*Asterias sp.*) predation. Previous studies have shown that only sea stars induce changes in abductor muscle morphology, while green crabs generally alter the shape and thickness of shells. In the Western Gulf of Maine, Blue mussels collected from wave protected sites with abundant green crab predators were shown to have significantly thicker shells and larger adductor muscles than mussels collected from wave exposed sites with few green crab predators. The phenotypes of mussels originating from wave-protected and high green crab abundance sites increased the handling time by *A. forbesi* compared to sites with low wave exposure and high green crab abundance. These results contradict the paradigm that shell thickness trades off with abductor morphology, and I propose that a likely candidate for increased energy allocation to these traits is a decrease in reproductive allocation. My results further suggest that the escalating “arms race” between invasive green crabs and blue mussels in the Western Gulf of Maine is leading to changes in the phenotypic response of mussel populations in ways that are likely impacting sea star foraging dynamics.

INTRODUCTION

Predators have direct impacts on their prey, damaging or killing individuals and altering the population size. However, predators also exert indirect effects on their prey that can drastically alter that prey's physiology and life history (Clark and Harvell, 1992). Some prey can ameliorate the impacts of predation through the use of inducible defenses, phenotypically plastic traits cued by exposure to predators or competitors that increase resistance to these threats but come at an energetic cost. If the risk of predation was predictable in time or space, an organism could use behavior to hide or other temporary methods to evade predation. If a particular defense strategy does not exert a high energetic cost, it may be beneficial for the organisms to have it regardless of its defense state; it protects an organism from predation, increasing the organism's fitness without a significant energy cost (Reissen, 1984). Thus, inducible defenses are found in organisms experiencing unpredictable risks of predation, and these defenses typically exert a high energetic cost (Clark and Harvell, 1992). This high energetic cost of inducible defenses can explain the ability of many species to fine-tune their defenses with respect to specific predation threat as a consequence of living in communities that have diverse predators and predator feeding modes (e.g. Smith and Jennings, 2000; Tollrian and Harvell, 1999).

An excellent system to study the effects of inducible defenses in response to a diverse guild of predators is the blue mussel *Mytilus edulis* in the Gulf of Maine. Blue mussels are a foundational species, creating a complex substratum for other organisms to inhabit (Seed, 1969; Menge, 1976) and supporting a diverse group of native and introduced predators in the Gulf of Maine. Unfortunately, the intertidal blue mussel population has declined by 60% in the Gulf of Maine since the 1970s (Sorte *et al.*, 2017) and an introduced crab predator, the European green crab (*Carcinus maenus*) is thought to be a key factor in this decline. Blue mussels are prolifically eaten by green crabs in Maine, a species originally from the Atlantic coasts of Europe. The global invasion of green crabs occurred in three main episodes, around 1800, in the 1850s-70s, and in the 1980s-90s (Carlton and Cohen, 2003). Green crabs can tolerate a wide range of salinities and temperatures, and thus have adjusted well to new habitats. The American green crab population initially ranged from New Jersey to Cape Cod, but by the 1890s, green crabs arrived in southern Maine, and by 1951, they were distributed throughout Maine and to the Canadian border (Carlton and Cohen, 2003). Dramatic increases in green crab populations

occurred in Maine in the 1930s, 1950s, and 1970s, during periods of sea-surface temperature warming (Welch, 1969). The crabs began to prey upon many intertidal bivalve species, with a significant decline in the hard-shell clam *Mercenaria arenaria* linked to the rising green crab population (Glude, 1955). Green crabs also began to eat blue mussels in the Gulf of Maine, which have been shown to use inducible defenses in areas of high green crab predation, thickening their shells and strengthening their byssal thread attachments (Leonard *et al.*, 1999). Thicker shells and stronger byssal threads have also been induced in several lab experiments through the exposure of blue mussels to green crab waterborne cues (Smith and Jennings, 2000; Freeman, 2007; Freeman *et al.*, 2009).

Table 1. Synthesis of experimental results examining the impacts of the presence of the effluent of sea stars (*Asterias rubens*), green crabs (*Carcinus maenus*), or both species, on phenotypic traits in the blue mussel (*Mytilus edulis*). 0 indicates no experimental effect, while empty cells indicated the effect has not been tested. Note that previous experiments have shown a species-specific response by abductor muscle morphology (grey shading).

Trait	<i>Sea stars</i>	<i>Green crabs</i>	Both
Abductor muscle size or weight	+/0*	0	0
Shell thickness	+/0*	+	0
Tissue weight/shell weight	+/0* (tissue weight)	+ (shell weight)	0
Size	-	0	

* Outcome depended on the study. See text for details.

Other native predators that feed on blue mussels are shorebirds and geese, seastars (primarily *Asterias forbesi* and *Asterias rubens*), and the dog whelk *Nucella lapillus*; each of these groups uses very different feeding strategies. Previous studies have shown that some phenotypic traits have species-specific responses to introduced and native predators (Table 1). In terms of the response to sea star feeding, Reimer and Tedengren (1996) found that *M. edulis* increased the size of their adductor muscles, thickened their shells, and had more meat per shell volume, but were overall smaller in the presence of *A. rubens* waterborne scent cues. Similarly, Freeman (2007) found that blue mussels developed thicker shells by decreasing their soft tissue growth,

and developed heavier abductor mussels, in the presence of sea star cues (*A. rubens*). In contrast, blue mussels have different responses to green crabs. In a complex experimental design, involving both sea stars and green crabs, Freeman et al. (2009) found that when raised in exposure to green crab cues (waterborne, downstream scent cues), blue mussels developed thicker shells but there was no significant change in adductor muscle weight compared to control mussels that were not exposed to predator cues as they were raised. This study also showed a different result from earlier experiments in terms of the blue mussel response to sea star cues, blue mussels developed heavier adductor muscles but there was no significant change in shell thickness (Table 1, +/0 effect). Further, when exposed to both green crabs and sea star cues, neither shell thickness or adductor muscle weight was significantly different. These results suggest that there are energetic trade-offs among traits (e.g. thicker shells are generally accompanied by smaller abductor muscles) and that spatial or temporal variability in predator communities will induce complex responses in blue mussel phenotypes. These induced responses can “feedback” into the predator community by impacting the feeding success and foraging rates of individual predators. For example, Freeman *et al.* (2009) could show that the induction of shell thickening in blue mussels deterred green crab predators regardless of the predator species that induced thicker shells, but only a larger abductor muscle deterred sea stars from feeding on blue mussels.

