**Biotic resistance on muddy shores: Non-parasitic native barnacles parasitise a non-native snail**

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**Abstract**

**Introduction**

Since the turn of the century, the number of marine non-indigenous species has continued to increase dramatically across the globe (Ruiz et al., 2015). Non-Indigenous Species (NIS) are organisms that have been transported deliberately or accidentally to areas beyond their native biogeographic range by humans by means of vectors such as shipping, aquaculture, fisheries and the construction of canals between previously unconnected water bodies, (Rilov & Crooks, 2010; Essl et al., 2018). These species may then establish and spread, which can lead to ecological or economic harm and a change of status from NIS to invasive (Sodhi & Ehrlich, 2011; NOAA, 2019). The impacts caused to marine environments from marine invasive species are increasing over time due to the accumulation of new introductions, and previously established NIS expanding their population densities and ranges (Parker et al., 1999).

The success of a NIS in a new marine environment can depend on characteristics of the species itself but also on the features of the recipient habitat. [Might add a sentence about species characteristics that favour invasiveness in marine environments here]. In addition, NIS establishment and/or spread may fail because of ‘resistance’ by the organisms that make up the recipient ecosystem (deRivera et al., 2005; Dick et al., 2013; Kimbro et al., 2013). This ‘biotic resistance’ arises from parasitism, predation, and/or competition from native species (deRivera et al., 2005; Dick et al., 2013). For example, resident European amphipods Gammarus pulex and G. duebeni celticus can effectively restrict the abundance and distribution of introduced North American amphipod Crangonyx pseudogracilis by preying on the latter (Dick et al., 2013). [would be good to add an example of competition providing biotic resistance]. Although native species can also parasitise NISs, there are far fewer (or any?) examples of this form of biotic resistance in marine environments.

The Japanese mud snail Batillaria attramentaria, native to northeast Asia, is now prevalent on the Pacific coast of North America. In the early 20th century, B. attramentaria was introduced as a hitchhiker on Pacific oyster imported from Japan for aquaculture (Galtsoff, 1932). A trematode parasite, Cercaria batillariae, which uses the snail as first intermediate host, was introduced at the same time and is still prevalent in North American populations (Torchin et al., 2005) [Need to say a little more about the snail – feeding mode, high densities reached, and especially lack of information about impact. In its native range, B. attramentaria is commonly found hosting little-cone limpets Patelloida conulus. The mobile limpets are thought to keep B. attramentaria shells free from encrusting barnacles and oysters (Noseworthy and Choi 2020). In the absence of a functionally similar limpet in habitats invaded by B. attramentaria, we asked whether native barnacles might contribute to biotic resistance. Our study therefore explores if native barnacles impose one or more costs on the mudsnails upon which they settle. We examined the effects of native barnacles on mudsnail movement, growth, and short-term survival. By understanding the influence of native organisms as biotic resistance to the Japanese mud snail, our study deepens our understanding of marine invasions, which that may help to predict the outcomes of future non-indigenous species introductions.

**Methods**

**Study site**

We performed experiments and collected *Batillaria attramentaria* at Blackie Spit (coordinates), Crescent Beach, British Columbia, in June and July 2021 . Blackie Spit is a sandy point that extends into Mud Bay at the mouth of the Nicomekl River (Figure 1). The site is characterised by a wide, muddy intertidal area that stretches as a shallow slope from a saltmarsh to the soft-bottom subtidal. *Batillaria attramentaria* was distributed across shallow ponds in the lower saltmarsh to the subtidal zone and was particularly abundant in areas with moist sand and mud.

**Population survey**

To establish the proportion of mud snails with barnacles , we ran three transects perpendicular to the shore, extending from at the edge of the saltmarsh to the water’s edge at low tide . Transect length varied from 129 to 150 m. Every 3 m along each transect, we counted the total number of mud snails in a 20 cm x 20 cm quadrat as well as the number of snails with barnacles on their shells. Using calipers, we measured the length and noted the presence of barnacles on up to 10 snails chosen at random in each quadrat.

**Behavioural observations**

To examine to the potential locomotion cost imposed by native barnacles on mud snails, we measured the speed of snails with and without barnacles. We observed 12 mud snails, selected haphazardly, for 5 min each in each of five ponds in the lower saltmarsh. Half of the mud snails were carrying at least one barnacle. We placed a wooden skewer near the posterior end of each snail’s shell to mark its starting location. We placed another skewer near the posterior end of the snail’s shell after 5 min. We then recorded the distance between the two skewers for each snail. We were able to track four mud snails simultaneously per observation. We then collected each snail in an individually labelled plastic bag, and froze the snails for later analysis.

In the laboratory, we used calipers to measure the maximum length, width and aperture diameter of each thawed snail. We counted and removed any barnacles, and obtained the wet weight (to the nearest 0.001 g) of the snail and, when present, its barnacles. Finally, we crushed each snail’s shell with a hammer to expose the posterior tissue for the presence of trematode cercariae.

