**Littorina littorea** Feeding Migration to Differing Algae Concentrations and Light Wavelengths

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**Introduction:**

Periwinkles (Phylum Mollusca, Class Gastropoda: *Littorina littorea* [Apolinario, 1999]) can be found along rocky coasts throughout the upper shore into the littoral zone (Jackson, 2005) and are very common along New England shores, with the exception of highly exposed areas. Periwinkles are distinguished anatomically by a round univalve shell (Biomescenter, 2005) which is usually dark in color, black or brown, and only about 3 cm in size.

PeriwinkleOs are a member of the Phylum Mollusca meaning they have a mantle, which secretes calcareous spicules used for making of the shell; a radula, a circular band of teeth used for feeding; and lastly a foot, which is made from ventral body wall muscles and usually used for locomotion (Pechenik, 2005). Periwinkles use their shell to protect themselves from dessication as well as from predators, and use an operculum, a proteinaceous shield, to cover the opening of the shell (Pechenik, 2005). *Littorina* is a strict herbivore and uses a radula for scraping algae from intertidal rocks. In some cases of *Littorina* overgrowth entire rocky shores can be scraped clean of algae (Biomescenter, 2005). The ventral foot is used for locomotion in the periwinkle and carries the organism and its shell along rocks for grazing.

*Littorina littorea* have tentacles as well as eye spots, both of which are exposed during feeding (Pechenik, 2005) and are possibly used for algae and light detection. In order to determine the role of periwinkle phototaxis and chemotaxis, experiments using *Littorina* and cold water algae were performed. First it was determined if a periwinkle could distinguish between areas of high algae concentration and low algae concentration. The hypothesis is if a periwinkle is given a choice to graze within an area of high food concentration versus low food then the periwinkle will choose to graze in an area of higher concentration. Next I hypothesized that periwinkles could distinguish between different colors of light, and if so, if they prefer to feed under a specific color of light. Four different types of light were tested; green, blue, white, and no light; it is assumed that a periwinkle will prefer to feed in a darker area than a lighter one because it will feel safer being hidden by being in the dark. Most marine animals with eye spots cannot distinguish between colors but only light and dark areas, so if any color of light is shown compared to a dark area the periwinkle will always migrate to the dark area. However it is also possible to see if the periwinkles can some colors and not others. Lastly, by combining food concentration and light color, one can determine which experimental factor influences periwinkle feeding, either algae abundance or light color. The last hypothesis is that a periwinkle will migrate to an area with a high algal concentration in a light that has been found that periwinkles stay out of, over an area with low algal concentration in a light that periwinkles migrate into, and that the preference for food overrides the need to be hidden.

By studying the use of these features on *Littorina* the method of feeding can be determined and can give insight into how *Littorina* finds food as well as identifying possibly dangerous feeding areas, such as feeding areas that have exposure to predators. Both of these factors can be used to speak about the periwinkle's adaptations to the littoral zone. The snail can be adapted to migrate to areas with a larger amount of algae as well as being adapted to migrate to dark areas. These are useful adaptations because if a snail migrates to areas of high algae it does not waste energy searching around for food.

Collaborators in this study were found among classmates. Serena Strydom researched the effect of light intensity on sea anemone orientation in "The Effects of Changing Light Intensity on Reorientation of Aiptasia palladia"(2005). Both projects deal with the effects of light on marine invertebrates, and comparing results revealed that snails orient themselves and migrate differently under different colors of light while sea anemones do not. Maris Madeira tested negative phototaxis on crabs in "Phototaxis vs. Coverage Preferences in Hemigrapsus sanguineus" (2005) which relates to this project in that marine organisms were given the option for shaded areas and their responses were measured.
Comparing results revealed that crabs preferred to be in a shaded area where they can be hidden compared to ambient white light, while my study revealed the same results.