In this thesis I focus on the consequences of a suite of phenotypic traits in blue mussels that are putatively induced by the presence of green crabs on the deterrence to sea star feeding. For experimental tests of feeding rates, I use mussels collected from field sites with high and low densities of green crabs (mediated by wave energy, e.g. Leonard *et al.* [1998, 1999]), and first document variation in key phenotypic traits shown to impact feeding by both green crab and sea star predators. Previous studies have observed how adductor muscle weight changes in response to predator cues. However, here I measure cross-sectional area of the adductor muscle because it is more directly related to the strength of a muscle to withstand the constant forces generated by sea star feeding (Wilkie, 2005). Specifically, with wild caught mussels that diverge in induced phenotypes I test three hypotheses: (1) Do the inducible traits of blue mussels living at sites with high and low green crab abundance match expectations of previous experimental studies? (2) Does shell thickness trade-off with adductor muscle size? (3) Do phenotypic traits putatively induced by green crabs also deter sea star feeding?

METHODS

Ecological Field Surveys and Mussel Collection:

In order to sample mussels exposed to high and low levels of green crab predation, I selected two sites that were wave exposed and two sites that were wave protected. Leonard *et al.* (1998, 1999) characterized sites in the Damariscotta River estuary as low flow, high green crab predation and high flow, low green crab predation, and found that blue mussels thickened their shells in areas characterized by low flow and high crab predation. Similarly, in the Maine rocky intertidal mobile consumers like the green crab are less common in high flow environments (Lubchenco and Menge, 1978). Thus, I assumed that more crabs would be present at wave protected sites. In addition to collecting blue mussels for downstream morphometrics and handling times from these sites, I confirmed that mussel and green crab abundances were associated with wave exposure as expected. The two protected sites were located on the westward, and less exposed shorelines of peninsulas in the townships of Harpswell (Potts Point, “PP”) or Bristol (Chamberlin Protected, “CP”); while the two exposed sites were located on the eastern shores of these peninsulas directly exposed to ocean swell (Chamberlin Exposed, “CE”; and Giant Steps, “GS”) (Fig. 1). At low tide and at 5 meters above the water line, I laid down a 100 m transect parallel to the shore. At every 10 meters I used a quadrat a 1 m² quadrat to count the blue mussel, green crab, and sea star abundances and to estimate percent cover of the algae. These surveys were carried out in July 2020, but mussels were collected in October, right before handling time experiments so they would not spend extended periods of time in the lab. At each site, I collected ~ 25 mussels of sizes ranging from 14 mm – 44 mm. Additionally, I only collected mussels from aggregation (greater than 9 mussels in a group), as mussels in aggregations display different growth and reproductive characteristics than single mussels (Okamura, 1986).

To test for the effects of exposure on species abundance, I used a nested one-way ANOVA model for each species. In these models, the site factor was nested within the exposure factor. I used the R environment for all ANOVA and ANCOVA models reported in this paper, and for the figures.

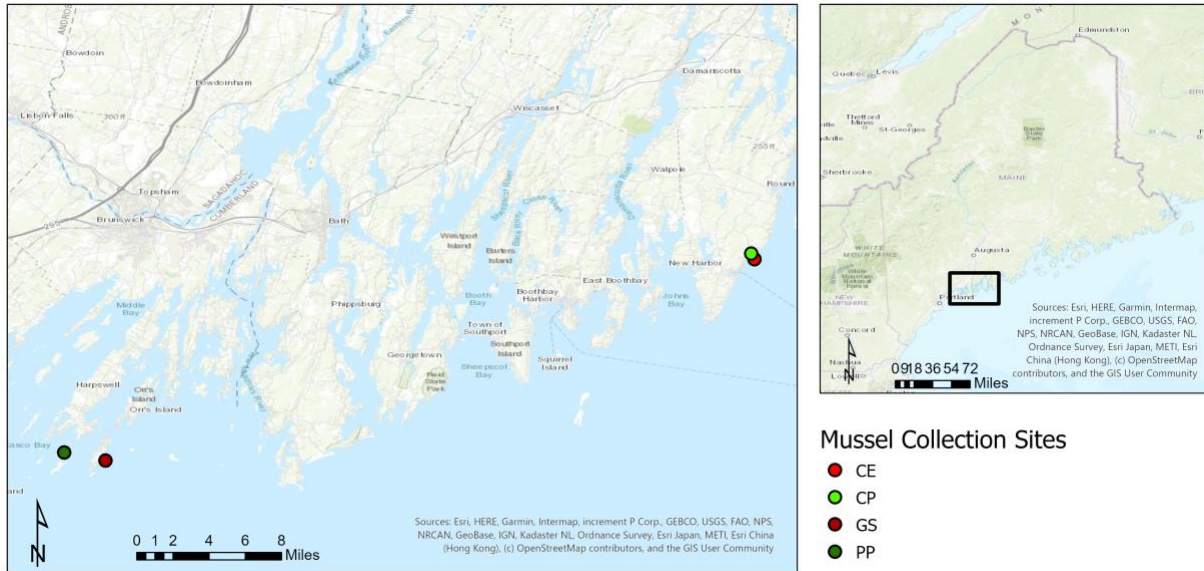


Figure 1. Locations of Mussel Collection sites in Harpswell and Chamberlin, ME shown with circles. Wave exposed sites are shown in red, and wave protected sites in green. Locator map shown on right of location of study area within ME. Maps were drawn with ArcGIS Pro.

