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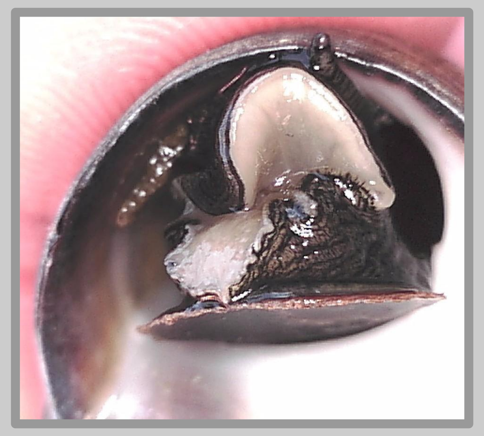

Effects of water quality on boldness and movement of the common periwinkle (*Littorina littorea)*

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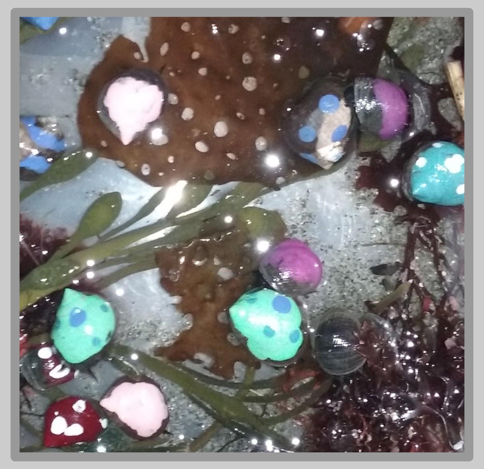

ABSTRACT

Stormwater runoff and sewage seepage are sources of chemicals and pathogens that lower immunity of marine organisms such as the common periwinkle (*Littorina littorea*), which can increase susceptibility to parasites. Periwinkles infected with parasites are less bold and show altered movements. The purpose of my study was to 1) identify pathogens in water that can affect marine organisms, 2) determine if water quality affects boldness in periwinkles, and 3) determine if water quality affects movement and substrate choice in periwinkles. I predicted that water samples vary in levels of fecal indicator bacteria because samples were collected from areas at high risk of sewage seepage and stormwater runoff. Based on previous work, I also predicted that periwinkles exposed to higher contamination levels are less bold and move shorter distances. I collected periwinkles from the same location and assigned them to 1 of 3 water quality groups. I tested water samples for *E. coli* and other bacteria, I measured boldness in periwinkles as latency to re-emerge from their shells, and I recorded rates of movement and choice of substrates. *E. coli* was present in 1 of the 2 expected contaminated samples. Periwinkles housed in water from the high sewage seepage area were more bold than those in the control tank and spent more time on substrate. Distance traveled did not differ among water treatments. In conclusion, bacteria may affect the behavior of marine organisms. My data add to research comparing water quality and behavior of marine organisms.

BACKGROUND INFORMATION

- Intertidal organisms are exposed to chemicals and pathogens from stormwater runoff and sewage seepage, which can lower their immunity (Lerman, 1986; Fig. 1).
- Periwinkles *(Littorina littorea)* infected with trematodes are less bold (Seaman & Briffa, 2015) and show altered movements (O'Dwyer, et al., 2014).
- Studies have focused on parasite effects on periwinkle behavior; however, the relationship between periwinkle behavior and exposure to fecal borne bacteria remains unexplored.
- I used periwinkles as my study species because they are abundant, resilient (Lerman, 1986), and exposed to fecal borne bacteria in their natural habitat (Spruce Creek Association, 2008).

