Green Means Go, Red means stop: Anti-predator behaviour in *Chlorostoma funebralis* to cues form the invasive green crab, *Carcinus maenas,* and native red rock crab, *Cancer productus*

by

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Abstract

The green crab, C*arcinus maenas*, is a non-native species on the coasts of British Columbia. Newly introduced invasive species may not be recognized as a predator by marine gastropods. Many marine gastropods have forms of anti-predatory behaviour, such as decreasing movement. This study determines how sympatric and allopatric populations of black turban snails, *Chlorostoma funebralis,* react to *C. maenas* compared to their common predator, *Cancer productus*. We investigated how *C. maenas* affected snail behaviour and foraging. We observed the movement of *C. funebralis* while exposed to different effluents. We found that both allopatric and sympatric snails moved more slowly in the presence of red rock crabs. When the crabs were fed conspecifics, the sympatric snails moved more slowly than allopatric snails. Both types of snails moved more slowly in the presence of green crabs when they were fed conspecifics. *C. funebralis* foraged less when Red Rock crabs were present but this result was on the borderline of significance. The results suggest that *C. funebralis* has not had enough time to adapt to *C. maenas* and does not recognize their chemical cue alone as a threat.

Keywords: Anti-predator behaviour, Black Turban snail, Green crab, Invasive, Red Rock crab, *Tegula funebralis*

Introduction

Invasive species thrive because they are able to efficiently exploit resources in new habitats. Their prey are unaccustomed to their presence and the chemical cues that these predators release may not trigger anti-predator behaviour (Keppel & Scrosati 2004). It has only been 10 years since the green crab, *Carcinus maenas*, invaded Pipestem Inlet (Gillespie et al. 2007). We wondered if this would be enough time for *Chlorostoma funebralis* to recognize *C. maenas* as a predator.

So far, green crabs have been found in Pipestem Inlet but have not yet invaded Grappler Inlet. In contrast, the Red Rock crab, *Cancer productus*, has coexisted with *C.*

funebralis for thousands of years (Gillespie et al. 2007). *C. productus* is a common predator but *C. maenas* is not, and because previous exposure and current risk can determine the strength of prey responses to predators (Keppel & Scrosati, 2004), gastropods may only respond to chemical cues from a familiar predator.

Many marine gastropods have some form of anti-predatory behaviour. Responses to a predator can include avoidance or escape behaviours, like climbing vertically to move above the water line. Climbing behaviour is triggered more often when predators are feeding on conspecifics (Jacobsen et al. 2004). Some species show the ability to detect and avoid predators that are feeding on conspecific versus congeneric prey (Ferland-Raymond & Murry 2008).

Avoidance behaviour is only well documented for *C. funebralis* in the presence of octopi, sea stars, and crabs of the genus *Cancer* (Keppel & Scrosati 2004) but researchers have not yet to fully investigated any anti-predator behaviour that *C. funebralis* exhibits in the presence of *C. maenas*. Here, we investigate how sympatric and allopatric populations of *C. funebralis* change their movement speed when exposed to chemical cues from the introduced *C. maenas*, compared to *C. productus.* Specifically, we ask if foraging and movement rates differ among allopatric and sympatric snails.

Because allopatric snails have never encountered *C. maenas* before, we predict that (i) allopatric and sympatric snails will behave differently in the presence of feeding green crabs. Similarly, we predict that (ii) the movement of allopatric *C. funebralis* will not be affected by effluent from Green crabs. They will also (iii) forage just as often in the Green crab treatment compared to a control or crushed food source treatment. We predict that (iv) the snails will move even more slowly and forage less when the crabs are feeding on consepcifics. Finally, we predict that (v) sympatric snails will decrease their movement speed and forage less in the presence of *C. maenas* just as they do in the presence of *C. productus*.

We found that both allopatric and sympatric snails moved more slowly in the presence of Red Rock crabs feeding on heterospecifics. There was a significant difference between the movement speed of allopatric and sympatric snails when the crabs were fed conspecifics. The sympatric snails moved more slowly than allopatric snails. When the crabs were feeding on conspecifics, both types of snails exposed to effluent from Green crabs and Red Rock crabs moved more slowly than the control. All snails moved more slowly in the presence of crushed conspecifics. However, this result was on the boarderline of significance. This suggests that *C. funebralis* has not had enough time to adapt to *C. maenas* and does not recognize their chemical cue alone as a threat.

Methods

We haphazardly collected 300 black turban snails from the Grappler Inlet (48°49.8' N, 125°07.1' W) and 300 from Pipestem Inlet (49°19.5' N, 125°19.4' W). We haphazardly collected all Red Rock crabs (11.61-13.30cm carapace width, 232-370 g) from Grappler Inlet and all Green crabs (7.70-8.87 cm, 132-194 g) from Pipestem Inlet.

