Shell Variability in *Littorina obtusata* Along the Eastern Canadian Intertidal in Response to Crab Predation and Novel Niches

By

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ABSTRACT

Predation is a pervasive biological process that shapes species-species interactions and morphological adaptations within populations and across ecosystems. Observing the dynamics of predator-prey interactions within the intertidal remains difficult, although not impossible.

In this study, I observed how crab-induced shell injuries of the Flat Periwinkle (*Littorina obtusata)* vary among abiotic and biotic conditions along the intertidal communities of Newfoundland and Nova Scotia. Extending from the observations of 21,613 shells retrieved from 92 localities upon various macrophytes (*Ascophyllum nodosum, Fucus vesiculosus, F. serratus, F. edentatus*), my results indicated the following. 1) *L. obtusata* in Nova Scotia exhibited a two-fold increase in injury prevalence compared to those found in Newfoundland. 2) Between localities, categories algae cover, and intertidal slope were not significant in explaining the differences observed in shell injury, however wave exposure did effect the outcome of shell injury depending on province. 3) Substrates varied in degrees of shell injury accumulation. 4) Shell injury prevalence was significantly dependant on macrophyte type in 14 localities, although, shell injuries overall were more prevalent if collected from the *F.serratus*.
5) The presence of shell injuries dependant on shell colour varied dependant on macrophyte type, with some localities exhibiting increased frequencies of injury dependant on shell colour and their fucoid background. Through these preliminary findings, I argue that select abiotic and biotic factors influence the outcomes of predator-prey interactions and highlight the complex nature of trait directionality.

Keywords: *L. obtusata*, Algal species, Unsuccessful crab-induced injuries, *F. vesiculosus, A. nodosum, F. serratus, F. edentatus,* Shell colour

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
NFLD	Newfoundland
NS	Nova Scotia
ASCO	*Ascophyllum nodosum*
FV	*Fucus vesiculosus*
FS	*Fucus serratus*
FE	*Fucus edentatus*

INTRODUCTION

Predator/Prey Dynamics

Predation is a dynamic biological interaction that spans various ecosystems, shaping species interactions and driving morphological plasticity within populations (Abrahams *et al.,* 2007; Arnett & Kinnison, 2017). By understanding the implications of predator-prey interactions, both the timing and mechanisms underlying morphological variations in prey organisms can be discerned.

In the context of marine gastropods, gathering information on predator-prey dynamics through direct observations in nature remains a challenge. However, valuable evidence regarding crab predation on gastropods can be gathered through analysis of scar frequencies incurred during shell regrowth from unsuccessful predation (Reimchen, 1974; Vermeji, 1980; 1983). As such, shell characteristics allow researchers to extrapolate how habitat variation affects the prevalence of interactions between gastropods and their predators. Along the coast of Newfoundland and Nova Scotia, quantitative field data regarding predation ecology is sparse. To address this gap, this thesis aims to investigate how environmental conditions can account for variations in shell injuries through analysis of Flat Periwinkle (*Littorina obtusata*) shells.

The Intertidal Zones of Newfoundland & Nova Scotia

The intertidal zones along the coastlines of Newfoundland and Nova Scotia exhibit remarkable diversity, with each area characterized by unique features and environmental conditions. From the substratum composition of rock, bedrock, mud, pebbles, and sand to the density of macrophyte species and the slope and levels of wave exposure of the beach itself, a variety of factors contribute to the distinctiveness of these intertidal communities. Native macrophytes, such as the commonly dark green *A. nodosum* and olive-green to dark brown *F. vesiculosus*, are dominant in many intertidal regions of Newfoundland and Nova Scotia (Wilbur & Steneck, 1999). These species have adapted to thrive in the challenging intertidal environment, playing crucial roles in ecosystem structure and function. However, within select shores of Nova Scotia, an invasive species, *F. serratus*, persists below the typical intertidal zonation of these native species and grows in higher densities throughout the lower intertidal (Figure 1).

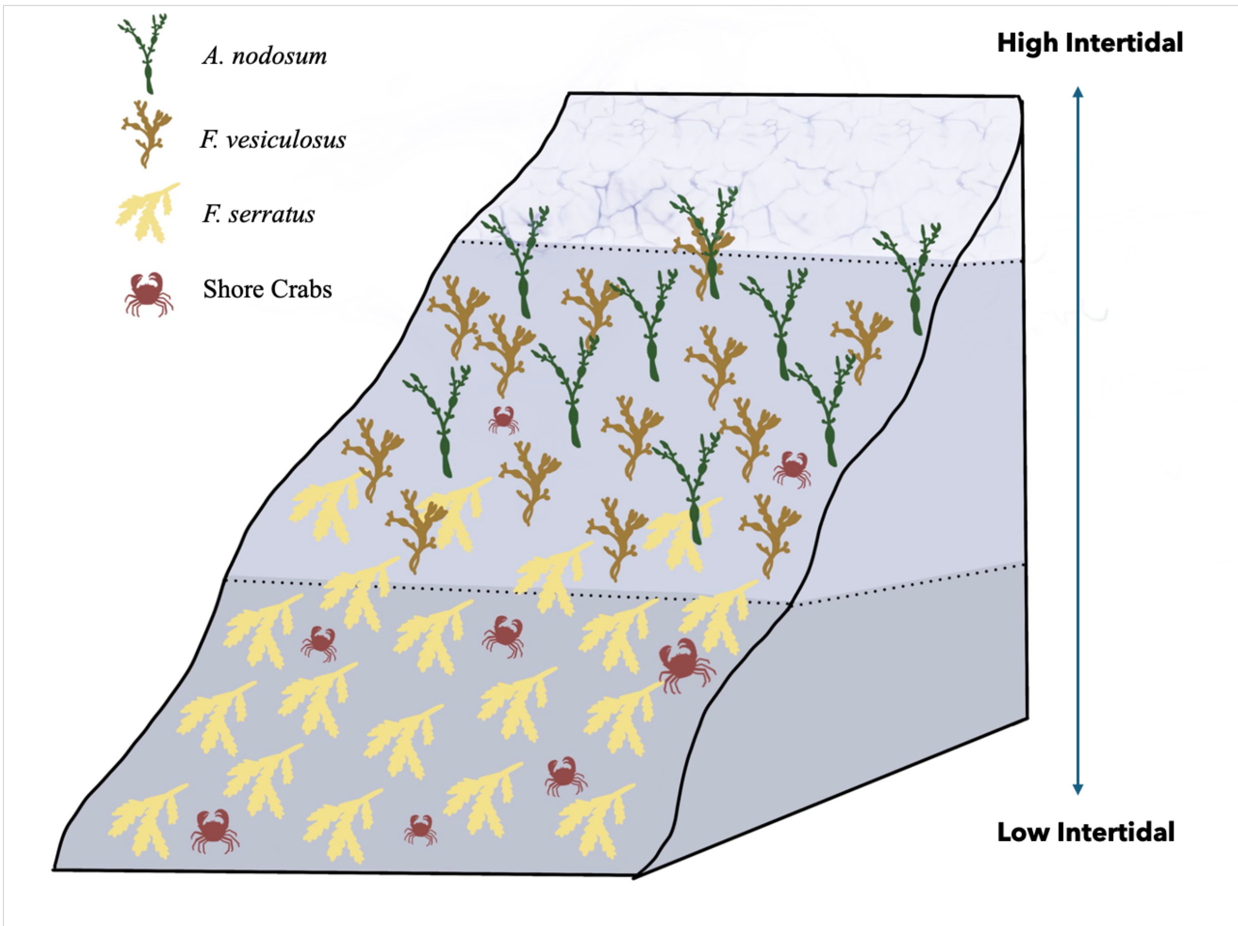

Figure 1. Simplified Illustration of an intehurtidal zone showing the native macrophytes and the lower invasive for the East Atlantic. Within Newfoundland, *F. serratus* was not found. Note that shore crab densities and size tend to increase lower in the intertidal zone where *F. serratus* predominantly zoned (Towne *et al.,* 2023). Additionally, *A. nodosum* and *F. vesiculosis* fronds project into the water column, whereas *F. serratus* lies directly against the substrate (Wahl *et al.*, 2011).

Littorina obtusata and its Predators

Amongst the fronds of these fucoids, *L. obtusata* inhabit, feed, and thrive (Watson & Norton, 1987). Belonging to the family *Littorinidae*, these intertidal gastropods are renowned for their remarkable adaptability and ecological significance (Sotelo *et al.,* 2020). Although hunted by *Hemigrapsus*, *Cancer,* and *Portunidae* crab predators, species of *Littorinidae* can exhibit a variety of shell defenses in order to 1) increase their ability to withstand predation and escape (Reimchen, 1982; Lowell *et al.,* 1994), and 2) improve camouflage against visual predators (Reimchen, 1979; Seeley, 1986). Thus, morphological changes in colour and size of an organism can confer a fitness advantage.

Research Objectives

In this study, I strove to explore the variations of *L. obtusata* injury frequencies in relation to different abiotic and biotic factors through the examination of characteristic crab-caused injury shell regrowth. Abiotic factors examined include shore exposure, crab habitat suitability, substrate type, and intertidal zone slope. Biotic factors included algal cover and type. In addition, I also considered how potential variations in shell colour and size arise in response to sub-lethal crab attacks within different macrophytes of the Flat Periwinkle (*L. obtusata*) habitat. At the onset of this research, my hypotheses were as follows:

1) Positive trends would be observed between intertidal factors favouring crab suitability and occurrence of shell injuries on *L. obtusata* as suggested in the current literature, with shore crab density being positively correlated with landscape factors such as increased beach slope, algal cover (Menge, 1978; Lucrezi, 2015), sheltered shores, and muddy substrates (Reimchen, 1982).

2) Prevalence of *L. obtusata* shell injuries will increase when found on the invasive lower-intertidal macrophyte, *F. serratus,* relative to its middle intertidal native counterparts *A. nodosum,* F. vesticulosus, and *F. edentatus*. This hypothesis is motivated by the literature, as shore crab density typically increase within lower intertidal zones (Reimchen, 1982; Wilbur & Steneck, 1999). Additionally, invasive species have been recognized for their role in driving evolutionary changes in native species, which are compelled to adapt to the shifting environments (Engel *et al*., 2011; Westley, 2011). Through these invasions, habitat complexity escalates and appears to display positive effects on increasing the density of crabs within the affected niche (Towne *et al.,* 2023).

3) Risk of crab predation will affect different shell colour and size variations within *L. obtusata* populations amongst its the various algal habitats. Previously, variations in shell colour and size in *Littorinidae* has been well documented through both *L. obtusata* and its sister species *L. fabilis* (Reimchen, 1979; 1982; Lowell, 1994; Eschweiler *et al.,* 2008). While research has explored shell differentiation regarding camouflage against macrophyte backgrounds and shell size polymorphisms as a potential adaptive response for survival, further study of direct crab-induced shell injury can be beneficial in solidifying these concepts.

METHODS

A descriptive and comparative research design was employed to analyze a collection of *L. obtusata* shells collected from live specimens throughout 92 localities spanning Nova Scotia and Newfoundland. Prior to the onset of this research, the collection of all *L. obtusata* specimens in 1994 by Dr. Thomas Reimchen was systematically conducted along the coastlines of Newfoundland and Nova Scotia to ensure adequate representation of *L. obtusata* populations across the two provinces. During shell collection at each locality, the presence of specific algal species (*F. vesiculosus, A. nodosum, F. edentatus,* or *F. serratus*) and their respective percentage algal cover was recorded through visual inspections of the intertidal zone. Coverage was grouped and recorded for each site: ≤ 30%, 30-70%, and ≥ 70%. To note, some sampled localities included multiple intertwined algal species. In such cases, percent algal cover was represented by the dominant macrophyte of the area.

Shell characteristics involving location and severity of shell injuries as well as size and colour were used to study predation trends. To remain conservative in my approach to sampling unsuccessful crab predation, any injuries that were "fresh" (without repair) were excluded from analysis along with injuries that were not indicative of crab predation (Figure 2A & 2B). Thus, my data was based on the remaining 21, 613 *L. obtusata* shells collected from the intertidal regions of Nova Scotia and Newfoundland. To avoid potential biases during subsequent scoring, the labels on the jars were changed, ensuring that when scored, the author was unaware of macrophyte species and locality.