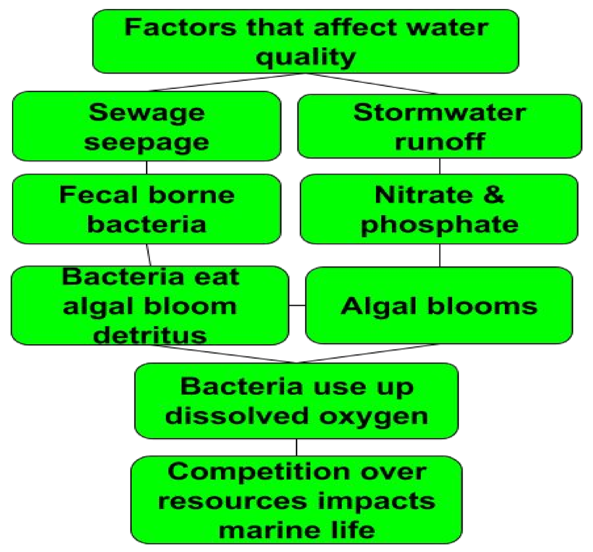

Figure 1. Factors that affect water quality, which in turn affect marine organisms.

OBJECTIVES

1. To identify pathogens in water that can affect marine organisms.
2. To determine if water quality affects boldness in periwinkles.
3. To determine if water quality affects movement and substrate choice in periwinkles.

PREDICTIONS

1. Water samples vary in levels of fecal borne bacteria because one area has low risk of pollution, another area has high risk of sewage seepage, and another area has high risk of stormwater runoff.
2. Periwinkles exposed to high levels of bacteria are less bold because competition over resources reduces overall health of periwinkles, making them more susceptible to pathogens; therefore, they are more cautious to re-emerge from safety of the shell (Seaman & Briffa, 2015).
3. Periwinkles exposed to higher levels of bacteria move shorter distances and choose different substrates than periwinkles exposed to lower contamination because periwinkles infected with parasites show different choice in habitat and move shorter distances (O'Dwyer, et al., 2014).

METHODS

I collected periwinkles and control water samples from Seapoint Beach, Kittery, ME (43.07°,-70.68°; Fig. 2), which I designated as area 1. I collected 2 additional water samples from Spruce Creek, ME, which has a history of pollution (Spruce Creek Association, 2008). Area 2 has high exposure to stormwater runoff because it is adjacent to U.S. Route 1. Area 3 has high exposure to sewage seepage because it is situated next to a densely populated (1,712 ha) residential area.

I placed 10 periwinkles in each of 3 tanks (20 x 37.5 x 20 cm) equipped with air stones and filled with 5 cm of room temperature water from different sites (Fig. 3). I painted snails with different colored waterproof nail polish to distinguish individuals. Every other day, I added water from respective areas to tanks to account for evaporation.

To test water samples for fecal borne bacteria, I used the COLISURE kit produced by Idexx to test for *Escherichia coli* because they are common fecal borne bacteria, and I used standard plate count method to identify total bacteria concentration.

I measured periwinkle boldness as time to re-emerge from the shell after I pushed the operculum back into its shell (Seaman & Briffa, 2015). I considered periwinkles re-emerged at the first sight of operculum, tentacles, or movement of shell (Fretter & Graham, 1994). I tested each periwinkle's boldness daily over a 7-day period.

I observed choice in substrate using continuous focal sampling for 2 min per individual with a stopwatch after re-emergence. I considered periwinkles positioned on rocks or the tank wall to be on substrate and periwinkles on tank floor or on other periwinkles not on substrate.

I used a 2.5 x 2.5 cm grid to record the vertical and horizontal distance traveled by each individual at 1 h after re-emergence (Fig. 3). I measured distance traveled from the initial position where I placed periwinkles after pushing the operculum. I initially placed all periwinkles on the tank floor where they were fully submerged in water and 5 cm from rocks.

I used Kruskal-Wallis tests followed by pairwise comparisons to examine differences in boldness, substrate choice, and distance traveled in different water samples. I used JMP 12.2 (SAS Institute, Inc., 2015) to analyze data using significance level of P < 0.05.