We used 32 identical apparatuses for all experiments (Figure 1). The bucket (H=22.5 cm; D=21 cm) held one snail per treatment and the clear container (L: 24 x

W:13 x H:17 cm) held the effluent source. The clear container with the treatment was elevated for down-flow into the snail buckets. In each experiment, we exposed both allopatric and sympatric snails to one of four treatments: no crab, a green crab with a crushed food source, a red rock crab with a crushed food source and the crushed food source.

Movement Experiment

Heterospecific trial

Crabs were kept together in a large sea table with flowing sea water and fed mussels, *Mytilus trossulus*, for 1 day before the experiment. We placed the crabs in the clear containers with crushed mussels. Before beginning a trial, we recorded each snail's height and weight to determine if these variables had an affect on snail behaviour. We observed initial snail movement for 15 minutes, recording their position at 5 minute intervals. After an additional 45 minutes, we recorded their final position as 1 of 5 possibilities: in the middle of the bucket, at the edge, on the wall of the bucket, at the water line (within 1 inch above or below the water line) and above the water line. After each trial, we placed the snails in marked containers to separate them by type and treatment for future experiments.

Conspecific trial

We moved the crabs back into their original sea table and fed them them snails for 1 day. We repeated the same procedure as the *Heterospecific trial*, except the observation period was reduced to 20 minutes rather than 60. We recorded their final position after 20 minutes.

Each treatment was replicated 32 times per food source. Trials using each food source were run on separate days.

Feeding Experiment

To compare the feeding rates of snails among effluent treatments, we tested the amount of kelp, *Nereocystis luetkeana*, the snails ate when they were exposed to cues from crabs feeding on conspecifics. We used the snails that had been previously exposed to a chemical cue during the *Conspecific trial.* We subjected them to the same cues in the feeding experiment. After measuring the height and weight of each snail and the original weight of the kelp, we placed 3 snails inside a small zip-lock container with the kelp and placed the container into the bucket. This ensured that the snails could not move above the water line, out of effluent. After 12.5 hours, we weighed the kelp and calculated the difference in grams.

Our data was analysed using R 2.5.0. (R Development Core Team 2007). We ran a non-parametric survival analysis of the time taken to leave the arena (bottom of the bucket) to determine if there were any differences in movement speed.

Results

Movement Experiment

Heterospecific trial

Both allopatric and sympatric snails took a greater time to reach the edge of the bucket when exposed to effluent from Red Rock crabs compared to all other treatments (coef=-0.7521; df=8; p=0.0049; Figure 2). Allopatric and sympatric snails moved at the same rate (coef=-0.3310; df=8; p=0.2100)

Conspecific trial

When the crabs were fed conspecifics, sympatric snails moved more slowly than allopatric snails (coef=-0.7447; df=8 ; p=0.0047; Figure 3a). Overall, both types of snails took a greater time to reach the edge of the bucket when exposed to effluent from Red Rock crabs compared to all other treatments (coef=-0.7163; df=8; p=0.0054; Figure 3b). All snails exposed to effluent from Green crabs and Red Rock crabs took longer to reach the edge than controls (Green crab: coef=-1.5873; df=8; p=0.0140. Red Rock crab: coef=-2.6883; df=8; p=0.0098). Effluent from crushed conspecifics also caused experimental snails to move more slowly but this result was near the 0.05 cut off (coef=-1.0349; df=8; p=0.0550). We found that the time it took the snails to reach the air-water interface was significantly longer for sympatric snails (coef=-1.6183; df=8; p=0.0150; Figure 4a). All snails were exposed to effluent from Red Rock crabs and green crabs left the water faster than the other treatments (Red Rock crab: coef=-2.6883; df=8; p=0.0098; Green crab: coef=-1.5873; df=8; p=0.0140; Figure 4b).

Feeding trial

The snails exposed to effluent from Red Rock crabs ate less than all other treatments but this result was near the 0.05 cut-off (t=-1.984; p=0.052; df=23).

Discussion

Allopatric and sympatric snails only behaved differently in the presence of *C. maenas* that were fed conspecifics. This result was not consistent with our hypothesis. Regardless of the food source, we expected allopatric *C. funebralis* to show no avoidance behaviour because they had not been exposed to *C. maenas* before (Keppel & Scrosati 2004) and sympatric snails to show an avoidance response. The sympatric snails behaved as the allopatric snails did when the crabs were feeding on mussels, suggesting that 10 years is not enough time for *C. funebralis* to recognize the green crab as a predator. This difference can also be explained by the differences among populations; not all responses to predators are the same and different populations can display different levels of evasive response to similar chemical cues (Keppel & Scrosati 2004).