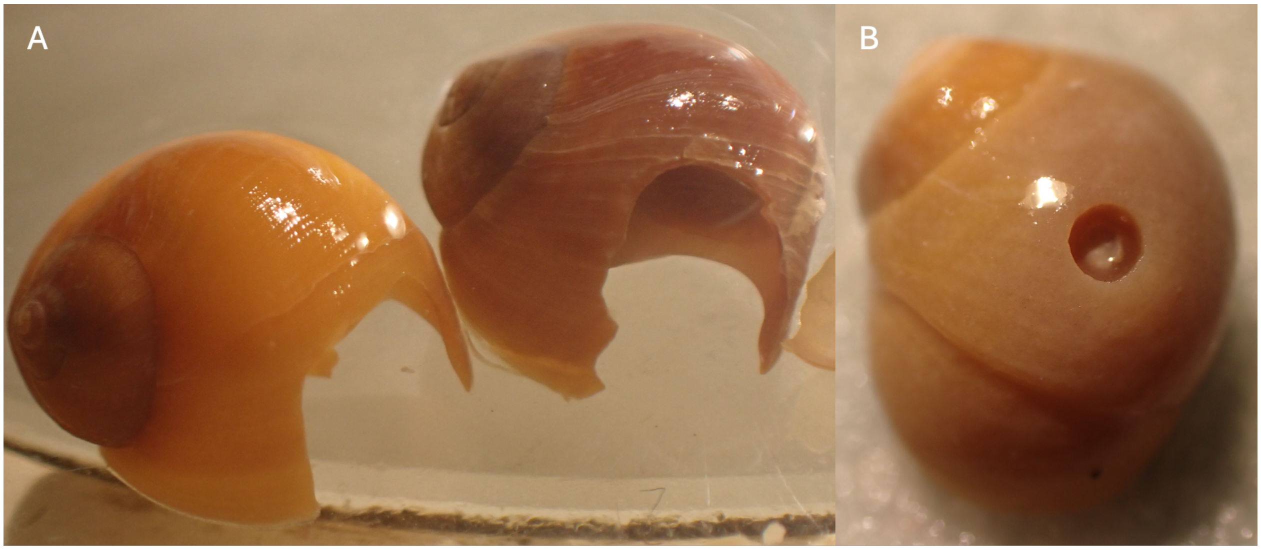

Figure 2. 'Ambiguous' cases of injury on *L. obtusata* shells. (A) Shell breakage with no regrowth = 'Fresh Injury'. (B) Shell injury indicative of a predatory drilling gastropod.

Shell Size Classification

Shells were categorized into three size groups: small (< 6.0 mm), medium (6.0 mm to 9.0 mm), and large (> 9.0 mm). Shells were assigned to a size group based on the length measurement from lip to opposite side of the body whorl.

Predation Severity

The shells of *L. obtusata* showcased diverse forms of accumulated damage, characterized by shell repair stemming from the distinctive crab peeling technique. This method involves crabs inserting their dactyl molar into the shell aperture, resulting in the breakage of shell pieces at the lip. These injuries were scored on a scale of 1 – 7; no injury (1), minor injuries (2-3), and major injuries (4-7) (Figure 3). Extraneous cases of crab-induced injuries displaying no regrowth or if determined to be injured by another predator such as the northern moon snail (*Euspira heros*) or dog whelk (*Nucella lapillus*) that bore holes into their prey marine gastropods, were categorized as 'ambiguous' (Figure 2B). For each locality, the mean injury was determined from the number of shells and the frequency of injury and in sections of my analysis, measurements of predation were also reduced to binary variables (0 = no predation, 1 = unsuccessful predation).

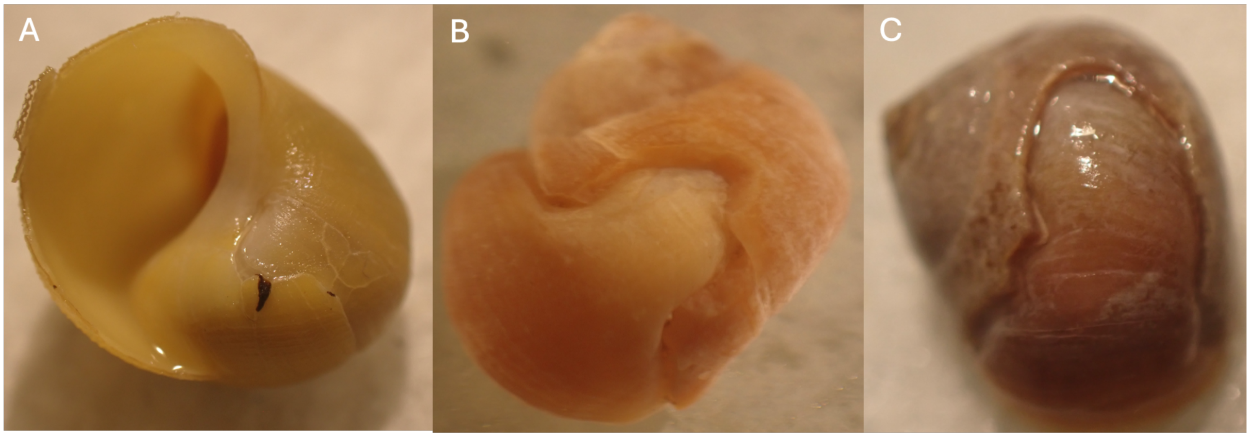

Figure 3. Unsuccessful crab predation on *L. obtusata* shells. (A) 'Minor' shell injury at the back whorl on a 'light' shell. (B) 'Major' shell injury at the front whorl on a 'medium' shell. (C) Major shell injury at the middle whorl on a 'dark' shell.

Location of Injury

The location of injury on the shells was also scored on a four-point categorical scale; with initial injuries from crabs beginning at the 1) lip, then the 2) front whorl, past the 3) middle whorl, and the 4) back whorl (Figure 4). Any injuries noted within the inner whorls of *L. obtusata* were ignored due to scoring ambiguity in this region, as regrowth often compresses the shell wounds and scars. For analysis, injuries were categorized based on their location on the shell into two groups: (1 & 2 = 'recent injuries'; 3 & 4 = 'past injuries'). This classification allowed for additional insights on injury ontogeny of *L. obtusata*. I attempted to determine when injuries occurred at different stages of shell growth and development, with hopes to examine the potential effects of size-dependent predation on shell size variations.

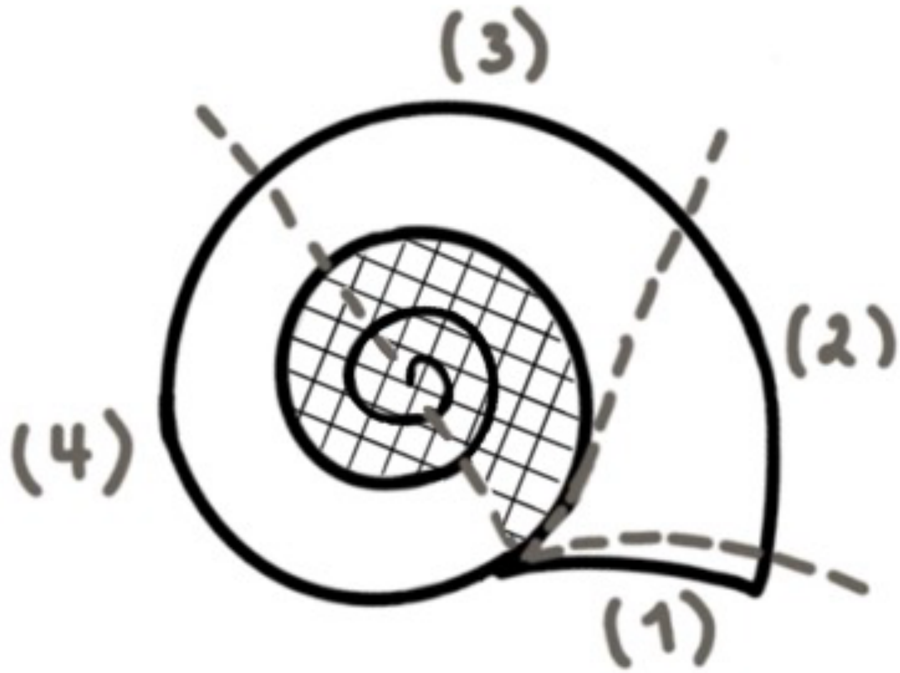

Figure 4. Illustration representation of location of unsuccessful crab-induced injuries. (1) 'Lip of shell,' (2) 'Front whorl,' (3) 'Middle whorl,' (4) 'Back whorl'. *Shaded region of the inner whorls not assessed for injuries.

Shell Colour Classification

Each *L. obtusata* shell was scored for colouration on a categorical scale: 'light' (yellow morphs), 'medium' (orange morphs), 'dark' (brown, black, olive) (Figure 5). When analyzing the effects of predation on shell colour, only sites with adequate sample sizes (n > 50) and injury representation of both dark and light shells were utilized.

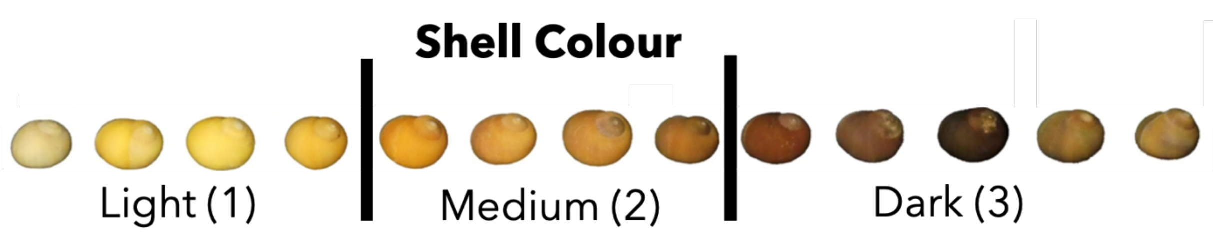

Figure 5. Categories of shell colour: (1) light (light-dark yellow morphs), (2) 'medium' (light-dark orange morphs), (3) 'dark' (brown, black, olive morphs). **This image was provided by Dr. Reimchen.*

Additional Relevant Ecological Data

Field environmental data regarding wave exposure, substrate type, algal type and percentage cover were combined to gain additional insights for each locality. Beach slopes were recorded and grouped for each site; ≤ 20º, 20-50º, and ≥ 50º. Furthermore, photos of all 92 localities were examined and the intertidal zone scored on a scale, 1 (least suitable) to 5 (most suitable), for crab habitat suitability based on rock size and percentage rock cover. Judgment for crab habitat suitability separately performed by myself and an additional contributor to enhance validity of scoring. A comparison between the two individuals scores yielded a correlation coefficient of $r = 0.654$, $p<0.001$, indicating a robust agreement between the two samplers based on predetermined criteria of rock cover, size, and algal cover. This alignment in the data suggests that despite potential variations in individual perspectives or methodologies, the samplers consistently identified similar classification of crab habitats.

Additionally, the 7 localities with *F. edentatus* as the most common macrophyte were excluded from this analysis as the majority of shells collected (97.8%) were classified as ‘dark’ in colour; thus, there was a weak representation of injuries of ‘light’ shells for meaningful statistical tests to be conducted comparing crab-induced predation on shell colour (pg.17).

Statistics

The data representing the weighted average of shell injury for individual sites adhered to the assumption of normality, as assessed through a QQ-Plot. While all data exhibited a normal distribution, some of our abiotic data, specifically the weighted average of shell injury to abiotic data, failed equality of variance tests. This violation increases the chances of a Type II error in the affected cases. To verify if this violation would impact our results, I conducted additional

Welch's ANOVA tests. Adjustments were made to the General Linear Model to align syntax which accommodates for unequal variances, effectively performing analyses analogous to Welch's ANOVA. Since none of the significance values were marginal, this type II error was considered acceptable. In loglinear testing, correlations were 'approaching significance' if the Z-value exceeded 1.0 or fell below minus 1.0. All morphometric univariate and multivariate analyses, including chi-squared, ANOVA, and log-linear analyses, were conducted using SPSS statistical package version 29.0.0. Significance was denoted as *p < 0.05, **p < 0.001.

RESULTS

Abiotic Factors Affecting Predation

Predation between Provinces

The characteristic 'peeling' of crab predators and the resulting breakages of the body whorl occurred in every locality along the coasts of Newfoundland and Nova Scotia, albeit, with dissimilar ranges and frequencies ($t = 4.7$, $P < 0.001$) (Table 1). A two-fold increase was observed between Nova Scotia relative to Newfoundland with regards to percentage of shell injury.