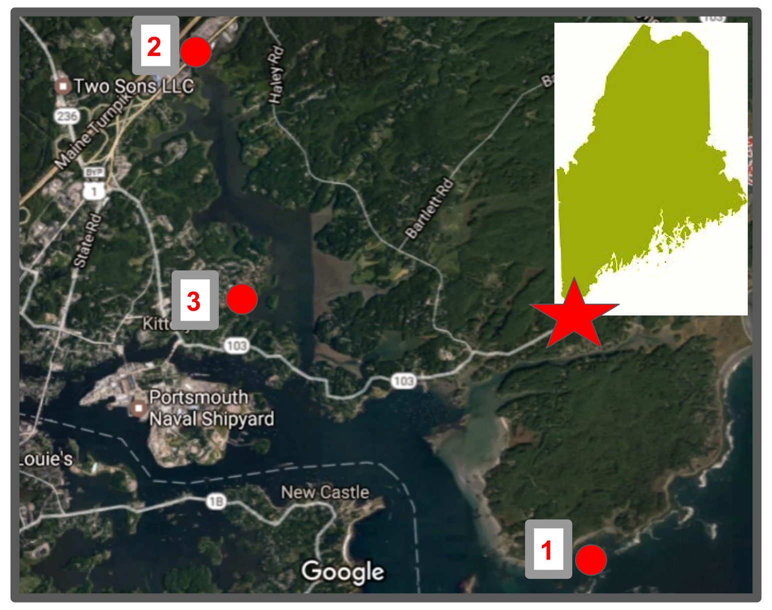

Figure 2. Spruce Creek watershed, Maine. Area 1 has low pollution risk, area 2 has high risk of stormwater runoff, and area 3 has high risk of sewage seepage.

Figure 3. Tanks housing periwinkles. Each tank contained 10 periwinkles, 1 air stone, and rocks and algae from respective water sample locations. Periwinkles grazed freely off algae on rocks and seaweed.

RESULTS

- I found fecal borne bacteria in water with high risk of stormwater runoff only; however, *E. coli* was not found in subsequent tests. Other, non-specified bacteria were found in all treatments (Table 1).
- Boldness, measured as re-emergence times, differed depending on the water source (χ^2 = 7.44, d.f. = 2, P = 0.024; Fig. 4). Periwinkles exposed to *E.coli* in water at high risk of stormwater runoff (area 2) re-emerged faster than snails in control water (area 1; P = 0.01). Boldness did not differ between snails housed in water from high risk of sewage seepage (area 3) and high risk of stormwater runoff or between snails housed in control water and high risk of sewage seepage (area 2 vs. area 3: P = 0.07; area 1 vs. area 3: P = 0.34).
- Distance traveled by snails housed in 3 different water treatments did not differ (χ^2 = 3.73, d.f. = 2, P = 0.15; Fig. 5).
- Mean time on substrate differed significantly across sites (χ^2 = 11.94, d.f. = 2, P = 0.0025; Fig. 6). Snails in water from high risk of stormwater runoff spent more time on substrate than snails in control water and water from high risk of sewage seepage (area 2 vs. area 1: P = 0.024; area 2 vs. area 3: P = 0.002; Fig. 6); however, snails in control water and snails in water at high risk of of sewage seepage did not differ (P = 0.062; Fig. 6).

Water source	*E.coli* present (cells/mL)	Colony forming units (cfu/mL)
control	0	12
stormwater runoff	0.110	290
sewage seepage	0	87

Table 1. *E.coli* and colony forming units in water samples from three areas.