Morphological variation among sites

I measured a number of morphological traits relevant to predator defense in blue mussels, including different measures of shell thickness and abductor muscle area. I made these measurements on N= 10 blue mussels for each site. To determine the cross-sectional area of the largest abductor mussel, I cross-sectioned the posterior adductor muscle at its midpoint and took digital images of the planar area under a dissecting microscope (1 image per sectioned muscle). From these images, area of the cross section was measured using ImageJ software. After removing all tissue from each valve, for each valve I measured the diameter of the adductor muscle scar, the thickness at the growing lip margin, the thickness of the center of the shell, and at the thickness of the umbo using sliding vernier calipers. These measurements were the same as those described in Leonard *et al.* (1999). I also measured the wet weight of the whole mussel by removing excess moisture from the dissected tissue and weighing to the nearest milligram.

Since all of these morphological traits scale positively with size, I used ANCOVA models to test for differences in each variable among sites or exposure, with shell length as a covariate. The ecological surveys indicated an association between wave exposure and crab abundance among the four sites, e.g. higher crab abundance at wave protected sites compared to protected sites (see RESULTS: Fig 2a, Table 2). To test for effects of different crab abundances

on attributes of shell morphology that are important in defending against predation, I used a series of ANCOVA models of shell traits with mussel size as a co-variate. In these models, the different sites (and associated differences in crab abundance) could impact morphological traits in several ways. First, the effect of the sites could scale differently with size (i.e. the slopes of the regressions would differ) as indicated by a significant interaction term in the ANCOVA model. Second, the effect could scale the same way with size (i.e. the slopes of the regression are the same) but impact the magnitude of the y-intercept. This outcome would be supported by a non-significant interaction term and a significant site term. Lastly, I calculated the slope values and standard errors for each regression individually, to assess the statistical difference among slope.

Effects of morphological variation on sea star handling time

I hypothesized that morphological traits induced by green crabs would also deter predation by sea stars by increasing handling time. To test this hypothesis, I used an experimental design with the sea star *Asterias forbesi* where I compared the effects of variation in the morphological traits among exposures (and therefore high and low green crab abundance) on consumption rates. I controlled for the effects of size differences between sea star predators and their mussel prey on handling time (e.g. Hummel *et al.* 2011) by using three categories of sized-matched pairs that produced the most efficient handling times reported in this paper. The three pair size-classes are listed in Table 2. Individual sea stars (*Asterias forbesi*) were reused in this experiment but were always given sufficient time between handling time trials without feeding to maintain foraging behavior (mean = 5 days). Handling times were quantified with cameras (GoPro, Inc., HERO8 Black model, San Mateo, California) taking time-lapse images every 60 seconds (on linear lens setting). From these time series I could determine the start and end time of feeding trials. I defined the start time as time at which the sea star first touches the mussel, and end time as the time at which the sea star releases and no longer contacts the mussel or mussel fragments. To test for the effects of size category and site on handling time, I used a two-way ANOVA model assuming size and site were fixed factors. Tukey's post-hoc method was used for means comparisons within each factor.

Table 2. The size ranges of mussels and sea stars within three pair size categories.

Pair size category	Mussel length (mm)	Sea star arm length (mm)
1	14-22	25-45
2	23-35	50-70
3	36-44	75-95

RESULTS

Ecological Field Surveys

The two exposed sites (CE and GS) had higher mussel abundances and lower crab abundances than the two protected sites (CP and PP), which had lower mussel abundances and higher crab abundances (Fig. 2a, b). For both green crab and mussel abundance data, the nested one-way ANOVA models showed a nonsignificant nested effect, but significant main effect (Table 3). These results indicate that wave exposure had a significant effect on abundance. In contrast both the nested one-way models for the kelp species *Ascophyllum nodosum*, and *Fucus sp.* revealed a significant nested effect that was apparent in their abundances at each site (Fig. 2c, d). For these data, a second analysis with one-way ANOVA models followed by post-hoc means comparisons tested for an effect of sites on abundance. The effect of site was significant in both one-way models (*A. nodosum*, $F_{3,36} = 58$, $P < 0.001$; *Fucus sp.* $F_{3,36}=27.24$, $P < 0.001$) and means comparisons revealed different significant groupings that depended on species (Fig. 2, Appendix A: Table 7). For *A. nodosum*, the two protected sites had similar and high abundance, while this group was different from both exposed sites, and there was more *A. nodosum* at Giant Stairs (GS) than Chamberlin exposed (CE). For *Fucus sp.* the two protected sites (CP and PP) had similar and low abundance, while this group was different from both exposed sites (GS and CE), and there was more *Fucus sp.* at Chamberlin exposed (CE) than Giant stairs (GS). No sea stars were counted at any of the four sites, but it is unknown if they occur sub-tidally at these sites.