Table 1. Percentage of injured shells among localities separated by province. SD = standard deviation.
Unpaired T-test: $T = 4.7$, $p < 0.001$.

Province	%	SD	Range (Localities)	N (Localities)	N (Shells)	$\bar{x}$ (Shells/Localities)
Newfoundland	8.6	2.8	2 - 45%	35	9267	265
Nova Scotia	19.0	3.9	2 - 71%	57	12246	215

Locality Exposure

Within Newfoundland, the highest frequencies of injury occurred in exposed localities. Nova Scotia, on the other hand, showed that injured shells were more prevalent in areas of lower-to-intermediate exposure; however, in both provinces, the difference between categories of exposure regarding shell injury was not statistically significant (Figure 6). Interestingly, the impact of exposure on the outcome of shell injury differs across both provinces.

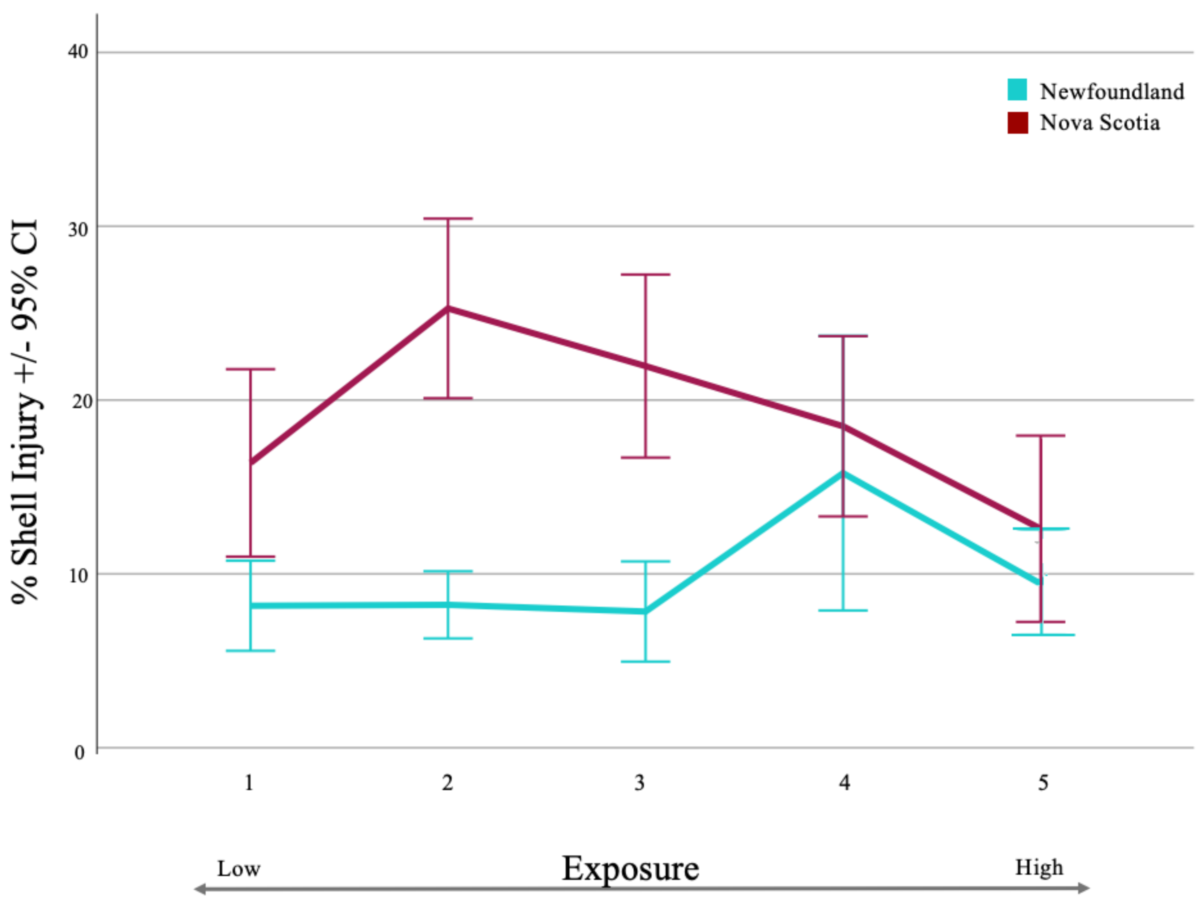

Figure 6. Percentage of repaired shell injuries in relation to shore exposure. Injuries based on total sample of localities. ANOVA (NFLD; F = 1.2, df = 4, P >0.05, N= 50: NS; F= 2.1, P >0.05, N= 88). Interaction effect (F = 4.1, df = 4, P < 0.05, N = 138).

Crab Habitat Suitability

Crab habitat suitability within individual localities was determined by examining images of sampled intertidal shorelines. If images showed extensive rocky substratum, formations, and cover, the habitat was deemed 'suitable' for residing shore crab species. No significant relationship was established between injury occurrence and crab habitat suitability among our localities (Figure 7).

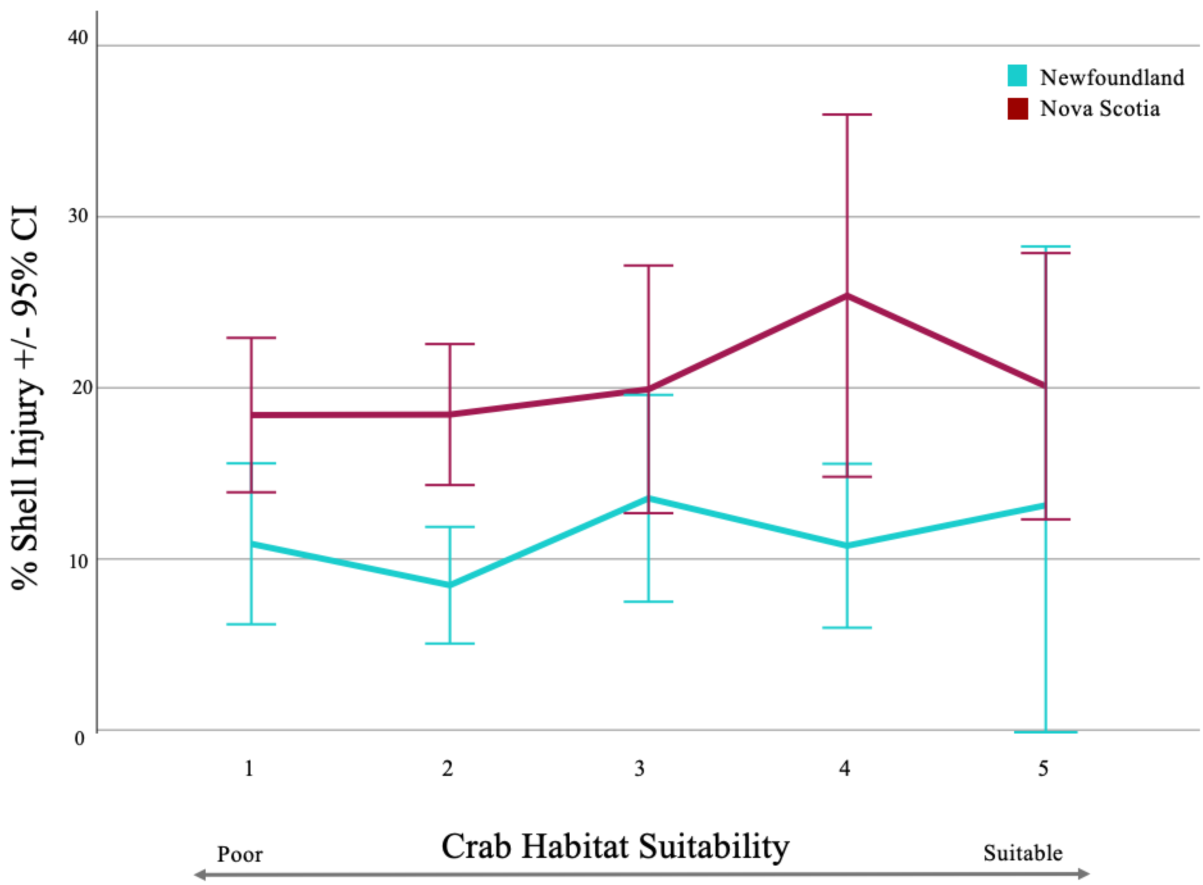

Figure 7. Percentage of repaired shell injuries in relation to habitat suitability. Injuries based on average samples of localities. ANOVA (NFLD; F = 0.53, P <0.05, N= 54: NS; F= 0.85, P >0.05, N= 83). Interaction effect (F = 0.40, df = 4, P > 0.05, N = 138).

Substrate Type

L.obtusata shells were collected from algae growing on five different substrates: sand, mud, pebble, rock, and bedrock. In Newfoundland, on sand substratum, shell injuries were significantly less common for *L. obtusata* relative to localities without sandy shores ($Z= -3.4$, $p< 0.001$) (Figure 8). Notably, mud and rock substrates approached statistical significance. Nova Scotia, however, showed that shell injuries were positively correlated with the presence of mud and pebble substrates while also being significantly less frequent when found on bedrock (log linear correlation; $Z= -3.2$, $p < 0.001$) (Figure 8). The remaining substrates of both provinces displayed no correlation between substrate type and shell injury.

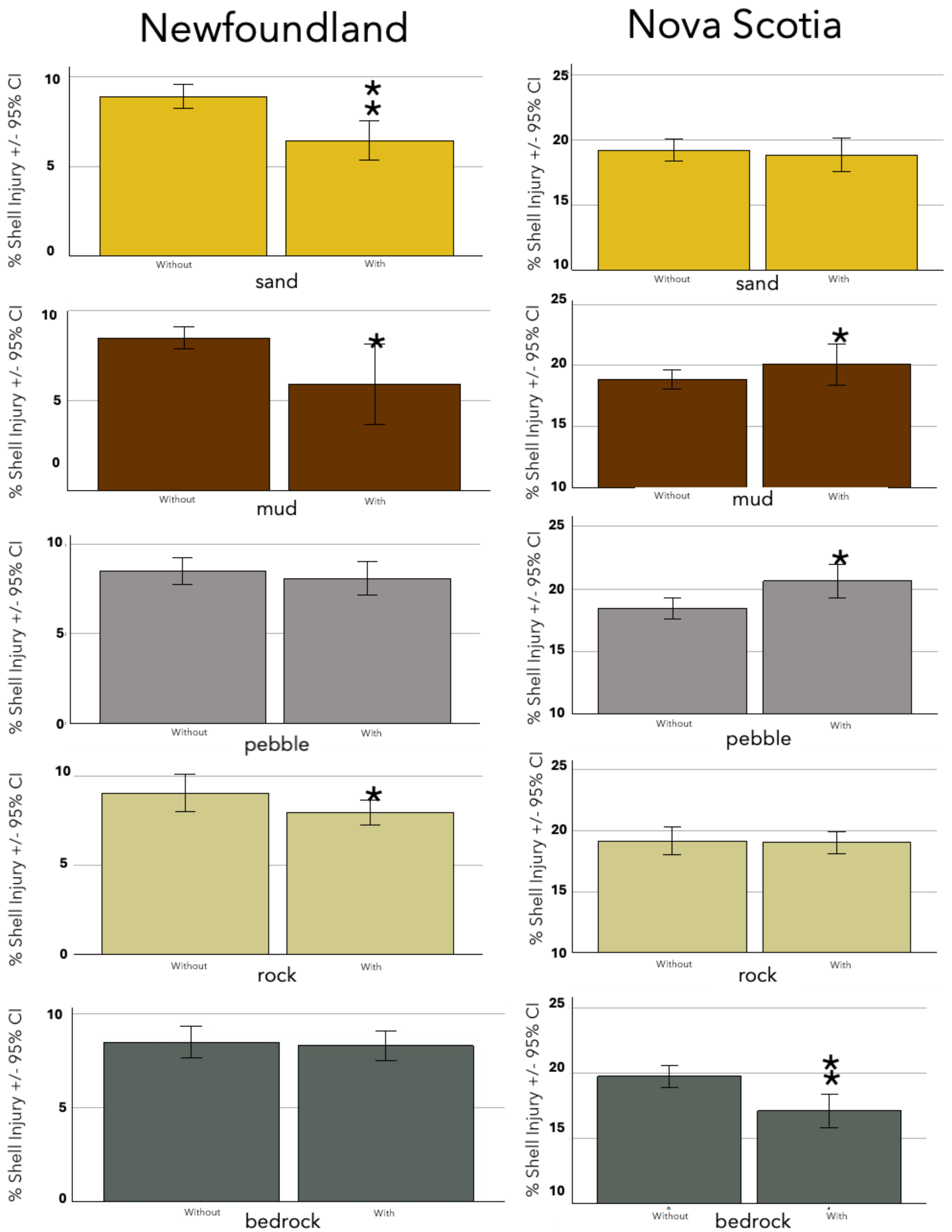

Figure 8. Substrate type and percentage of shells injured. Loglinear (* Z > 1.0, or *Z < -1.0); (** Z > 1.96, or **Z < -1.96).