**Materials and Methods**

**Materials:**

- **Animals employed:** 6 periwinkles (*Littorina littorea*) of approximately the same size
- Equipment to hold animals: 10 gallon tank kept in the cold room on the 2nd floor of the Science Center (15 C), ~10 gallons of 35 pps water to be used for the tank and for the experimental tray.
- **Materials to collect data:** Under gravel filter from the bottom of a well-established tank that is kept at 15 C and thus covered in cold water algae, a large plastic tray that is large enough so that half of the under-gravel filter can fit easily inside, glass plates, a black garbage bag, tape, a hydrometer to measure salt content, a light stand, a lamp, a blue light bulb, and a green light bulb.
- **Materials to analyze data:** Epson Photo PC time-lapse camera to help decipher where certain periwinkles are at the end of the testing time. This is because it can be difficult to count periwinkles while they are still grazing, and this can change data because they can move while you are counting. By taking a picture at the end of the testing period it will decrease the variability of snail migration when the testing time is over.

**Methods:**

**Setting Up the Tank:**

1. A 10 gallon tank was obtained.
2. Sea water was made up in a tub (which was found in the cold room) by using Instant Ocean sea salt and distilled water.
3. A hydrometer was used to test the salinity of the water.
4. Salinity was kept at 35 pps
5. The tank was kept in the cold room on the 2nd floor in the Science Center and the filter apparatus was turned on.
6. Periwinkles were placed in the tank, being careful to not let snails cling to the tank outside of the water for more than a day because it is very common for periwinkles to perish by clinging to the dry side of a tank waiting for "high tide" and then drying out.

**Setting Up the Experiment to Study Periwinkle Migration to Different Algae Concentrations:**

1. All steps done in the cold room, 15 C, of the 2nd floor of the Science Center at Wheaton College in Norton, Massachusetts.
2. Animals were removed from the tank in the cold room and placed in a white bucket filled with 35 pps sea water temporarily.
3. The under gravel filter, it is blue in appearance with grating and lines found on the tank floor, was carefully lifted up and the two plastic rings on the bottom of the filter holding the two halves together were removed. The under gravel filter was almost completely covered in algae, about 1 mm high, except for some grazing lines the snails had left.
4. The left half of the under gravel filter was removed and placed in a large tray that was filled with about 2 inches of sea water.

5. The half of the filter that contains the most algae was determined, because this filter has already been used for grazing in the tank and the algae distribution is a bit uneven.

6. On the half of the filter with the most algae eight consistent and alternating vertical bars of algae/no algae/algae/etc about 3 cm in width were made. A sponge was used to scrape off algae in order to make even bars on the filter.

7. Two large glass plates were placed flat over the half of the filter that was not being used, these glass plates served to section off the test area from other areas of algae as well as to sink the filter in the water.

Conducting Experiment to Study Periwinkle Migration to Different Algae Concentrations:

1. Six periwinkles were placed on different vertical boundaries on the test filter. The vertical boundaries were the lines that separated the areas of high algae concentration from low algae concentration. Each periwinkle was placed on a different boundary in order to prevent interaction between organisms. I placed periwinkles on the boundaries so there was an equal chance of migration to either area, high or low algae concentration.

2. When I was placing periwinkles I had to make sure the posterior end of the shell was facing the top of the tray, with the operculum/foot area facing the southern/bottom area of the tray. By placing all periwinkles in this position it decreased variability between organisms.

3. After periwinkles were placed in the accurate positions they were left for 10 minutes in the cold room under the normal light in the cold room.

4. After 10 minutes I returned to the cold room, and took a picture with an Epson Photo PC time-lapse camera in order to go back and accurately analyze data.

5. Steps 1-4 were repeated 7 more times.

Analyzing Data On Periwinkle Migration to Different Algae Concentrations:

1. From the pictures taken at the end of each trial it was determined whether each periwinkle moved into an area of high algae concentration or low algae concentration.

2. To score as "a move into an area of high algae concentration", all of the periwinkles' body parts were completely past the straight vertical boundary line that formerly separated the region of algae overgrowth from the region where algae had been removed, into a strip of high algae concentration.

3. To score as "movement into an area of low algae concentration", all of the periwinkles' body parts were completely past the straight vertical boundary line that separates the region of algae overgrowth from the region where algae had been removed, into a strip where algae had been removed.