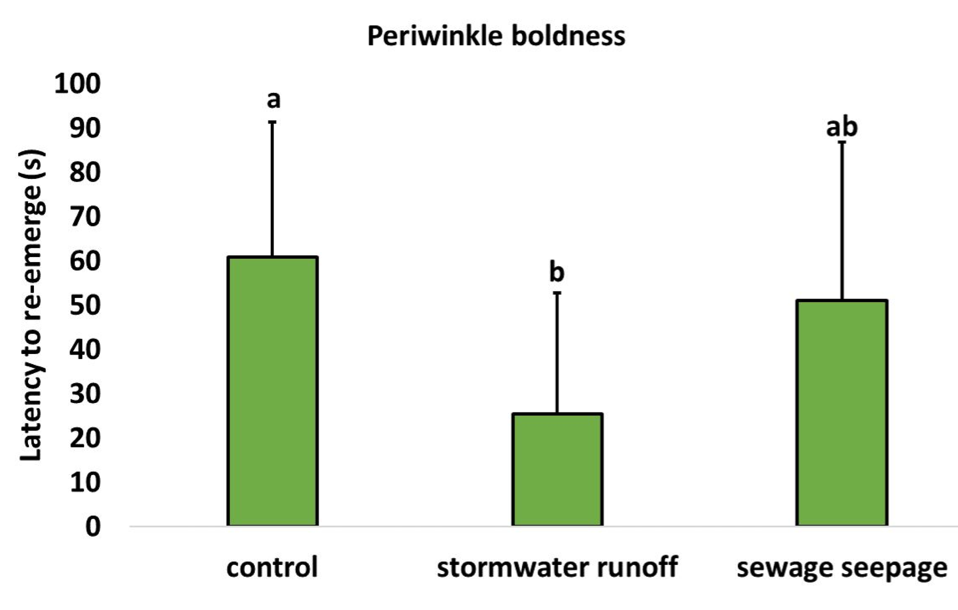

Figure 4. Mean (+SD) time (s) for periwinkles to re-emerge from shell in different water treatments. Different letters above bars indicate statistically significant differences between samples (P < 0.05; n = 10 snails per treatment).

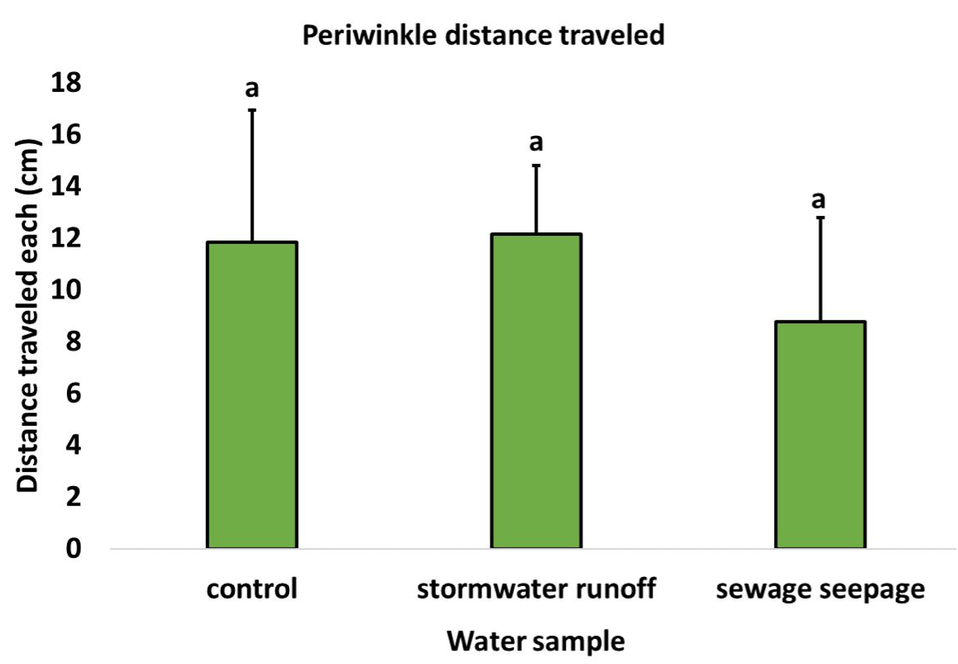

Figure 5. Mean (+SD) distance traveled by periwinkles in different water treatments. Same letters above bars indicate no significant differences between samples (P < 0.05; n = 10 snails per treatment).