Allopatric *C. funebralis* moved significantly slower when exposed to effluent from Red Rock crabs but not Green crabs. This was consistent with our hypothesis. Allopatric *C. funebralis* only moved more slowly when exposed to effluent from green crabs feeding on conspecifics.

Both sympatric and allopatric snails foraged less in the presence of *C. productus*, which is consistent with our hypothesis. However, there was no strong statistical significance. This may be due to the long-term exposure of both types of snails; constant exposure to high predation risk does not allow them to utilize foraging less as an anit-predator response (Hamilton et al 2001).

The data we collected implies that crushed conspecifics had an effect on anti-predator behaviour. This makes sense as the mere presence of predators does not mean there is an immediate predation threat (Keppel & Scrosati 2004). This could explain why allopatric snails responded to Green crabs feeding on conspecifics. Our results suggest that cues from crushed conspecifics can be used to create an association between a novel predator and danger.

The snails were not as hesitant to move when the crabs were feeding on crushed mussels. However, we cannot directly compare the data because the trials using mussels and those using conspecifics were run at different times, making them independent. In future studies, we would divide our set up so that the trials with crabs feeding on heterospecifics and those feeding on conspecifics are run at the same time. This would allow us to compare the two with more confidence.

We noticed that many of the snails from Pipstem Inlet were covered with epibionts like B*alanus glandula.* We wondered if this was a defence mechanism, as some predators, such as *Pisaster orchraceus*, prefer snails with bare shells (Thornber 2007). In future studies, one could look at effectiveness of epibionts in deterring *C. maenas*.

Our results show that *C. funebralis* had the strongest response to *C. productus*. They slowed their movement speed in response to chemical cues from a common predator. Anti-predator behaviour could be due to genetics or could be a learned behaviour (Geller 1982). Our study suggests that anti-predator behaviour is genetic, as the sympatric snails did not behave dramatically different from the allopatric snails. Not enough time has passed for *C. funebralis* to recognize *C. maenas* as a predator. Snails from an area in which predators are present should illicit the strongest response (Geller 1982), but this was not true in our experiment. This result suggests that *C. funebralis* takes more than 10 years to develop associations with their defence mechanisms and the chemical cue alone of a new predator.

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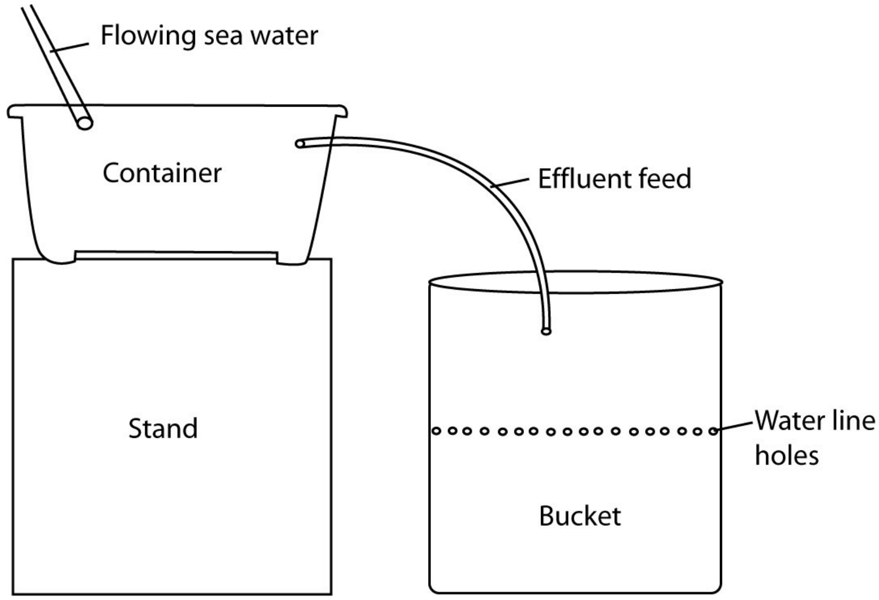

Figure 1. Schematic of experimental chamber (8 per sea table, in 4 tables total) used in all experiments. The effluent from the container travelled into the bucket via a plastic tube at a rate no greater than 1 L/min.