4. Periwinkles whose heads physically touched another snail during the test period, who were physically attached onto another snail ('piling'), who had moved out of the algae testing area, either off the filter or onto the side of the tray, or who had not moved from the straight vertical boundary line that separated the region of algae overgrowth from the region where algae had been removed were all excluded.

5. I then counted the number of snails that moved to each scored area on the filter (high concentration or low concentration).

6. From these data I calculated the percent migration to an area of high algae concentration and the percent migration to an area where algae had been removed.
Running Control for Periwinkle Migration to Different Algae Concentrations:

1. To run this control an under gravel filter that had been scraped clean of algae was used.
2. Tape was placed along the bottom of the tray in 8 vertical sections about 3cm apart to segment the filter into sections such as in the previous experiment.
3. I designated certain areas to be "high algae concentration" and "low algae concentration".
4. The rest of the experiment was done in "How to Set Up Periwinkle Migration to Different Algae Concentrations".
5. I placed 3 periwinkles on different vertical boundaries on the test filter. The same placement of periwinkles as in the "Conducting Experiment to Study Periwinkle Migration to Different Algae Concentrations" was used.
6. After periwinkles were placed in the accurate positions they were left for 10 minutes in the cold room under the normal light in the cold room.
7. After 10 minutes I returned to the cold room, and took a picture with an Epson Photo PC time-lapse camera in order to go back and accurately analyze data.
8. Score movement into designated areas as was done in "Analyzing Periwinkle Migration to Different Algae Concentrations Data" keeping in mind that there are not really areas of high/low algae concentration but the entire plate has been stripped of algae. This control will be able to determine whether or not any features of the experimental set up or under gravel filter were a factor in periwinkle migration.

Setting Up Experiment to Study Periwinkle Migration to Different Light Wavelengths:

Part I: White light vs. Shade

1. All steps were done in the cold room of the 2\textsuperscript{nd} floor of the Science Center
2. I removed animals from the tank in the cold room and placed them in a white bucket filled with 35 pps sea water temporarily.
3. I carefully lifted up the under gravel filter (it is blue with grating and lines on the tank floor) and removed the two plastic rings on the bottom of the filter holding the two halves together.
4. The right half of the under gravel filter was taken and placed in a large tray, this tray was a dark color so that light could not penetrate, that was filled with about 2 inches of sea water.
5. I placed the filter in the middle of the tray, and wedged the filter into the middle of the tray so it would stay sunken and in place without the use of weights.
6. I took a black garbage bag and taped the bag over the right half of the tray to create a dark, hidden area for snails. There were equal amounts of the under gravel filter under the dark area as the uncovered area.
7. There were approximately the same concentrations of algae on both halves of the filter, if one side had more algae I scraped some off to make the algae concentration the same for both variables (light vs. dark).

Part II: Light Wavelengths vs. Shade

1. Follow steps 1-7 of Part I (How to Set Up Periwinkle Migration to Different Light Colors Experiment).
2. A lamp was set up on the other side of the filter, the side that was not shaded.
3. Use a green light bulb first and then a blue light bulb.

**Conducting Experiment to Study Periwinkle Migration to Different Light Wavelengths:**

1. I placed 6 periwinkles on the under gravel filter. They were placed on the border of the garbage bag shaded area and the unshaded area, so they had an equal chance of migrating either direction. I placed periwinkles vertically along the border and as far apart as possible to decrease the snails interacting with one another.

2. I placed periwinkles with the posterior end of the shell facing the top of the tray, and with the operculum/foot area facing the southern/bottom area of the tray. By placing all periwinkles in this position it decreased variability between organisms.

3. After snails were placed correctly the tray was left in the cold room for 10 minutes so the natural light in the cold room is on only one half of the filter, while the other half of the filter was kept dark by the garbage bag.

4. After 10 minutes I returned to the cold room, took a picture with an Epson Photo PC time-lapse camera in order to go back and accurately analyze data.

5. I repeated steps 1-4 two more times.

6. I repeated this experiment (steps 1-5) using a green light instead of white light.

7. I repeated this experiment (steps 1-5) using a blue light instead of a green light.

**Analyzing Data On Periwinkle Migration to Different Light Wavelengths:**

1. From pictures taken at the end of each trial determine if each periwinkle moved into an area with a certain light wavelength or into the shade.