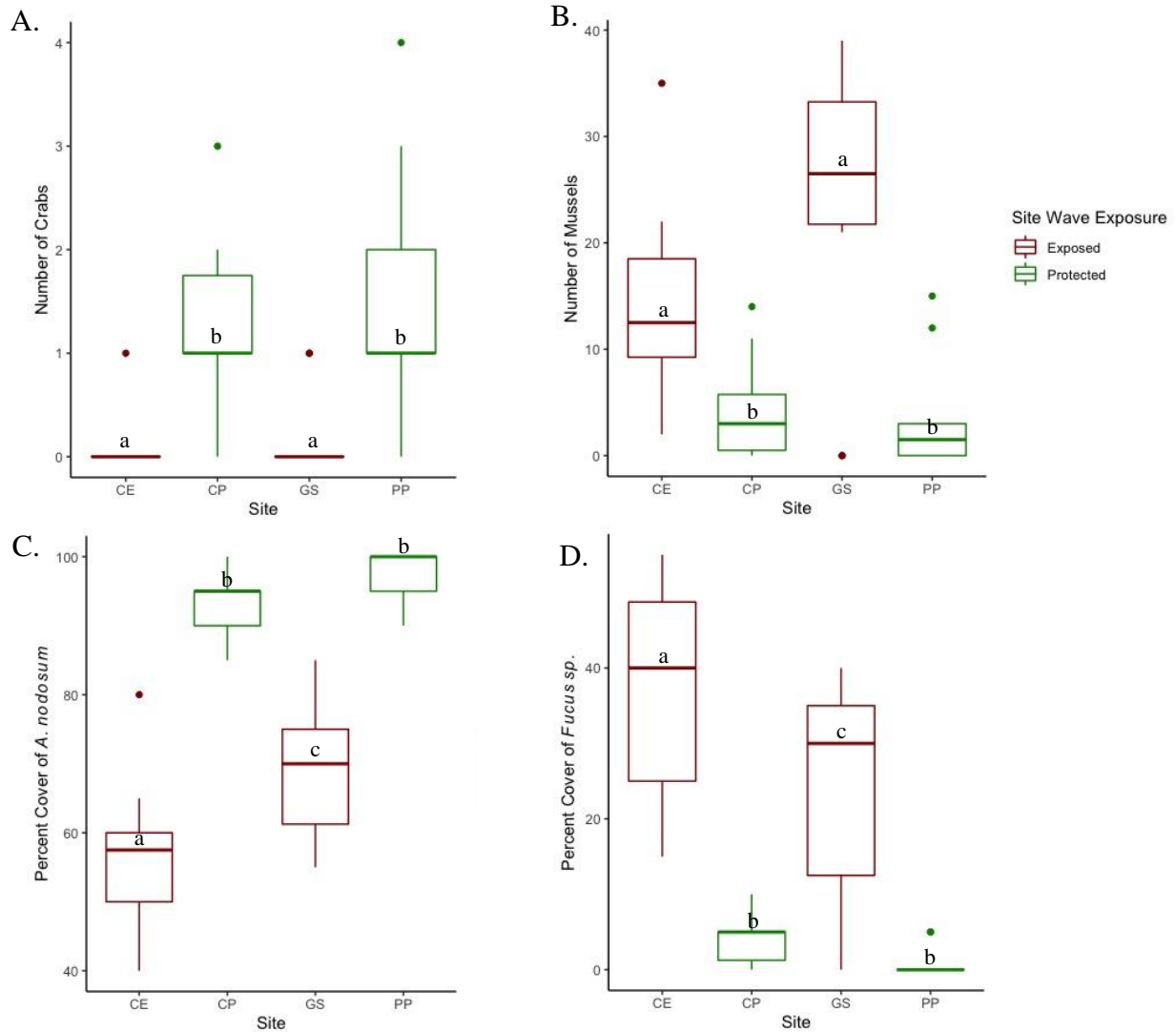


Figure 2. Box plots of the abundance of a. *Carcinus maenus*, b. *Mytilus edulis*, c. *Ascophyllum nodosum*, and d. *Fucus sp.*. Counts were recorded from quadrat sampling at each site (number of individuals for crabs and mussels, percent cover for the algal species). Unique lower case letters above median bars indicate groups that are significantly different from one another. There were N= 10 quadrats counted at each site.

Table 3. Results of Nested ANOVA analysis to compare the number/percent cover of species measured in quadrats along a 100m transect at each site (number of crabs and mussels counted in each quadrat, percent cover estimated for algal species). Since the nested factor was significant for *A. nodosum* and *Fucus sp.* percent cover at each site, the data were also analyzed with a one-way ANOVA followed by post-hoc means comparisons. See Appendix A for the ANOVA tables and results of the means comparisons.

Species	Source	df	SS	MS	F	P
<i>C. maenus</i>	exposure	1	14.4	14.4	21.073	< 0.001
	site (exposure)	2	0.5	0.25	0.366	0.696
<i>M. edulis</i>	exposure	1	2295.2	2295.2	27.58	< 0.001
	site (exposure)	2	454.4	227.2	2.73	0.0787
<i>A. nodosum</i>	exposure	1	10401	10401	159.498	< 0.001
	site (exposure)	2	946	473	7.256	0.00225
	site	3	11347	3782	58	< 0.001
<i>Fucus sp.</i>	exposure	1	8266	8266	73.246	< 0.001
	site (exposure)	2	956	478	4.237	0.0223
	site	3	9222	3074	27.24	< 0.001

Morphological variation among sites

Shell Thickness

For all three measures of shell thickness (umbo, center of shell, and growing lip margin), mussels from sites with more green crabs, had greater shell thickness for a given shell size than mussels from sites where green crabs were rare (Fig. 3 a - c). The Shell length (SL) x Site term was significant in all three ANCOVAs involving shell thickness variables (Table 4), and inspection of the plots in Figure 3 a – c indicates that in general the slope values were greater for the low-crab abundance sites (CP and PP) than the high-crab density sites (GS and CE). Further, the magnitudes of the slopes for the two low-crab abundance sites were generally significantly different than the magnitude of the slopes for the two high-crab abundance sites (Table 5).

Table 4. ANCOVA results of the site effect for three measurements of shell thickness and adductor cross-sectional area, with shell length as the covariate N= 10 mussels from each site. There is also a second set of ANCOVA results for adductor cross-sectional area, with adductor weight as the covariate. Note significant interaction term (shaded) in each model.