**Length-weight allometry**

To examine the potential effect of native barnacles on mud snail growth, we compared the length-weight allometry of mud snails with and without barnacles. We collected snails along four 9-m-long transects running perpendicular to shore from the water’s edge. At each metre we collected haphazardly six to eight mud snails, three to four with barnacles size-matched with three to four without. After collection, we froze the snails in transect\*location labelled bags and processed them in the laboratory as described above.

**Mark-recapture study**

Finally, we conducted two mark-recapture studies aimed at determining if barnacles affect Batillaria survival. In the first study (18 June 2021), we haphazardly collected 10 mud snails with barnacles and 10 mud snails without in each of the five ponds used for behavioural observations. We used red nail polish (Sally Hanssen Insta-Dry, ‘Rapid Red’) to paint the posterior half of each snail and, after allowing 3-4 min for drying, placed the mud snails back into their original pond. Six days later, we revisited the ponds and recorded the number of marked mud snails remaining with and without barnacles.

We repeated the mark-recapture study on 16 July 2021, expanding the marking to eight ponds (including four from the first study).

**Statistical analysis**

- Standardised and centered all continuous variables (distance from water and length) to allow comparison of effect sizes.

Distribution and prevalence

- Used GLMER with negative binomial distribution to examine relationship between snail abundance and distance from the water because of overdispersion of the data. Used transect as random effect. Removed three data points that were outliers.

total\_num\_glmer <- glmer(total\_num ~ scale\_distance\_from\_water + (1|Transect),

data = snail\_distribution\_noNA, family = poisson())

- Used LMER (gaussian distribution) to examine relationship between snail length and distance from the water. Used quadrat nested within transect as random effect

snail\_length\_lmer <- lmer(scale\_length ~ distance\_from\_water + (1|Transect/quadrat),

data = snail\_length\_noNA)

- Used glmer with binomial distribution to examine likelihood of having one or more barnacles in relation to distance from water, snail length and the interaction between the two, as well as transect as a main effect because fit was singular if used as a random effect. Quadrat included as random effect

prob\_barn\_glmer4 <- glmer(barnacled\_num ~ scale\_distance\_from\_water \* scale\_length + Transect + (1|quadrat), data = snail\_distribution\_nozero\_scaled, family = binomial())

**Snail movement**

- Used hurdle models to examine first the effect of barnacles of whether snails moved at all (using binomial distribution), then second on the distance covered in 5 min for those snails that did move (using gamma distribution with a log link). We conduct three separate models to examine (as fixed factor) the effect of presence/absence of barnacles, the effect of total barnacle weight, and the effect of barnacle weight as a proportion of snail weight. Snail length and presence of trematodes were included as fixed effects in all models, as was pond as a random effect.

hurdle\_mod1 <- glmer(distance\_logistic ~ barnacled + length +

trematodes + (1|pond), family = binomial, data = snail\_movement\_logistic)

hurdle\_mod2 <- glmer(distance ~ barnacled + length +

trematodes + (1|pond), family = Gamma(link = "log"), data = snail\_movement\_gamma)

**Shell allometry**

- Used lmer model to examine differences in length-weight relationships between snails with and without barnacles. Included length, presence of barnacles, the interaction between the two, as well as presence of trematodes as fixed effects. Pond as random effect.

mod\_allometry <- lmer(wet\_wgt\_snail ~ length \* barnacled + trematodes + (1|pond),

data = snail\_movement)

Checked VIF, which indicated that predictors were highly correlated (VIF > 10 or 2.5?). Not sure what to do about this.

length barnacled trematodes length:barnacled

4.131578 158.133429 2.767827 177.001833

**Recapture**

- Used GLMER with Poisson distribution to compare the numbers of marked snails recaptured after marking in relation to whether they had barnacles or not. Used pond nested within date (June vs July) as random effect.

recapture\_glmer <- glmer(num\_barnacled ~ barnacled + (1|date/pond),

data = snail\_recapture, family = poisson())

**Results**

**Distribution and prevalence of barnacles on *Batillaria***

We surveyed 144 quadrats and encountered 560 *B.* *attramentaria*. The number of Ba per quadrat ranged from zero to 46 (mean ± 1 SD: 3.9 ± 5.6 individuals per 400 cm2). Snail length ranged from 5 to 41 mm (mean ± 1 SD: 23.4 mm ± 6.3 mm, n = 477). Nearly one-third (32.9%, n = 184) of all Ba were carrying one or more barnacles.

Snail abundance increased slightly (estimate: 0.17008, SE: 0.07857, Z = 2.165, p = 0.03; Fig 2), while snail length decreased slightly (estimate: -0.003092, SE: 0.001745, t = -1.77, p = ??; Fig 3) with distance away from the water. In both cases, the effect size was very small.