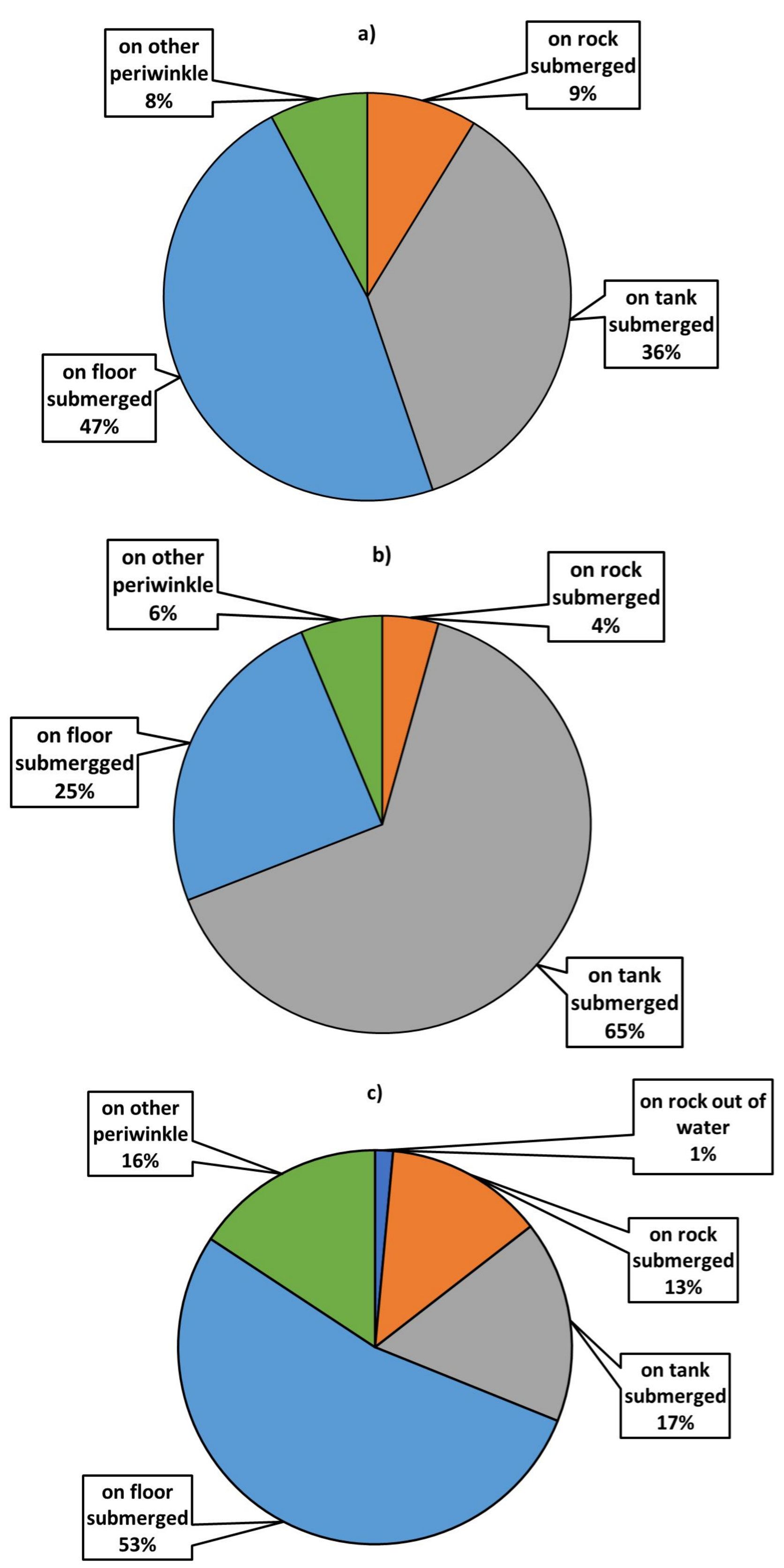

Figure 6. Mean percentage of time that periwinkles spent on different features of a microhabitat in 3 water treatments. Total time per tank = 8,400 s. a) Control, b) High risk of sewage seepage, c) High risk of stormwater runoff (n = 10 snails per treatment).