2. The guidelines for scoring were the same as in "Analyzing Data On Periwinkle Migration to Different Algae Concentrations", (steps 1-4) except the boundary lines dealt with different light wavelengths rather than algae abundance.

3. I counted the number of snails that moved to either the shade or an illuminated area for each trial.

4. From this data I calculated the percent migration to each different variable (white light, green light, blue light, and shade).

**Running Controls for Experiment On Periwinkle Migration to Different Light Wavelengths:**

1. I used an under gravel filter that had been scraped clean of algae.

2. I placed the plate in a tray filled with about 2 inches of sea water and designated a left side and a right side by using a piece of tape on the bottom of the filter to mark off one side from the other, both sides were the same in length.

3. One trial was done with the entire tray being exposed to ambient light in the cold room, and using only 3 periwinkles. The set up and placement on the boundary line was the same as in all other experiments.

4. After periwinkles were placed in the accurate positions I left the tray in the cold room for 10 minutes under the white light.

5. After 10 minutes I returned to the cold room, took a picture with an Epson Photo PC time-lapse camera in order to go back and accurately analyze data.

6. Movement was scored into designated areas as was done in "Analyzing Periwinkle Migration to Different Light
Colors’ keeping in mind that there were not really areas of light/shade and the entire plate had been stripped of algae. This control was done to be able to determine whether or not any features of the experimental set up or under gravel filter were a factor in periwinkle migration.

7. Repeat this experiment with the entire tray kept dark by a garbage bag.

**Setting Up and Running Algae Concentration versus Light Color Experiment:**

1. Use the same experimental set up as was used in "Periwinkle Migration to Different Light Wavelengths Experiment" except the under gravel filter was scraped clean of algae on one side while the other was covered in algae.

2. A blue light, which was found to be the wavelength that was migrated to the least, was set up over the half of the filter that was covered in algae.

3. The half of the filter that was scraped clean of algae was covered with a garbage bag.

4. Six snails were placed on the boundary line as in "Periwinkle Migration to Different Light Wavelengths" experiment.

5. The snails were left for 10 minutes in the cold room and after 10 minutes a picture was taken with an Epson Photo PC camera.

6. Steps 4-5 were repeated two more times.

**Analyzing Data On Periwinkle Migration to Different Light Wavelengths:**

1. Movement was scored into designated areas as was done in "Analyzing Periwinkle Migration to Different Light Colors".

2. I counted the number of snails that moved to either the shaded area or the area under the blue light for each trial.

3. From these data I calculated the percent migration to the blue light and the shade.

**Results:**

The results from these studies indicate that periwinkles will migrate to an area of high algae concentration rather than low algae concentration (See Figure 1). The control for this experiment also was successful, giving a standard deviation (See Figure 1) that was not statistically significant, therefore the control was successful.

For the second part of the experiment, Periwinkle Migration to Different Light Wavelengths, it was found that there was a trend in moving into shade away from white light. However, it was also found that periwinkles would move towards green light when given a choice between green light and shade. Lastly, and unexpectedly, periwinkles did not migrate at all to blue light and instead migrated to a shaded area. The controls for this experiment showed that there was not a significant difference in migration due to the standard deviation.

For the last experimental testing, Algae Concentration vs. Light Wavelengths, it was found that snails would migrate to an area of high algae concentration even though it was under a wavelength of light they were previously found to migrate away from.
Figure 1 (above):

For the first experiment, periwinkle migration to areas of high algae concentration vs. low algae concentration, it was found a trend of movement towards areas of high algae concentration. Column 1 designates movement into areas of high algae concentration while Column 2 designates movement into areas of low algae concentration. This data was compiled from eight 10 minute trials using 6 snails each, within these eight trials only six snails total were excluded. For this experiment there was a standard deviation of 1.85.