Intertidal Slope

The intertidal zones of Newfoundland and Nova Scotia varied in slope from 0˚ to 90˚. When assessing the relationship between intertidal slope and shell injury, no significant difference was found between the two variables across both provinces (Figure 9).

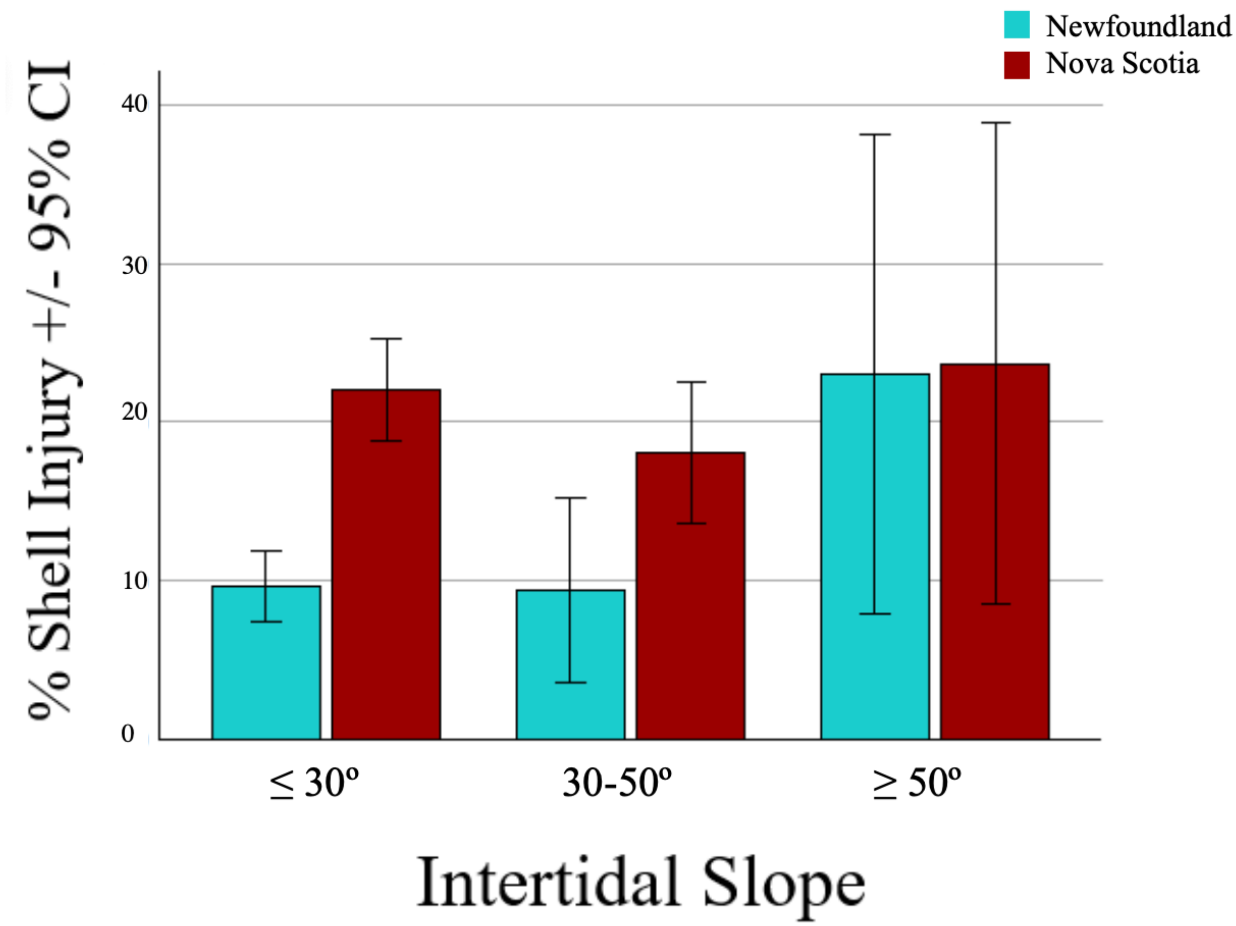

Figure 9. Intertidal slope and percentage of injured shells between both Newfoundland and Nova Scotia. ANOVA (NFLD: $F = 3.0$, $p > 0.05$, n=50; NS: $F= 1.0$, $p > 0.05$, n=87). Interaction effect ($F = 0.481$, $df = 2$, $P > 0.05$, $N = 137$).

Biotic Factors

Macrophyte Cover

Dependent on locality, intertidal surfaces were covered from a range of 5 to 100 % by residing macrophyte species. Between localities respective of their province, macrophyte cover did not exemplify statistical significance determining the frequency of shell injury. (Figure 10).

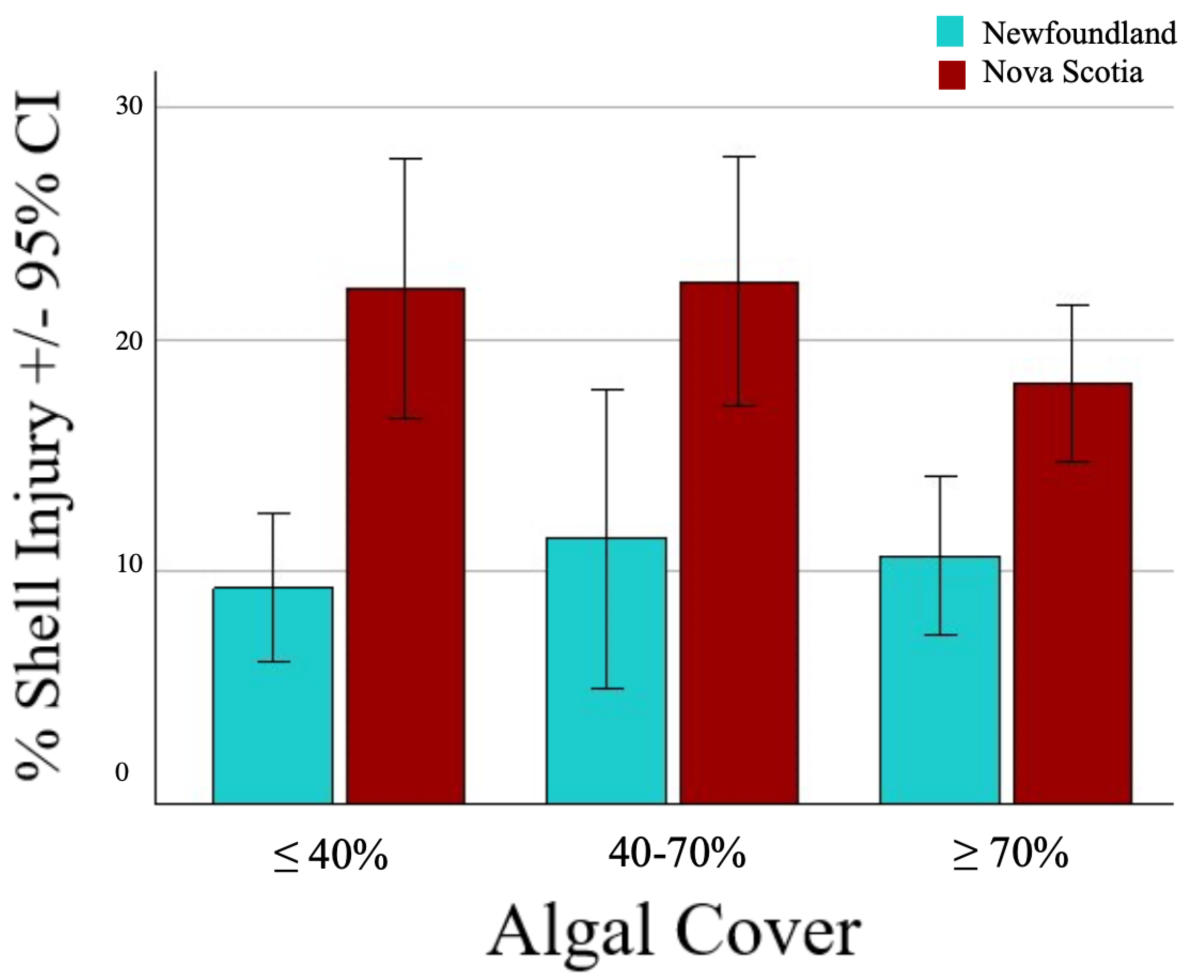

Figure 10. Percent algal cover and percentage of injured shells between both Newfoundland and Nova Scotia. ANOVA (NFLD: F= 0.22, $p > 0.05$, N= 53; NS: F =1.3, $p > 0.05$, n= 87). Interaction effect (F = 0.481, df = 2, P > 0.05, N = 13).

Macrophyte Type & Shell Injury

Localities containing two or more algal species were analyzed to explore the relationship between shell injury among different macrophyte types. Several distinct localities demonstrated significant deviation from expected injury frequencies:

1) Shell injuries were significantly more frequent on *A. nodosum* in 6 sites (Arisaig, East Ferry, Liscomb, Mill Bay, Mushaboom, Norris Point Pier) compared to other nearby macrophytes (Table 2).
2) Shell injuries were significantly more frequent on *F. vesiculosis* in 4 sites (Arichat Point, Bottle Cove, Lobster Cove, Torr Bay) compared to other nearby macrophytes (Table 2).
3) Shell injuries were significantly more frequent on *F. serratus* in 4 sites (Bayfield, Cape John, Main a Deux, Tidnish Park) containing the algal substrate compared to other nearby macrophytes (Table 2).

Table 2. Multiple Chi-Squared(X^2) analysis of the 14 localities that displayed associations between shells with injury on algae type. X^2 test shows departure from 1:l ratio. Probabilities are * $p < 0.05$ and ** $p < 0.001$

	A. nodosum		*F. vesiculosis*		*F. serratus*		
Localities	Injured	No Injury	Injured	No Injury	Injured	No Injury	N
Arichat Point	9	71	31**	54	9	56	230
Bayfield	32	82	81	299	61*	139	694
Cape John	6	128	5	120	39**	204	502
Main a Deux	33	110	13	49	61**	25	291
Tidnish Park	0	0	10	105	16*	66	197
Arisaig	105*	150	0	0	70	159	484
Mill Bay	2*	9	0	0	3	81	263
Norris Point Pier	23*	195	0	0	8	181	407
Liscomb	31**	60	0	0	33	226	350
East Ferry	76**	128	0	0	116	445	765
Torr Bay	5	102	15*	91	0	0	213
Lobster Cove	3	317	16*	229	0	0	567
Bottle Cove	10	120	23**	45	0	0	198
Mushaboom	12**	47	12	114	0	0	185
# of Sites Sampled	Of 36 Sites		Of 34 Sites		Of 11 Sites		

Shell Colour, Macrophyte & Injury

Shell Colour & Predation within Different Algal Environments

Scored on a scale from 'light' (1) to 'dark' (3), *L. obtusata* shell colour varied greatly among localities containing different macrophyte species (Table 3).

In Nova Scotia, in localities with *F. vesiculosus,* darker shells were weakly correlated with increased injury, albeit this correlation was negligible in Newfoundland. Localities with elevated injury rates on *F. serratus* were typically darker-shelled populations. In contrast, lighter-shelled populations showed elevated injury rates on *A. nodosum* in both provinces, although weakly correlated (Figure 11).

Table 3. Shell colour variations among various macrophytes (X^2=1922.9, df = 6, p<0.001).