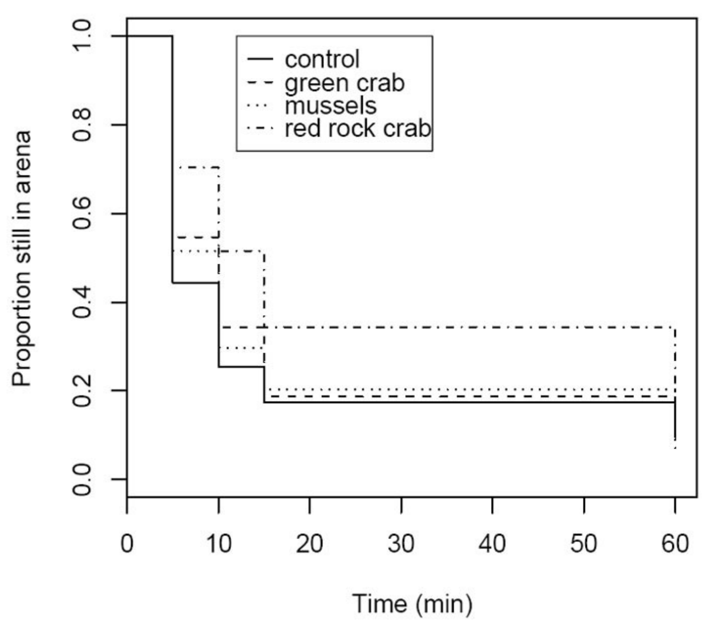

Figure 2. Proportion of allopatric and sympatric *C. funebralis* still in arena, in the bucket, when exposed to effluent from 4 different treatments.

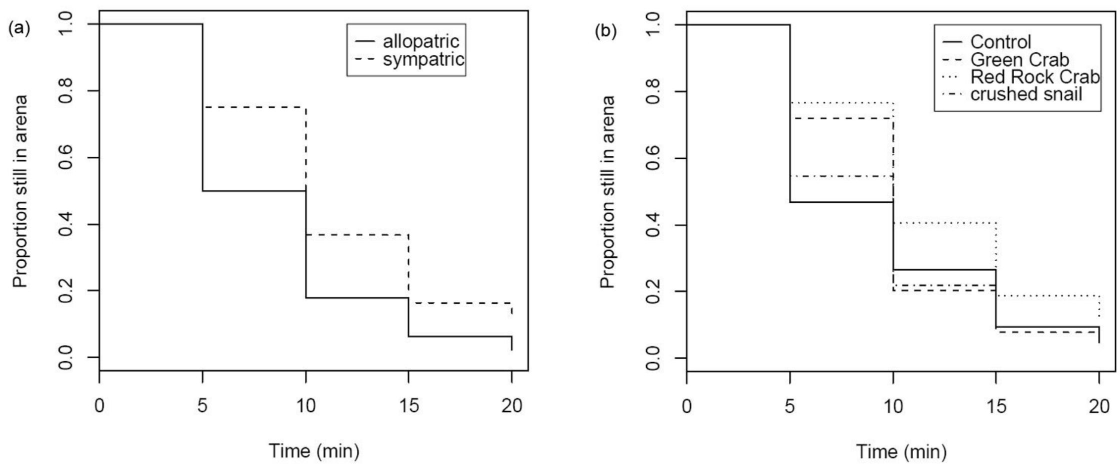

Figure 3. (a) Proportion of allopatric and sympatric *C. funebralis* still in arena (b) Proportion of allopatric and sympatric *C. funebralis* still in arena, in the bucket, when exposed to effluent from 4 different treatments .

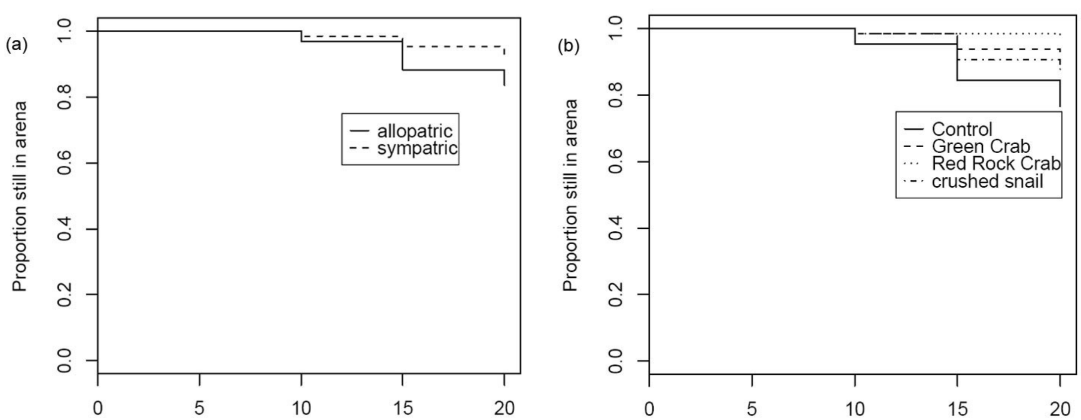

Figure 4. (a) Proportion of allopatric and sympatric *C. funebralis* still in arena, not at the water line. (b) Proportion of allopatric and sympatric *C. funebralis* still in arena, not at the water line, when exposed to effluent from 4 different treatments.