Variable	Source	df	SS	MS	F	P
Umbo	Shell length (SL)	1	5.546	5.546	252.4	<0.001
	Site	3	2.396	0.799	36.35	<0.001
	SL x Site	3	0.383	0.128	5.814	0.00264
	Residuals	33	0.725	0.022		
Center of Shell	SL	1	1.446	1.446	120.4	<0.001
	Site	3	1.729	0.5764	48.01	<0.001
	SL x Site	3	0.2582	0.0861	7.167	<0.001
	Residuals	33	0.3962	0.0120		
Growing Lip Margin	SL	1	0.3692	0.3692	78.89	<0.001
	Site	3	0.7772	0.2591	55.35	<0.001
	SL x Site	3	0.0693	0.0231	4.932	0.00613
	Residuals	33	0.1545	0.0047		
Adductor Cross-Sectional Area	SL	1	5230	5230	311.9	<0.001
	Site	3	1648	549	32.76	<0.001
	SL x Site	3	260	87	5.171	0.00486
	Residuals	33	553	17		
Adductor Cross-Sectional Area	Adductor Weight (AW)	1	6191	6191	260.6	<0.001
	Site	3	639	213	8.962	<0.001
	AW x Site	3	78	26	1.093	0.3657
	Residuals	33	784	24		

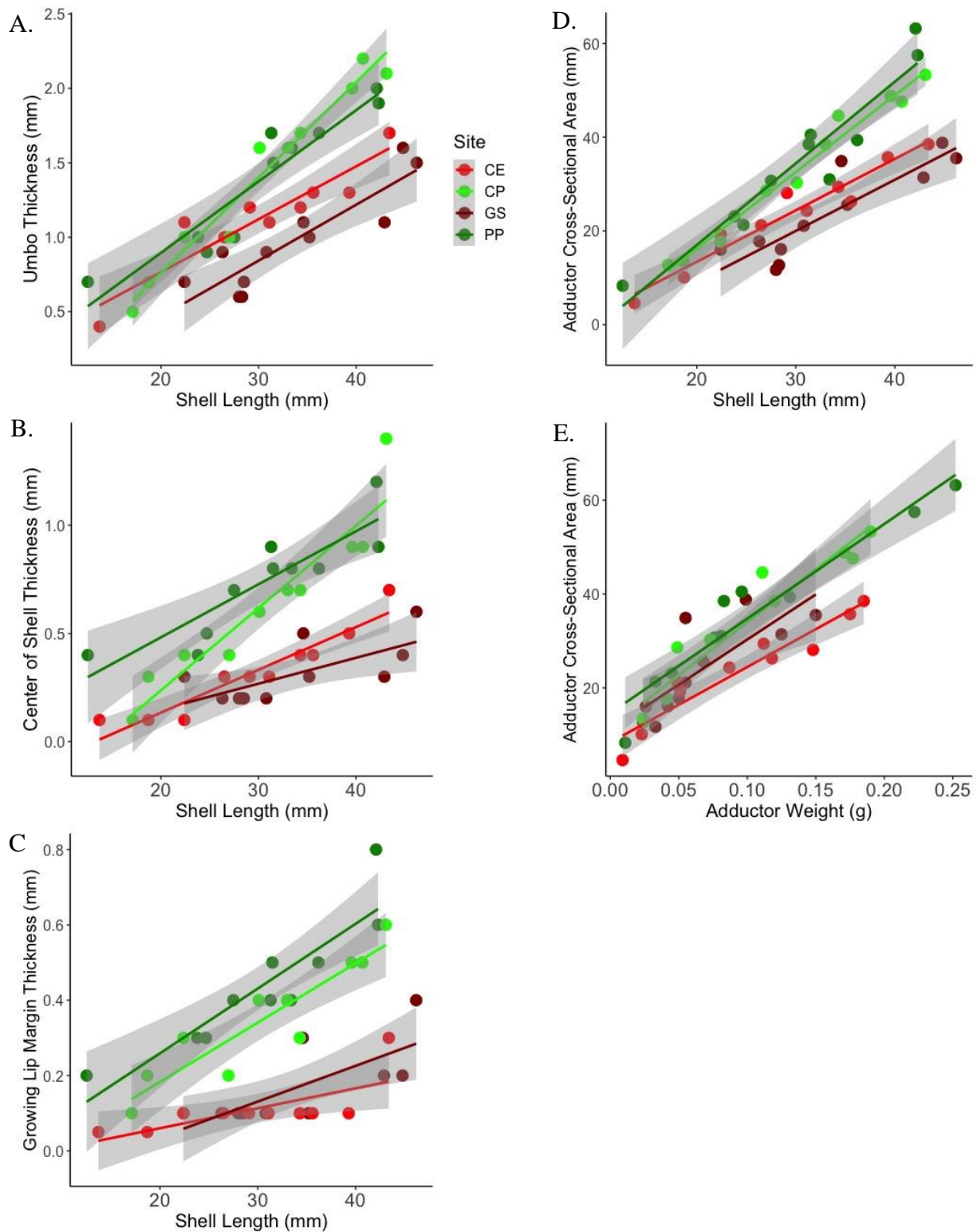


Figure 3. Shell thickness variables (a-c) and adductor cross-sectional area (d) versus shell length. The relationship between adductor cross-sectional area and weight is shown in panel e. Lines of best fit and SE around lines shown for each site. Colors of lines and circles indicate wave exposure: red - exposed, green - protected). N=10 mussels for each site.

Table 5. Regression coefficients and their error. See figure 3 for scatter plots and best fit line.

Variable	Site	Slope	95% CIs
Umbo	CE	0.0355	0.0261-0.0449
	GS	0.0376	0.0250-0.0502
	CP	0.0641	0.0549-0.0733
	PP	0.0479	0.0353-0.0605
Center of Shell	CE	0.0198	0.0152-0.0244
	GS	0.0120	0.00382-0.0202
	CP	0.0380	0.0284-0.0476
	PP	0.0245	0.0169-0.0330
Growing Lip Margin	CE	0.00530	0.00152-0.00908
	GS	0.00949	0.00379-0.0152
	CP	0.0157	0.0109-0.0205
	PP	0.0172	0.0114-0.0230
Adductor Cross-Sectional Area (w/ shell length as dependent variable)	CE	1.097	0.899-1.295
	GS	1.091	0.711-1.471
	CP	1.636	1.472-1.808
	PP	1.741	1.541-2.041
Adductor Cross-Sectional Area (w/ Adductor Weight as dependent variable)	CE	161.0	124.18-197.82
	GS	192.1	97.42-286.78
	CP	225.7	179.18-272.22
	PP	201.7	161.6-241.8