DISCUSSION

I found *E. coli* in water collected near U.S. Route 1; however, I did not find *E.coli* in water from area 3, where I expected to find the highest concentration of fecal borne bacteria due to the close proximity to Admiralty Village, a densely populated area in Kittery. I found other, non-specified bacteria in all three areas, with the highest concentration from area 2, followed by area 3. The unexpected lack of *E. coli* in area 3 may be due to local initiatives to decrease pollution from sewage seepage and stormwater runoff in Spruce Creek (Spruce Creek Association, 2008).

Data do not support the prediction that bacterial presence reduces periwinkle boldness; rather, snails exposed to bacteria were significantly more bold than those that were not exposed. Contrary to previous research that suggests boldness indicates overall health (Dantzer, 2001, cited in Seaman & Briffa, 2015), my data suggest that boldness may be altered in response to predation. Periwinkles hide in their shells to avoid predation (Cain, et al., 2014); however, parasites may increase host boldness to encourage predation and transfer the parasite to the next host (Reisinger, et al., 2015). Like trematodes, *E. coli* commonly occur in animal digestive systems and are dispersed through feces; therefore, *E. coli* may benefit from host consumption to increase their exposure and range. More research is needed to investigate possible mechanisms that enable *E.coli* to affect animal behavior.

Data do not support the prediction that bacterial presence in water decreases periwinkle movement. Distance traveled did not differ across groups, perhaps due to the microhabitat in the tanks. I provided periwinkles with unlimited food, and snails were never at risk of desiccation. In their natural environment, periwinkles must forgo food and water for hours waiting for the tide to come in (Fretter & Graham, 1994), or they move toward water. Periwinkles infected by parasites show less movement toward the ocean than those that are not infected, which increases exposure to predators and decreases exposure to food (O'Dwyer, et al., 2014). To test the possibility that periwinkles move shorter distances when conditions are favorable, I could place periwinkles on a rock out of water in the center of the tank after pushing back their operculum, rather than placing them on the tank floor where they are fully submerged, thus simulating the decision to follow the water or to wait for the tide to return.

Periwinkles exposed to *E. coli* and high levels of bacteria spent more time on substrate than the control group, which supports the hypothesis that water quality affects habitat choice. Previous studies found periwinkles infected by trematodes were less likely to choose "safe" substrates such as under rocks, in crevices, and in the lower intertidal zone, where predation is less likely (O'Dwyer, et al., 2014). Periwinkles exposed to bacteria showed a similar pattern, as they spent more time on substrate close to the surface of water and less time on the tank floor. To determine if bacteria affect periwinkles' choice in substrate, I could set up a field study in a natural habitat with high levels of bacteria. I could use similar methods; however, I would leave snails undisturbed and log the position of each snail daily to determine whether they choose favorable locations and whether position is related to likelihood of predation.

CONCLUSIONS

- Bacteria levels were high in areas at high risk of pollution. However, only small amounts of fecal indicator bacteria were found in the area at high risk of stormwater runoff, which indicates that bacteria other than fecal borne bacteria may affect marine life.
- The presence of bacteria appears to have adverse effects on periwinkles, possibly by increasing exposure to predators; however, the possible mechanisms by which *E. coli* and other bacteria manipulate snail behavior are unknown.
- More research is needed to determine whether bacterial presence directly affects snail health and behavior.
- Bacteria levels were highest in areas exposed to stormwater runoff and sewage seepage; therefore, anthropogenic sources of pollution may alter the behavior of marine organisms.

ACKNOWLEDGMENTS

Thanks to Idexx Laboratories, Inc for the free supplies donated to USM, R. Larsen for assistance with water quality analysis, A. Perry & K. Weber for laboratory access, and C. Maher for constructive criticism and revision.

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