The likelihood that a snail had one or more barnacles did not vary with distance from the water (estimate: -0.01904, SE: 0.17875, z: -0.107, p = 0.915) but increased with snail length (estimate: 1.04323, SE: 0.17370, z: 6.006, p < 0.001; Fig 4). There was no interaction between distance from the water and snail length (estimate: -0.20214, SE: 0.17948, z: -1.126, P = 0.26).

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Fig 2. Relationship between snail abundance and distance from the water. (prob redo figure – uses Poisson distribution instead of neg binomial) N = 139 quadrats

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Fig 3. Relationship between snail length and distance from the water. N = 477 snails

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Fig 4. Likelihood of Ba carrying one or more barnacles in relation to snail length. Snail length was scaled and centred in the analysis but is shown untransformed here. N = 477 snails

**Snail movement**

The presence of barnacles on a snail tended to reduce the likelihood that a snail would move at all during the observation period, but had no effect on the distance moved (Table 1). No other predictors (length, presence of trematodes) influenced snail movement (Table 1). In contrast, the weight of barnacles carried had no effect on the likelihood that a snail would move, but reduced the distance moved (Table 1, Fig 5). The weight of barnacles relative to snail weight tended to reduce the likelihood that a snail would move, and decreased significantly the distance moved (Table 1, Fig 6).

Table 1. Results of hurdle models examining the effect of barnacles on snail movement. Hurdle 1 refers to movement vs no movement. Hurdle 2 refers to the distance moved by those snails that did move.

**Barnacle metric Estimate Std. Error z value P**

*Presence/absence*

Hurdle 1

(Intercept) 0.67583 4.10178 0.165 0.869

barnacledyes -1.28950 0.71171 -1.812 0.070 .

length 0.06092 0.17093 0.356 0.722

trematodesyes -0.93197 1.97399 -0.472 0.637

Hurdle 2

(Intercept) 4.36510 1.97907 2.206 0.0274 \*

barnacledyes 0.02657 0.24544 0.108 0.9138

length -0.09812 0.08366 -1.173 0.2409

trematodesyes 0.77904 1.09306 0.713 0.4760

*Weight of barnacles carried*

Hurdle 1

(Intercept) -5.2730 5.5027 -0.958 0.338

wgt\_barnacles -0.7855 0.5124 -1.533 0.125

length 0.2668 0.2341 1.140 0.254

trematodesyes -0.2033 2.2805 -0.089 0.929

Hurdle 2

(Intercept) 1.40596 3.04357 0.462 0.6441

wgt\_barnacles -0.41716 0.19594 -2.129 0.0333 \*

length 0.02797 0.12670 0.221 0.8253

trematodesyes -0.01805 1.63523 -0.011 0.9912

*Ratio of barnacle weight to snail weight*

Hurdle 1

(Intercept) -0.39099 5.45196 -0.072 0.9428

wgt\_ratio -2.42518 1.37781 -1.760 0.0784 .

length 0.08892 0.22241 0.400 0.6893

trematodesyes 1.23915 2.77432 0.447 0.6551

Hurdle 2

(Intercept) 3.3367 2.9957 1.114 0.26535

wgt\_ratio -1.2614 0.4800 -2.628 0.00859 \*\*

length -0.0455 0.1236 -0.368 0.71275

trematodesyes 0.4815 1.6186 0.297 0.76609

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Fig 5. Distance moved in 5 min by Ba in relation to weight of barnacles carried. All snails that did not move omitted. N = 20 snails – NEEDS TO BE REDONE PROPERLY

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Fig 6. Distance moved in 5 min by Ba in relation to weight of barnacles carried relative to snail weight. All snails that did not move omitted. N = 20 snails – NEEDS TO BE REDONE PROPERLY

**Snail shell allometry**

Shell weight was unsurprisingly highly correlated with shell length, but the slope of the relationship varied depending on whether a snail carried barnacles or not (Fig 7). Shell weight increased faster in relation to shell length for snails that carried barnacles. Need to get p values. Should centre and standardise the data to compare the effect sizes

(Intercept) -1.11318 0.38277 -2.908

length 0.09240 0.01609 5.744

barnacledyes -1.21474 0.53373 -2.276

trematodesyes 0.15345 0.16199 0.947

length:barnacledyes 0.05132 0.02222 2.310

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Fig 7. Shell allometry in Ba, shown for snail that carried (red dots and line) or did not carry (black dots and line) barnacles. N = 60 snails Need to change ‘Barnacled’ for something better.

**Mark-recapture**

Across the two replicate experiments, 40% ( ± 20%, SD) of marked snails were recaptured 5 days after release, with 59% (± 13%) recaptured in June and 30% (± 16%) in July. The presence of barnacles had no effect on the proportion of marked snails recaptured (estimate: -0.2063, SE: 0.1935, z: -1.067, p = 0.286 ).

**Discussion**

Acknowledgements

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References