Abductor Muscle Size

The cross-sectional area of the adductor muscle is of primary interest because it determines the strength of the muscle, and therefore the resistance to prying open the shell by crab predators (e.g. both crabs and sea stars, see Introduction). ANCOVA analysis comparing adductor cross sectional area between the four sampling sites with adductor weight as a covariate showed a nonsignificant adductor weight x site interaction (Table 4), and inspection of the slopes between all sites shows no statically significant difference (Table 5). Thus, the relationship between adductor weight and cross-sectional area was not significant different between the sites, and an

increase in cross-sectional area can be assumed to correspond to an increase in weight for adductor muscles from mussels at all sites sampled. For cross-sectional area with shell length as a covariate, ANCOVA analysis shows a significant size x site interaction (Table 4), and inspection of the slopes between the high- and low-crab density sites suggests a significantly greater slope at the wave protected sites (CP and PP) than at the wave exposed sites (CE and GS; Table 5). I tested this hypothesis by combining data from the two sites within each wave exposure, and running an ANCOVA model. This analysis also indicated a significant crab-density (or wave exposure) x surface area interaction ($F=32.76$, $P<0.001$), indicating that surface areas are increasing at faster rate with mussel size at sites where green crabs are common (Fig 3d).

Effects of morphological variation on sea star handling time

There was a clear effect of location on handling times, and also an effect of sea star-mussel size categories (Fig. 4b). A two-way ANOVA model testing the effects of sea star-mussel size categories and location revealed that both of these factors significantly impacted handling time, but there was no significant interaction between these factors (Table 6). Further means comparisons revealed that the largest pairs of sea stars and mussels (size category 3) had the longest sea star handling time, and this effect was particularly significant for mussels collected from the high green crab abundance locations (Fig. 4b, 4c). In terms of the differences in locations, the two high crab abundance locations (PP and CP) had significantly higher handling times than the two low crab abundance locations (GS and CE; Fig 4a). Thus, sea stars took significant longer to consume mussels from the two high crab abundance sites than mussels from the two low crab abundance sites.

Table 6. Two-way ANOVA model of handling time with location and size category as factors. N=40 (10 mussels from each site).

Variable	Source	df	SS	MS	F	P
Handling Time	Location	3	987300	329100	54.938	< 0.001
	Size Category	2	104200	52120	8.701	0.0012
	Location x Size Category	6	78130	13020	2.174	0.07595
	Residuals	28	167700	5990		

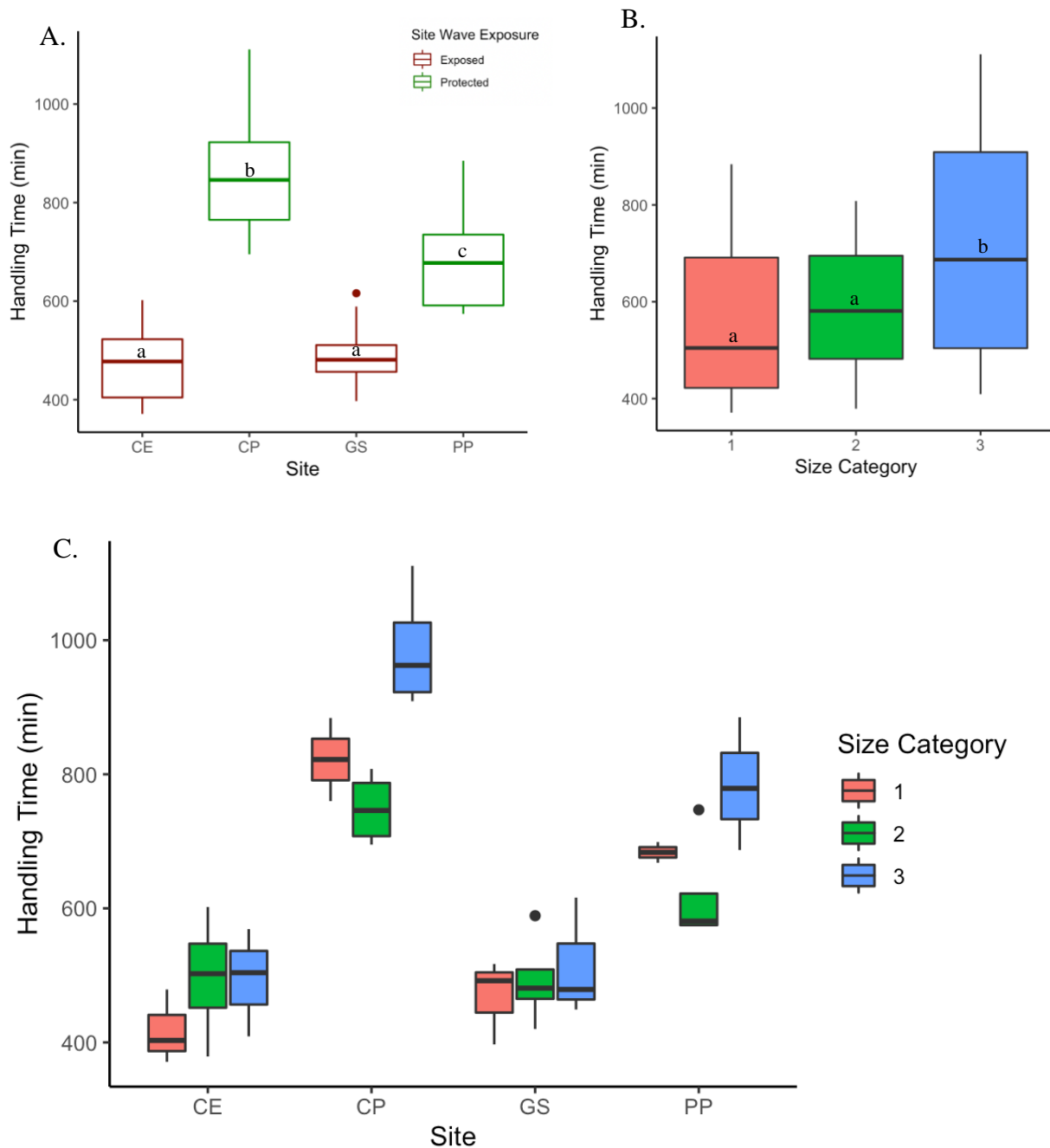


Figure 4. Box and Whisker plots of handling time of mussels from each site by the sea star *Asterias forbesi*. Panel A represents handling time of mussels from each site, with the color of each box plot representing its wave exposure (or number of crabs present). Panel B shows handling time for each site broken down into the three size categories used to assign mussels to sea stars. Unique lower case letters above median bars indicate groups that are significantly different from one another. N=40 (10 quadrat samples performed at each site). See text for interpretation of the size category differences.