Algae Type	'Light'	'Medium'	'Dark'	(N)Localities	% Dark
A. nodosum	785	2486	2790	42	46.0%
F. vesiculosus	1983	4792	5952	79	46.7%
F. serratus	655	720	380	11	21.6%
F. edentatus	4	20	1046	5	98%

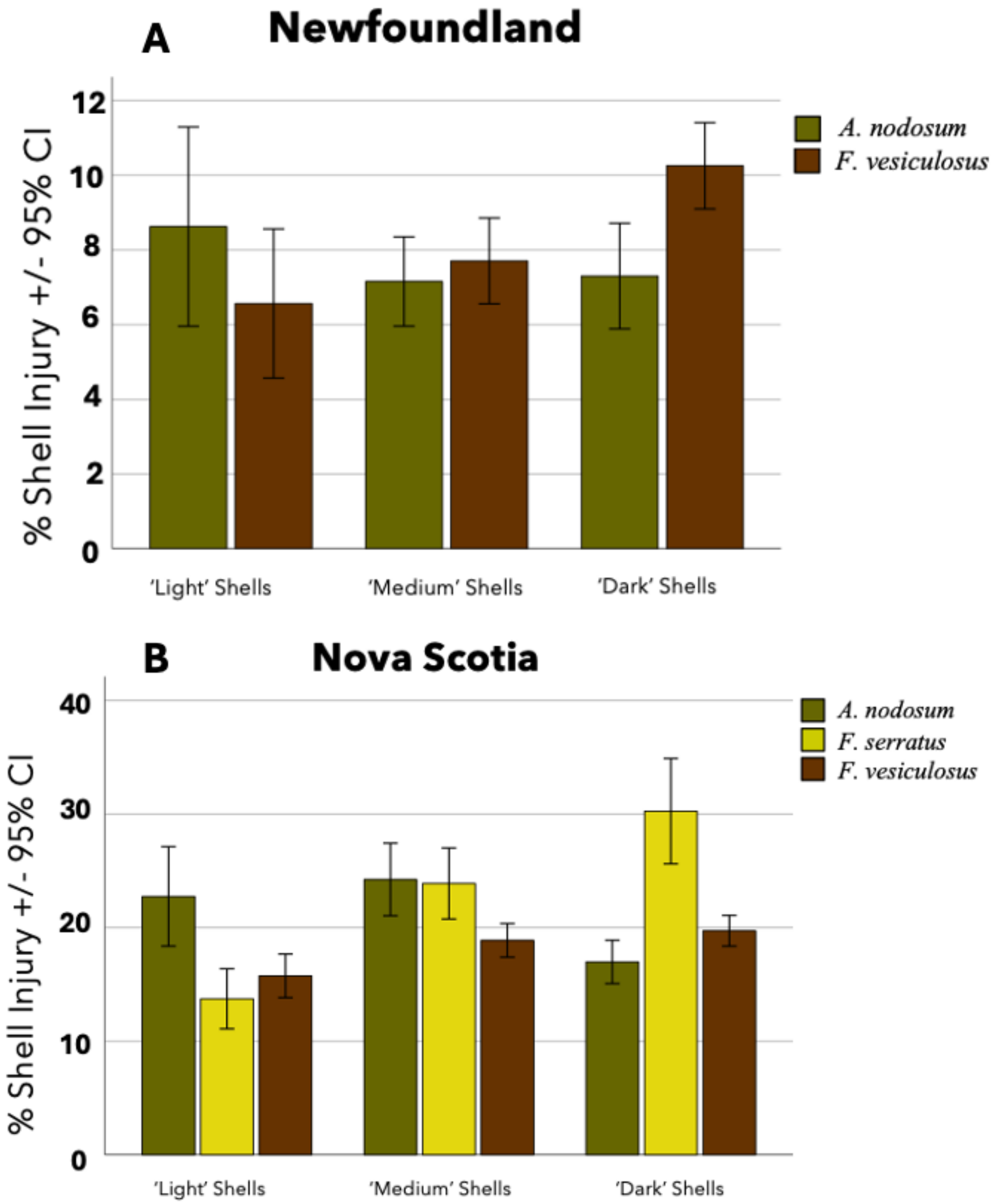

Figure 11. Percentage of repaired shell injuries in relation to shell colour and macrophyte species. Correlation coefficients for A) Newfoundland (ASCO: $r = -0.33$, $P > 0.05$; FV: $r = 0.30$, $P > 0.05$), for B) Nova Scotia (ASCO: $r = -0.42$, $P > 0.05$; FV: $r = 0.11$, $P > 0.05$; *FS: $r = 0.61$, $P<0.05$).

To determine the possibility of directional shell colour variation to crab predation within localities, shell injuries for the external groups of shell colour 'light' and 'dark' were explored against their corresponding host macrophyte species.

A. nodosum

In Newfoundland, four localities displayed greater incidence of injury on 'dark' shells, with one of these localities (Dover) approaching significance. Loglinear analysis revealed no significant correlation between unsuccessful shell predation and the outcome of light or dark morphs (Figure 12).

Meanwhile, In Nova Scotia, five localities showed a greater incidence of injury on 'dark' shells, with two of these localities (Briar Island & Cape John) approaching significance. Additionally, four localities showed higher injury incidence among 'light' shells, with two localities approaching significance (East Ferry & Mushaboom). Loglinear analysis also revealed no significant overall correlation between unsuccessful shell predation and the outcome of 'light' or 'dark' shells (Figure 13).

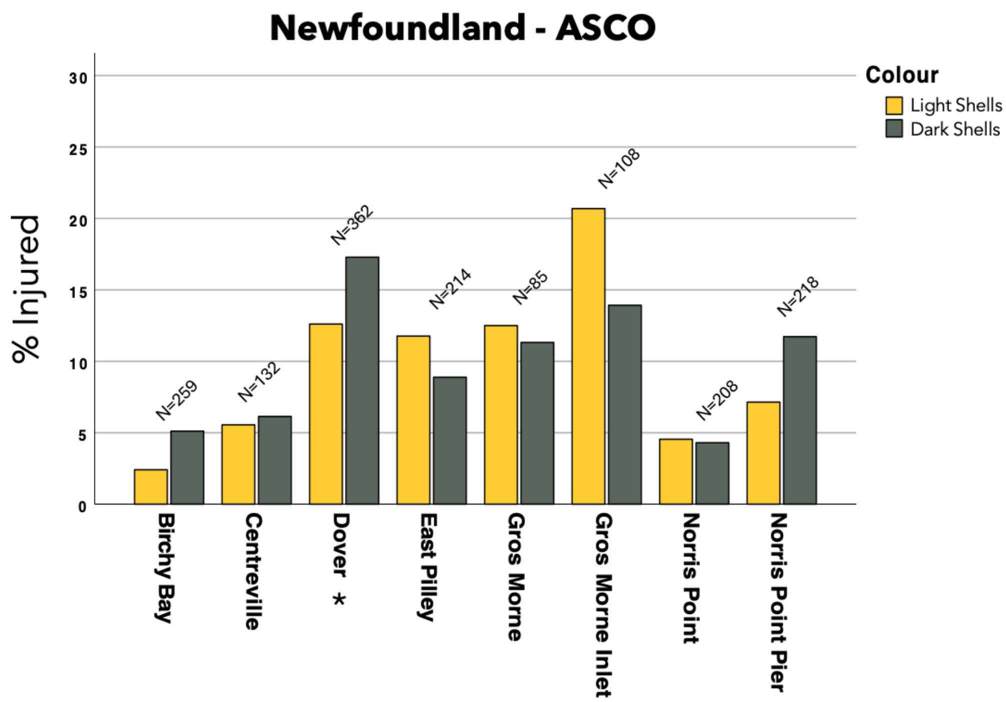

Figure 12. Unsuccessful predation for both light and dark *L. obtusata* shells found within Newfoundland on *A. nodosum*. Probability (* Z > 1.0, or *Z < -1.0) based on log linear comparison of light shells (1) to dark shells (3).

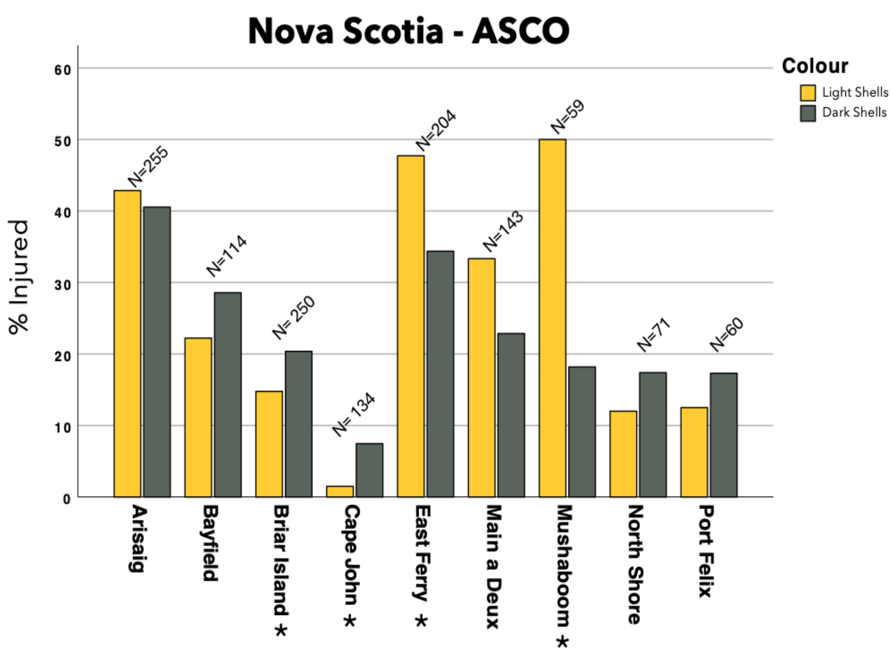

Figure 13. Unsuccessful predation for both light and dark *L. obtusata* shells found within Nova Scotia on *A. nodosum*. Stars indicate probability (* Z > 1.0, or *Z < -1.0) based on log linear comparison of light shells (1) to dark shells (3).

F. vesiculosus

In Newfoundland, eight localities displayed greater incidence of injury on 'dark' shells, with two of these localities (Birchy Bay & Boyds Cove) approaching significance. In the remaining four localities where shell injuries were more abundant on 'light' shells, one locality (Gros Morne) also approached significance (Figure 14).

In Nova Scotia, sixteen localities displayed greater incidence of injury on 'dark' shells, with two of these localities (Gulf Shore & Cheticamp 2) being significant, meanwhile five localities (Heather Beach, Cheticamp South, Havre Bouche, Pomquet, Queensland) approached significance. In the remaining nine localities where shell injuries were more abundant on 'light' shells, one locality (Merigomish) was significant, while additionally, two localities (Arichat Point & Marie Joseph) approached significance (Figure 15).

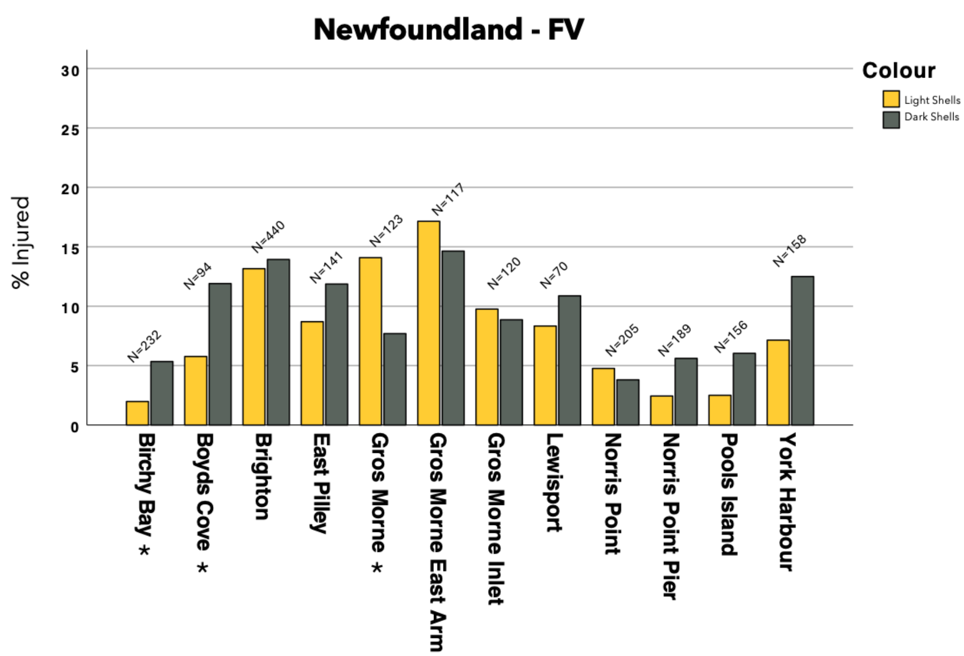

Figure 14. Evidence of unsuccessful predation for both light and dark *L. obtusata* shells found within Nova Scotia on *F. vesiculosus*. Probability (* Z > 1.0, or *Z < -1.0) based on log-linear comparison of light shells (1) to dark shells (3).

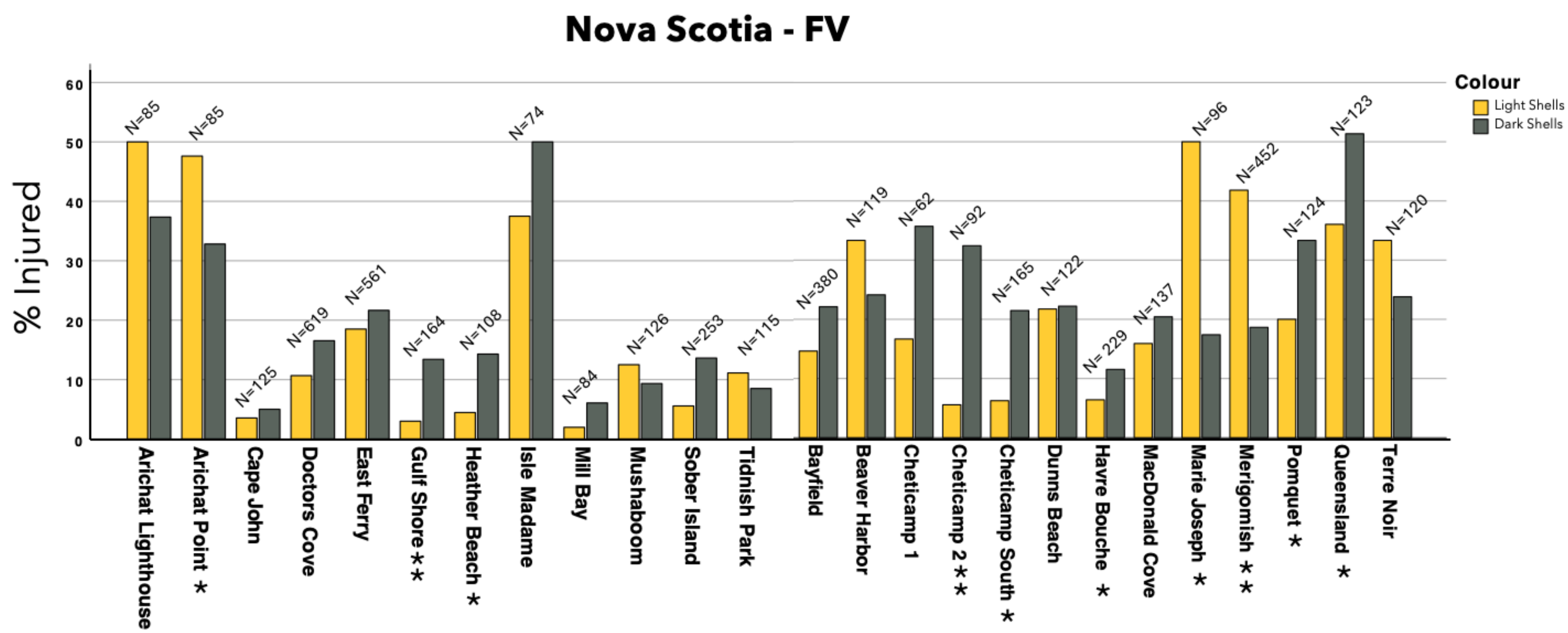

Figure 15. Evidence of unsuccessful predation for both light and dark *L. obtusata* shells found within Nova Scotia on *F. vesiculosus*. Probability (* $Z > 1.0$, or *$Z < -1.0$) ; (** $Z > 1.96$, or **$Z < -1.96$) based on log-linear comparison of light shells (1) to dark shells (3).

F. serratus

Exclusively found in Nova Scotia, eight localities displayed an increased incidence of injuries on ‘dark' shells, with one locality (Cape John) largely accounting for this correlation. An additional locality (Gulf Shore) approached significance. Of the two remaining localities where injuries were more common on ‘light' shells, one locality (Tidnish Park) approached significance (Figure 16).

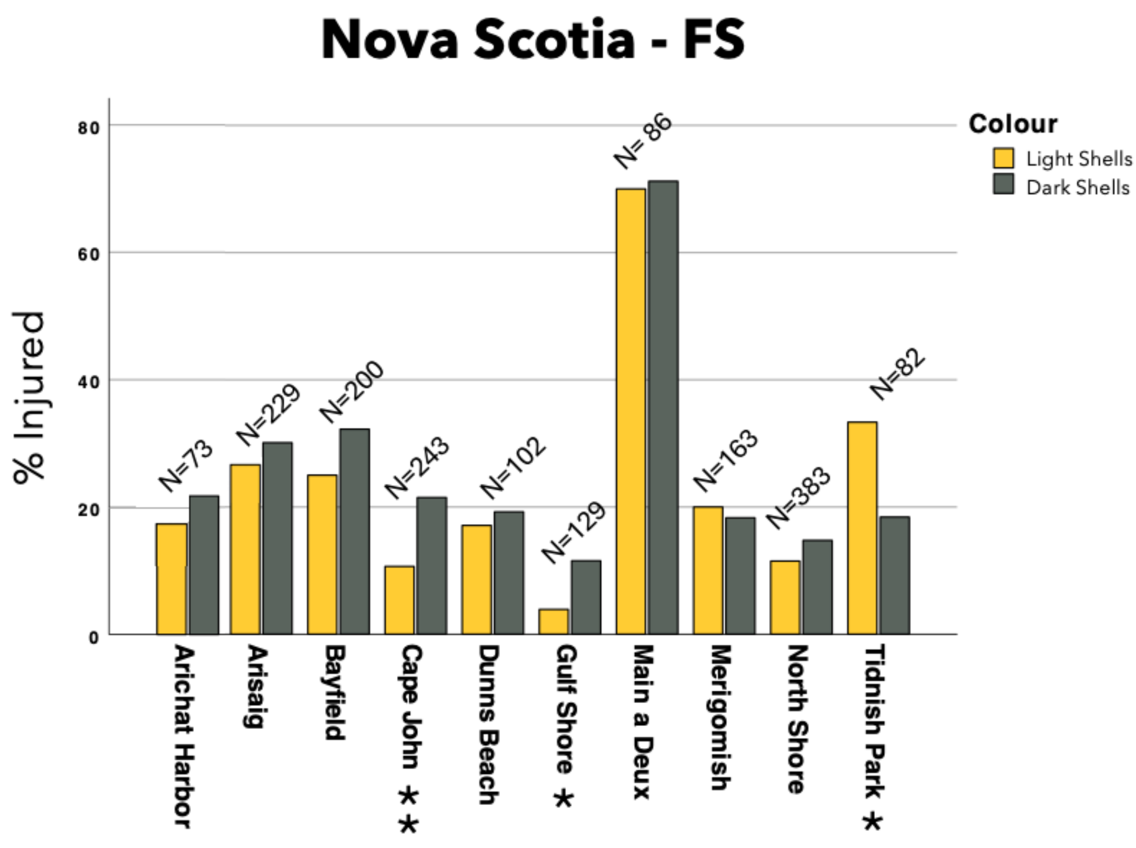

Figure 16. **Evidence of unsuccessful predation for both light and dark *L. obtusata* shells found within Nova Scotia on *F. serratus*.** Probability (* Z > 1.0, or *Z < -1.0) ; (** Z > 1.96, or **Z < -1.96) based on log linear comparison of light shells (1) to dark shells (3).

Shell Size, Macrophyte & Injury

Scored on a 3-point categorical scale from 'small' (1) to 'large' (3), shell size varied greatly across the different macrophyte species of localities (Table 4). However, frequencies of shell injury were positively correlated with an increase in shell size on all three analyzed macrophyte species (Figure 17).

Table 4. Shell size variations among various macrophytes (X^2=116.6, df = 6, p<0.001).

Algae Type	<6.0mm	6.0-9.0mm	>9.0mm	(N)Localities	% >9.0mm
A. nodosum	2789	2355	917	42	15.1%
F. vesiculosus	5075	5507	2145	79	16.8%
F. serratus	700	684	371	11	21.1%
F. edentatus	392	438	240	5	22.4%

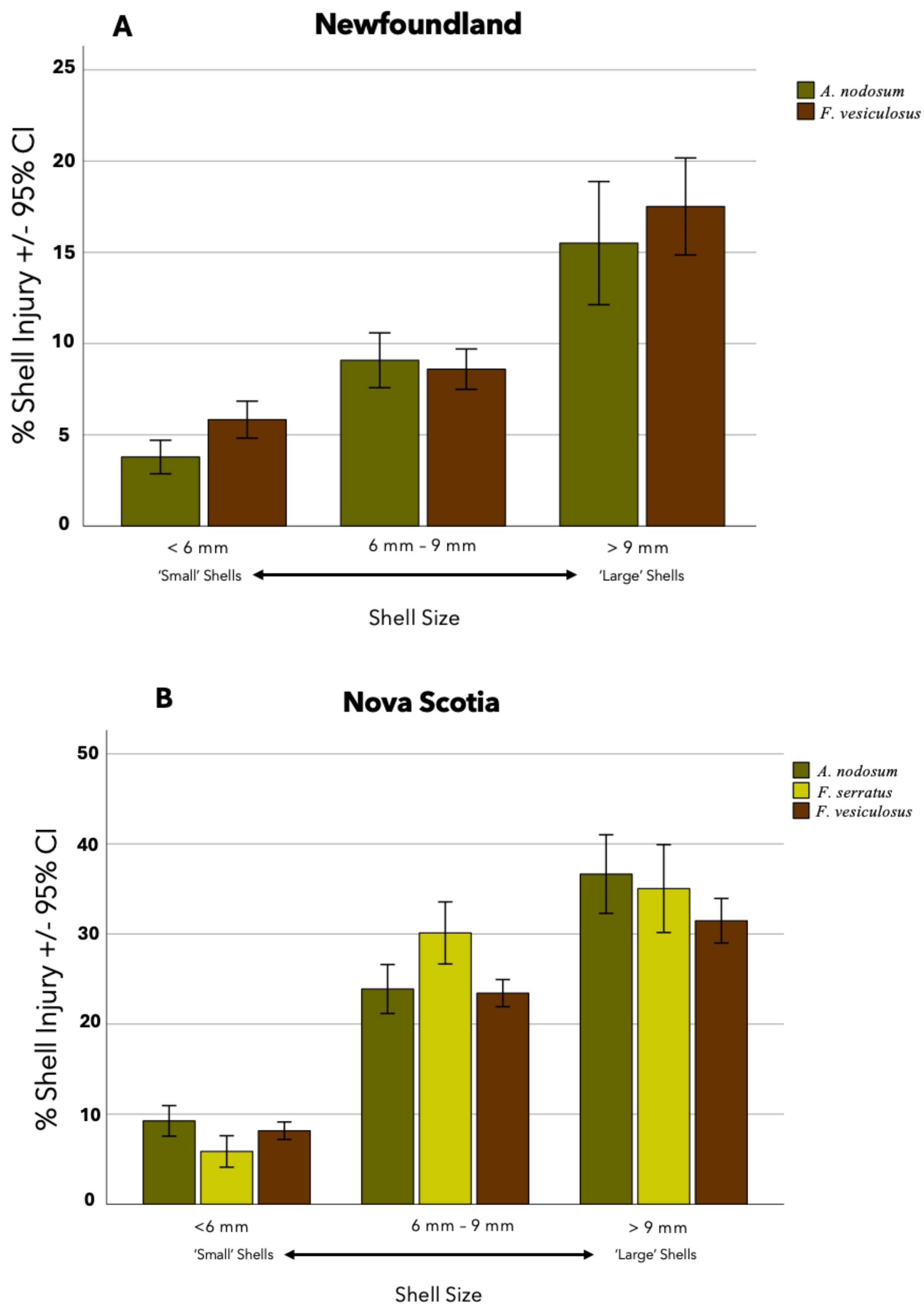

Figure 17. Percentage of repaired shell injuries in relation to shell size and macrophyte species. Correlation coefficients for A) Newfoundland (ASCO: r = 0.56, P < 0.05; FV: r = 0.70, P < 0.001), for B) Nova Scotia (ASCO: r = 0.43, P < 0.05; FV: r = 0.61, P <0.001; FS: r = 0.76, P < 0.01).