To confirm the efficacy of the sea star-mussel size category sorting, regression analysis was performed on handling time results for mussels from each location versus sea star length, and versus mussel shell length (Table 7). If the size categories were effective, then the slopes of these regression lines would not be significantly different from zero, the confidence interval would include zero. This proved true for all the handling time versus size relationships at all sites except the handling time of mussels from CE versus mussel size. In this case, the lower end of the possible range of slopes based on the calculated standard error was 0.702, so small, but not significant different at zero. Thus, there was a small size effect at this site. This data is shown graphically in Figure 5.

Table 7. Slope and slope standard error from regression analysis of handling time versus sea star length and mussel shell length. N=40 (10 per site). Left mussel shell length used for used for analysis involving mussel length.

Variable	Site	Slope	95% CIs
Sea Star Length	CE	3.677	-0.901-8.255
	GS	1.842	-0.998-4.682
	CP	2.502	-1.188-6.462
	PP	1.094	-1.404-3.592
Mussel Shell Length	CE	7.246	-1.844-16.336
	GS	5.894	0.702-11.085
	CP	3.830	-3.228-10.888
	PP	1.615	-3.345-6.575

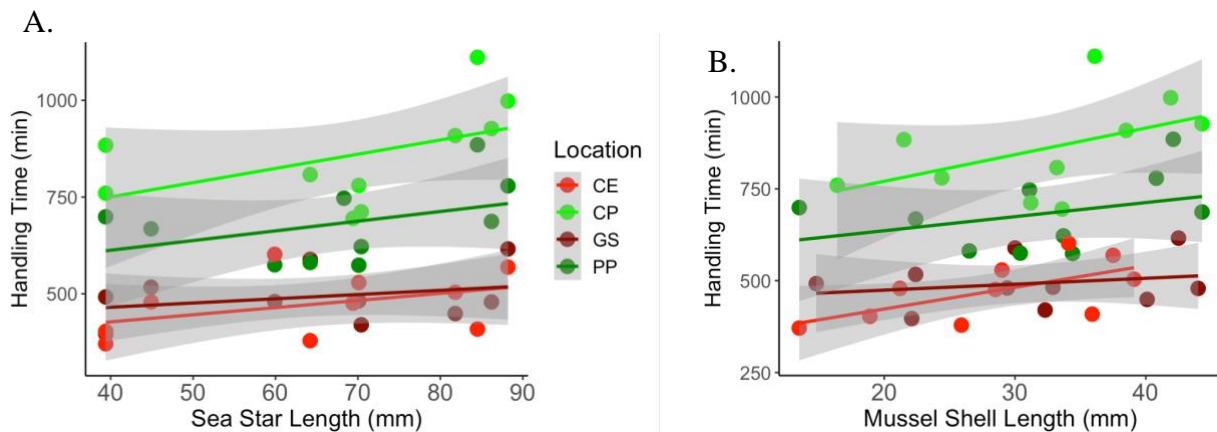


Figure 5. Sea star handling time of mussels versus sea star (A) and mussel (B) length. Lines of best fit and SE around lines shown for each site. Colors of lines and circles indicate wave exposure: red is wave exposed, green is wave protected). N=40.

DISCUSSION

My results suggest that high abundance of green crabs is inducing changes in both shell shape and abductor muscle morphology, in ways that also have strong positive effects on defense from sea stars by increasing their handling time. While it is unknown how both of these traits influence defense from green crab predators, previous studies demonstrated that shell thickness deterred chipping green crab predators (Boulding, 1984; Freeman, 2007) and larger abductor surface areas are known to increase the force required to pry bivalve valves open (Reimer and Tenendren, 1996; Freeman, 2007). These patterns suggest that in the escalating arms race between blue mussels and green crabs in the Gulf of Maine, influenced by warmer seawater temperatures in the last 20 years (Pershing *et al.*, 2015) and higher green crab abundance (Tan and Beal 2015), adductor muscles may now be playing more of a role in preventing green crab predation than they did 14 years ago. By holding the shell together more tightly, it makes it more difficult for a crab to access the mussel tissue even after the shell is cracked. Thus, blue mussels may have advanced their defense, inducing larger adductor muscles as well as thicker shells to combat green crab predation. It is also possible that sea stars may be present at these sites subtidally, influencing the results. Further, I did not account for the presence of *Nucella lapillus*. However, it is unlikely either are the sole cause for the significant difference in shell thickness and adductor muscle area seen between mussels from sites with high and low crab abundance. When *Asterias forbesi* abundance is high in the subtidal, these sea stars are also often found in the lower intertidal (Menge, 1979) and I failed to count any *A. forbesi* in the low intertidal at any of my sites. Additionally, Freeman (2007) found that *M. edulis* did not have significantly thicker shells or heavier adductor muscles when exposed to green crabs and sea star cues simultaneously than control mussels exposed to no predator cues. Further, Freeman (2007) found no changes in significant adductor muscle weight in response to *N. lapillus* cues. Therefore, this indicates that the thicker shells and larger adductor muscles found in mussels from Potts Point and Chamberlin Protected are unlikely caused by the presence of green crabs and sea stars simultaneously or by *N. lapillus*, and rather predominately by the presence of green crabs.