DISCUSSION

Shells of *L. obtusata* displayed extensive geographical variation in the frequencies of shell injuries inflicted by residing crab species. These differences ranged among localities; in Main a Deux, injury frequencies were as high as 71% but were less than 5% at Norris Point Pier. Although these injuries do not provide robust information regarding the mortality rate of *L. obtusata*, they can shed light on the prevalence of crab attacks between a variety of intertidal regions (Reimchen, 1979; Vermeij *et al.,* 1980; 1983). In this study, I examined how locality exposure, habitat suitability, substrate type and intertidal slope can affect the prevalence of shell injury. Overall, the quantitative survey indicated that populations of *L. obtusata* incur a 'doubling' effect in shell injury frequencies in the maritime province of Nova Scotia compared to Newfoundland. This effect is likely attributed to variations in local crustacean species. The Snow Crab, for instance, is predominantly found in deeper waters (> 20 m) past the intertidal region of fucoid in Newfoundland which could play a role in the reduction of observed shell injuries between the two provinces as Snow Crab are of greater abundance in Newfoundland (Cote *et al.*, 2019), while the *Hemigrapsus* and *Cancer* crab species are abundant throughout all regions of the intertidal zone in both Newfoundland and Nova Scotia. Furthermore, the vast intertidal ranges of Nova Scotia, notably exemplified by the Bay of Fundy with the world's largest tidal variations, are certainly conducive to a higher abundance of various shore crab species, the primary predators of *L. obtusata*.

The introduction of an invasive crab species, *Carcinus maenas*, also known as the European Shore Crab, to Nova Scotia in the 1960s (Audet *et al.,* 2003), might explain the differences observed in injury rates between the provinces as *C. maenas* arrived on the shores of Newfoundland in 2007, 47 years after their arrival in Nova Scotia, and 13 years after the collection of *L. obtusata* specimens used in this study, which occurred in 1994. This timing discrepancy may explain the decreased incidence of shell damage observed in *L. obtusata* across the sampled intertidal areas of Newfoundland, as this species of crustacean had yet to colonize Newfoundland's intertidal zones. Considering this possibility, future studies investigating shell differentiation in *Littoridae* species should consider the differences in local predator species across geographical regions (Kang, 2017).

Exposure levels were classified on the same scale used by Dr. Reimchen in previous works (1979, 1982). Within Nova Scotia, *L. obtusata* specimens collected on semi-sheltered localities incurred relatively higher levels of injury compared to more 'exposed' shores (Figure 6) and this is further supported by the greater abundance of various shore crab species within sheltered beaches (Hunter & Naylor, 1993; Rochette & Dill, 2000; Audet *et al.*, 2008; Dudgeon & Petraitis, 2019). However, variation in injury frequencies at the localities prevented the establishment of meaningful relationship between shell injury and exposure levels. *L. obtusata* shells collected from localities within Newfoundland had a greater occurrence of injuries with increasing levels of 'exposure,' albeit not significantly. Nonetheless, this prompts speculation as to how increasing shore exposure could potentially heighten the prevalence of shell injury. I suspect that this phenomenon may be attributed to a behavioral change in *L. obtusata*. In the UK and Ireland, a sister species, *L. fabilis*, is commonly found on macrophyte lamina in sheltered waters, while in wave-battered locales, they cling to the stems of macrophytes (Reimchen, 1979). It's plausible then, that *L. obtusata* adjust their location on the macrophyte in response to environmental pressures associated with exposure levels. My observations support this premise, as I noticed a shell darkening effect associated with *L. obtusata* from high-exposure localities, indicative of shells being found on darker stems, as noted by Reimchen (1979) (Figure S2& S3), and this behavioural shift is likely shared among Atlantic *L. obtusata* populations. However, the change in macrophyte position may inadvertently render the flat periwinkle more susceptible to predation by crab species, particularly when they inhabit lower sections of their residing macrophytes, hence increasing their proximity to the substrate and potentially heightening the risk of encounters with crabs. The correlations noted between substrate type and shell injury likely elucidate the minimal variance in shell injury across our categories of crab habitat suitability. My predefined criteria, which predominantly centered on the presence of rocks as indicators of suitable crab habitat, is likely a poor indicator for the presence of shore crab populations.

Previous studies have suggested that crab predation may increase due to landscape factors such as increased beach slope and algal cover (Menge, 1978; Lucrezi, 2015). Among both provinces, localities with steeper intertidal zones displayed a higher frequency of shell injuries relative to gentler slopes. The positive relationship between slope and injury prevalence can be indicative of a greater abundance of crabs due to increased moisture, shelter, and food availability typically associated with steeper intertidal zones (Silva *et al.*, 2010).

The intertidal zones of Newfoundland and Nova Scotia are composed of a variety of substrate types. Although, rocky zones are the most abundant within both provinces, localities can also consist of sand, bedrock, pebbles, and mud (Figure S3). Among the localities of Nova Scotia, shell injuries were more common on mud and pebble substrates and significantly less common on algae growing on bedrock. Whilst in Newfoundland, shell injuries were less common in localities with substrates of mud and rock, and significantly less common on sandy areas. Supported by previous research (Reimchen, 1982) and observed within our Nova Scotia samples, injuries were common on muddy substrate, most likely due to shore crab species' preference for muddy shores (Towne *et al*., 2023). However, the observed reduction in shell injuries on macrophytes collected from bedrock in Nova Scotia and sand in Newfoundland is curious. I speculate that the reduction in shell injury on bedrock and sand substrates is influenced by the lack of habitat suitable for local crab species relative to other available substrates. Without support from related literature, this speculation remains conjecture.

In localities characterized by extensive algal cover, I observed a nonsignificant decrease in shell injuries among localities for *L. obtusata* populations in Nova Scotia, suggesting greater protection from crab predators in areas with abundant algae. While the observed decrease in shell injuries was not statistically significant, it aligns with the notion that dense algal cover can provide refuge and concealment for intertidal organisms, reducing their vulnerability to predation. Notably, this was not the case for the localities of Newfoundland, which showed no significant variations among shell injuries and algae cover. However, in localities where shell injury exceeded expectations on a select macrophyte species, such as *L. obtusata* found on *F. serratus*, this indicates that shells are more predated when residing on the invasive macrophyte. Explanations for the increase in shell injuries found on this macrophyte could be a potential increase in shore crab density in the low intertidal zone of where this macrophyte grows (Reimchen, 1982; Wilbur & Steneck, 1999). Additionally, the slight increase in shell injuries on this invasive macrophyte may be due to its position against the substrate. While *A. nodosum* and *F. vesticulosis* project their fronds into the water column, fronds of *F. serratus* rest in contact with their substrate (Reimchen, 1979), thus it is reasonable to assume that a bottom dwelling crab would have better access to *L. obtusata* that reside on the fronds of *F. serratus* (Figure 1).

Analysis of shell injuries of snails on the common macrophytes of both provinces, showed that ‘light’ shells (yellow colouration) were seemingly less targeted by crabs when residing on *F. serratus* in the localities of Nova Scotia, possibly indicating that intertidal crustaceans are better able to visually detect darker shelled *L.obtusata* against their respective macrophytes. Previous papers have explained similar colour polymorphisms upon the fronds of *F.serratus* with the *F. fabilis* sister species (Reimchen, 1979), yet there is no explanation for a similar shell differentiation on *F. vesticulosis*, which transmits light poorly through its olive-green to dark brown fronds. I suspect that the lower rates of shell injury found on yellow morphs of *L. obtusata* could be due to improved camouflage against the air bladders structures on *F. vesticulosis*, as they are often lighter than the algal fronds (personal observation). *L. obtusata* collected from *A. nodusom* accumulated less shell injuries on brown and olive-coloured shells, indicating that on this macrophyte, 'dark' shells are likely less recognized by visual predators against the background of the macrophyte. Despite the typically associated costs of shell color displays (Ahlgren *et al.*, 2013), my research suggests that the reduction in accumulated shell injuries, contingent upon coloration against macrophyte backgrounds, indicates heightened fitness for *L. obtusata* through evasion of crab predation (Gefaell *et al*., 2023).

L. obtusata shells observed within this study consisted of a range of sizes (Table 4). Regardless of macrophyte type, higher frequencies of shell injuries occurred to 'large' shell classes compared to the two smaller classes. Although this may be a sign of a defensive adaptation to shell-breaking predators (Bertness & Cunningham, 1981; Reimchen, 1982), more likely it is due to confounding survivorship biases likely contributing to the trend of larger shells having greater accumulations of injury. The 'invisible fraction' effect, as proposed by Hadfield (2008), suggests that juveniles of a species face higher mortality risks. Therefore, the fewer shell injuries observed in smaller shells likely indicates that if predation occurred, mortality was more common among smaller classes of shell. To counter this confounding principle for the future, further measurements focusing on shell lip thickness would provide a stronger indication of the potential effects of predation on shell size, as suggested by Brookes & Rochette (2007).

This thesis aimed to address gaps in our understanding of predator-prey dynamics within the family *Littorinidae* by providing ecological insights into shell variations of *L. obtusata* in response to local crustacean predators in the intertidal ranges of Newfoundland and Nova Scotia. The trends observed in this study demonstrated that the accumulation of shell injuries in *L. obtusata* are influenced by environmental factors, where certain shifts in abiotic and biotic conditions can influence predation pressures and the prevalence of shell injuries.

FUTURE DIRECTIONS

The analysis of shells offers versatile applications across ecological research, conservation efforts, and evolutionary studies. Shells serve as invaluable tools for deciphering predator-prey interactions, illuminating ecosystem dynamics, and identifying key players in food webs. Understanding how damage patterns vary depending on abiotic and biotic factors can prove useful in formulating management strategies for effective conservation. Furthermore, comparative analyses of shell morphology also provide insights into evolutionary processes, including speciation events and adaptive radiations. By tracing changes in shell traits over time, researchers can infer evolutionary trajectories and to better understand the mechanisms driving morphological variation. In essence, shell analysis contributes to a holistic understanding of ecological systems, with the ability to inform conservation efforts and shed light on the evolutionary history of organisms.

Despite my best efforts to uncover relevant information concerning shell injury ontogeny interactions based on the location of shell injuries, my findings were inconclusive. Nonetheless, I maintain that such measurements hold promise in providing valuable ecological insights such as crab size and species through observations of shell injuries and could potentially offer insights into the optimal shell size of *L.obtusata* to evade predation.

Comparing my own observations of *L.obtusata* shell size to that of literature (Reimchen, 1981; 1982) suggests that there is differentiation of shell sizes of *L.obtusata* between the UK and the Canadian intertidal range. My range of shell sizes for *L.obtusata* closely resembles that of its sister species *L. fabilis* within the UK. It is plausible that this difference is driven by the size of their local crab predators or through temperature, salinity, and calcium availability (Doyle *et al.*, 2010; Phifer-Rixey *et al*., 2023). Conducting comprehensive surveys of various intertidal crab species belonging to Canada and the UK could help determine how and if crab size is affecting this phenomenon.

Regarding shell colouration, the high prevalence of 'dark' shells on *F. edentatus* hindered the establishment of a correlation between potential predator-mediated effects and shell colour outcomes due to the limited representation of 'lighter' shells. I hypothesize that these 'dark' morphs are providing effective camouflage against visual predators against the dark brown colouration that is typical of this algal species. At the present, there is no explanation in the literature as to why darker shells are more prevalent on this alga. Further research is required.

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Competing interests

The author declares no competing interests.

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APPENDIX

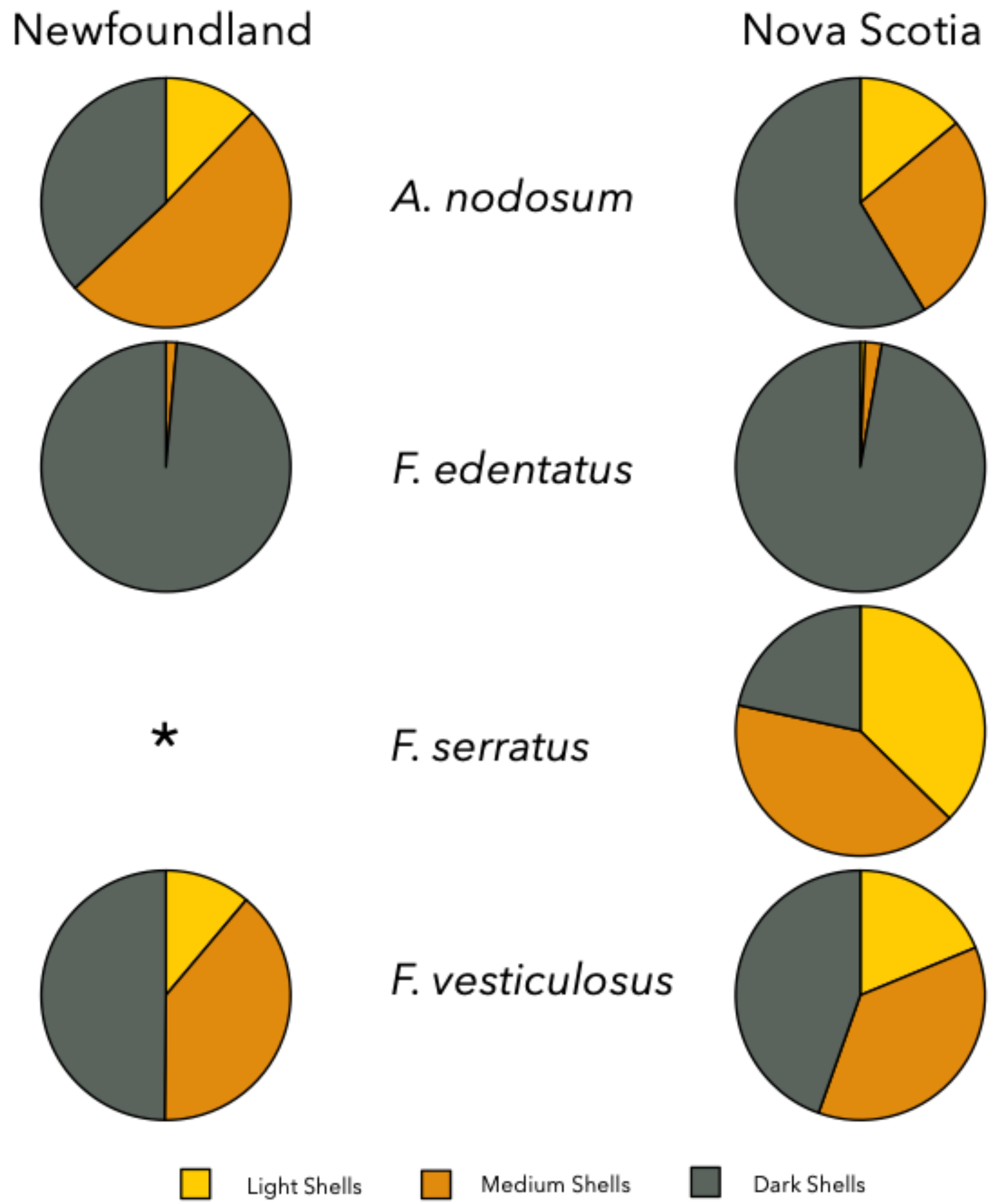

Figure S1. Newfoundland and Nova Scotia colour trends for the algal species found within each province. *No FS was observed in NFLD at the time of specimen collection.

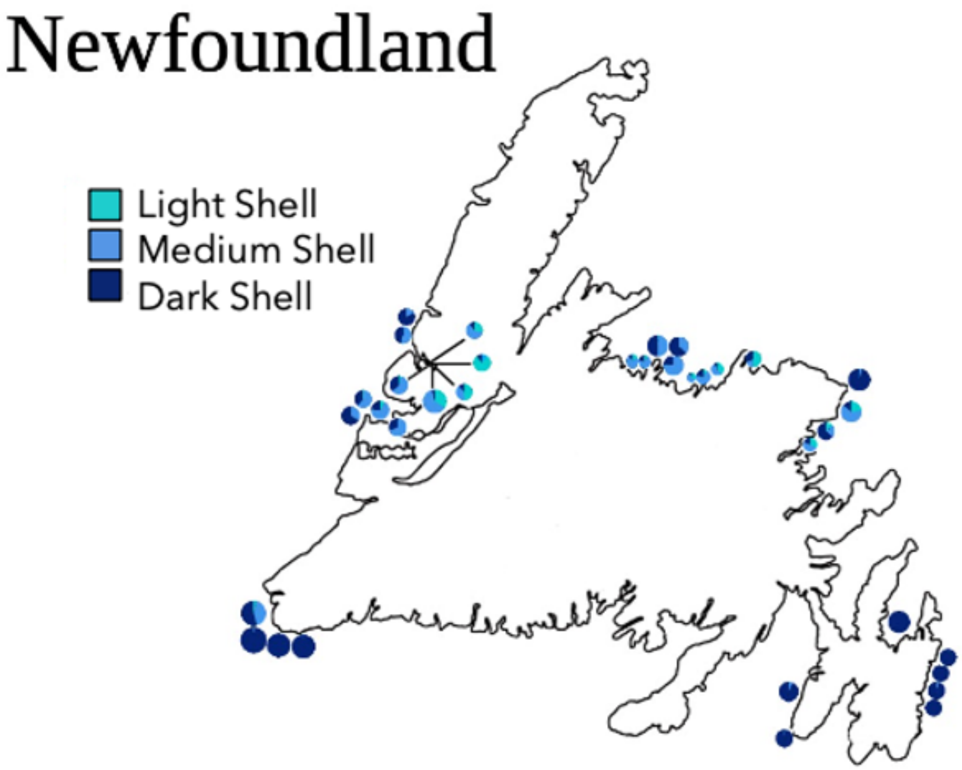

Figure S2. Shell colour differentiation of 36 localities in response to increasing wave exposure within Newfoundland.

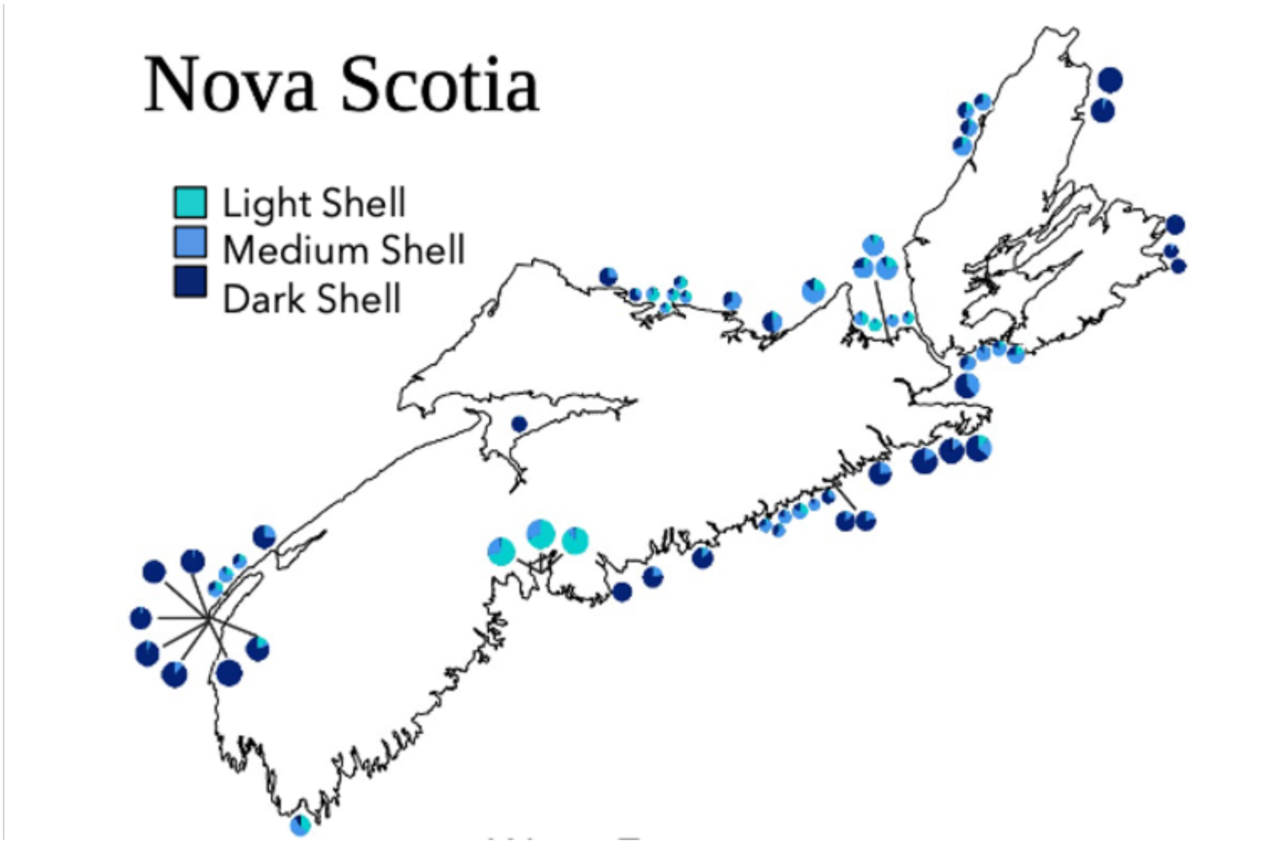

Figure S3. Shell colour differentiation of 62 localities in response to increasing wave exposure within Nova Scotia.

Locality Exposure and Shell Colour

Loglinear analysis revealed a significant positive correlation between high levels of wave exposure and the outcome of 'dark' shells in both provinces (NFLD & NS: $Z > 4.5$, $p < 0.001$) (Figure S2 & S3).

Locality Exposure & Shell size

With respect to shell size, no significant difference was observed between groups of locality exposure and mean size (NFLD: $F=2.34$, $p>0.5$, $n=54$; NS: $F = 1.31$, $p >0.05$, $n=83$).

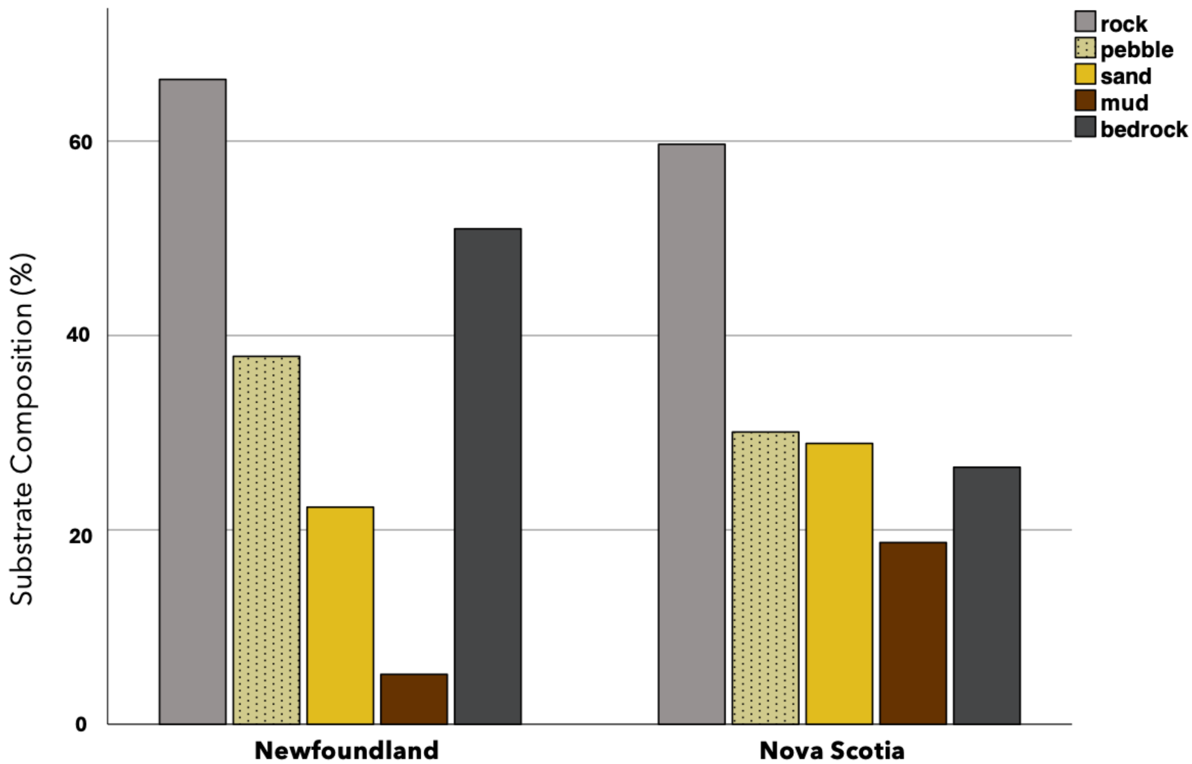

Figure S4. Percent composition of substratum of Newfoundland & Nova Scotia. Note that Nova Scotia is has more muddy and sandy beaches on average compared to that of Newfoundland.