Both Freeman (2007), and Reimer and Tedengren (1996), found that strengthened adductor muscles were an effective defense against sea star predation: when mussels induced larger adductor muscles in the presence of *A. rubens*, the handling time increased. My results also show

that adductor muscle size is significantly larger (when measured as surface area) in the mussels with thicker shells, collected from the sites with more *C. maenus* present. Further, given the larger adductor muscles, sea stars (*A. forbesi*) took significantly longer to eat blue mussels from the two sites with more crabs (CP and PP; Fig 2a; Table 3; Fig 4). Further, blue mussels and sea stars were matched using size categories based on sea star predation preference for mussel sizes (Table 1; Hummel *et al.*, 2011). While there was no significant difference in the handling time between size categories 1 and 2, the largest mussels (size category 3) took significantly longer to eat (Fig 4b). Interestingly, this effect seems strongest at the sites with more crabs (Potts Point and Chamberlin Protected; Fig 4c). Figure 3d and Table 4 show that the cross-sectional area of adductor muscles from mussels collected from the protected sites increases faster proportional to mussel length than the adductor area of mussels from the exposed sites. Mussels at the high crab abundance sites had bigger adductor muscles proportional to their shell length, and therefore stronger adductor muscles at a given shell length than mussels from low crab sites. Figure 3d also reveals that the adductor area does not appear much larger in mussels from protected sites in the smaller shell sizes. However, at a shell size on the high end of the overall range (~ 40 mm length), the adductor area is ~ 1.5 times larger in surface area in mussels from Potts Point and Chamberlin Protected than in mussels from Giant Steps and Chamberlin Exposed. This effect may explain the results discussed above and shown in Figure 4c: mussels from Potts Point and Chamberlin Protected in the largest size category have the largest adductor muscles proportional to their shell lengths, and therefore take much longer to eat by sea stars and perhaps by green crabs as well.

Other environmental factors, beyond diverse predator communities, influence shell shape and thickness. For example, varying flow speed has been suggested to change the shape of *Mytilus galloprovincialis*, a mussel closely related to *M. edulis* (Steffani and Branch, 2003). This seems likely to have ramifications for handling time that have yet to be examined. Steffani and Branch (2003) also did not separate the effects of flow and predation on shell thickness. On the other hand, the evidence of shell thickening as an inducible defense to predation—in blue mussels and other species—is widely supported by literature (see studies cited in Leonard *et al.* 1999). While experimental studies have clearly validated the idea that predators and their cues modulate key shell morphological traits (references as above), water flow has yet to be rigorously incorporated

into an experimental design that can parse the relative effects of these two important environmental factors.

While it is known that most inducible defenses have “costs” to other traits (e.g. Harvell, 1992; Clark and Harvell, 1992; Selden *et al.*, 2009; Tollrian, 1995), the results of this study indicate that the increased energy required to build thicker shells is not compensated by reductions in adductor muscle size. On the other hand, reproduction becomes the most likely candidate to be a “compensator” in the Gulf of Maine arms race, and allocation to reproduction may be decreasing in order to create thicker shells *and* stronger adductor muscles in the presence of green crabs. In declining intertidal blue mussel populations in the Gulf of Maine (Sorte *et al.*, 2017), indirect impacts on reproduction are important to consider. Less energy for reproduction may cause a shorter spawning season, lower fecundity, and/or less energy investment into each gamete. This could have catastrophic effects for the blue mussel population, quickening their decline. Additionally, the impacts of the induction of this array of traits by invasive green crabs may have cascading effects on populations of sea star consumers like *A. forbesi*. A once common prey item is now becoming more rare, more difficult to consume, and sea stars must compete with a rapidly growing green crab population for these prey. Thus, I would predict lower sea star growth rates at protected sites where green crabs are common.

In summary, my study indicates that a general inducible response that is likely to be largely driven by green crab predators is also increasing defense to sea stars. Given that the introduction of green crabs to the Gulf of Maine and New England is driving an arm race in many prey species that can be observed on experimental (e.g. phenotypic plasticity; Smith and Jennings, 2000; Reimer and Harms-Ringdahl, 2001; Freeman, 2007; Freeman *et al.*, 2009) and evolutionary time scales of a few hundred years (Leonard *et al.*, 1999), this multi-predator system continues to provide a number of insights into the speed and diversity of evolutionary responses. Other environmental factors (e.g. flow regimes, increasing ocean acidity) must also introduce complexity into phenotypic variation, its consequences on energy allocation, and the feeding rates of different functional groups. Consequently, blue mussels provide a tractable experimental model to further parse these important environmental effects on ecological performance.

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APPENDIX A

Table 7. Means Comparison calculated for *Ascophyllum nodosum* and *Fucus sp.* percent cover at each site. N=10 quadrat samples per site.

Site Comparison	Species	diff	lwr	upr	P
CP-CE	<i>A. nodosum</i>	36.5	26.77	46.23	<0.001
GS-CE	<i>A. nodosum</i>	13.0	3.274	22.73	0.005039
PP-CE	<i>A. nodosum</i>	41.0	31.27	50.73	<0.001
GS-CP	<i>A. nodosum</i>	-23.5	-33.23	-13.77	<0.001
PP-CP	<i>A. nodosum</i>	4.5	-5.226	14.23	0.6023
PP-GS	<i>A. nodosum</i>	28.0	18.27	37.73	<0.001
CP-CE	<i>Fucus sp.</i>	-34.0	-46.79	-21.21	<0.001
GS-CE	<i>Fucus sp.</i>	-13.5	-26.29	-0.7052	0.03534
PP-CE	<i>Fucus sp.</i>	-37.0	-49.79	-24.21	<0.001
GS-CP	<i>Fucus sp.</i>	20.5	7.705	33.29	<0.001
PP-CP	<i>Fucus sp.</i>	-3.0	-15.79	9.795	0.9212
PP-GS	<i>Fucus sp.</i>	-23.5	-36.29	-10.71	<0.001