Figure 2 (above):

The control for this experiment shows that 40% of periwinkles will migrate to an area designated as "algae" while 60% will migrate to an area designated as "no algae". This control was done on a blank filter (with no algae, but designated areas instead) using 3 snails for a trial of 10 minutes. Column 1 designates movement into an area designated as "no algae" while Column 2 designates movement into an area designated as "algae". For this control there was a standard deviation of 0.81.
Figure 3 (above):

Column 1 designates the use of white light, column 2 designates the use of green light and column 3 designates the use of blue light. In the second half of the experiment, Fig. 3 shows that the majority of periwinkles will move towards a shaded area compared to snails who will move towards an area under white light. In this experiment there were 7 periwinkles, out of the total 18, that were excluded from the data set, due to reasons mentioned in the methods. When a green light is used, rather than the normal white light in the cold room, the majority of periwinkles migrate towards the light is recorded, while the minority move away from the green light. Only 2 snails were excluded from this experiment. In the last part of this experiment a blue light was used instead of a green light and it was found that 100% of snails migrated away from the blue light and into the shaded area. Ten snails were excluded from this round due to no movement or moving off the test plate. Each of these experiments was done three times for 10 minutes each, using 6 snails for each trial. The standard deviation for white light was 0.75, for green light was 1.21, and for the blue light was 1.50.

Figure 4 (above):

The controls for this experiment yielded equal 50% values for a filter, whose algae had been removed, under normal room light split into two halves- one designated as no light and one designated as light. The values for the same plate kept in the dark by a garbage bag, split into the same designated areas, were similar with 57.20% of periwinkles migrating to the left side of the plate and 42.8% migrating to the right side of the plate. For the control all under shade the standard deviation was 0.75 and the control all under light had a standard deviation of 0.63. Column 1 designates movement to the left side of the plate while column 2 designates movement to the right side of the plate.
Figure 5 (above):

For the last experiment it was found that 100% of periwinkles would migrate to an area with a high algae concentration under blue light rather than a dark shaded area with no algae. This experiment was done for three 10 minute trials using 6 snails each. Three snails total were excluded from these trials. The standard deviation of this experiment was 2.73. Column 1 designates movement into the no algae/shade area while column 2 designates migration to the high algae/blue light area.

Discussion and Conclusions:

In the "Periwinkle Migration to Different Algae Concentrations Experiment" the results show that the majority (76.2%) of periwinkles will migrate to an area with a higher concentration of algae when given a choice between high and low concentrations of algae. This shows that a periwinkle can detect and distinguish areas of high algae concentration from areas of low algae concentration. From this data it can be assumed that periwinkles do not graze along rocks 'blindly' but rather are able to sense where food is present and most abundance. Because the under gravel filter in this experiment was not scraped 100% clean of algae, and some algae remained in the grooves of the filter, the snails had to distinguish between areas of more algae versus areas of less algae. This shows that the periwinkles can distinguish between food abundance, and not just presence. The control for this experiment showed a 40% migration to an area designated "high algae" versus 60% migration to an area deemed "low algae" when in fact there was no algae on the filter at all. The standard deviation was high so there was no factor on the filter or within the experimental set up that influenced the periwinkle movement. In order to get better control data in further experiments it would be helpful to use more snails and more trials to increase the n value. This was a preliminary study and that is why all standard deviations are relatively high.

The data for this experiment clearly supports my hypothesis that snails will be able to detect a difference in algal concentration and will migrate towards the higher concentration. The actual anatomical feature responsible for this movement is not known, but given these data one possibility would be the organisms tentacles are used for food and food abundance detection. It was observed that the snails usually had their tentacles sticking out of their shells while grazing. This observation supports the assumption that they are involved in food detection.

One source of error within this experiment is that the same plate was used for each of the 8 trials, so grazing along the high algal/low algal boundaries disrupted the boundaries after each trial. To be more accurate a new filter with new boundaries could be used after each trial to eliminate the re-use of ingested boundary lines.
In the second part of the current study, "Periwinkle Migration to Different Light Wavelengths Experiment", it was shown that periwinkles will migrate to a shaded area when given a choice between a shaded area and well lit area by 63.6% compared to 36.3%. This result is most likely due to the fact that most organisms that are prey to a large amount of other predators would prefer to graze in a darker area because it keeps them hidden and out of view. However, the periwinkles had definite reactions to colored light. The green light was migrated to more often by the periwinkles by 68.75% vs. 31.25% migrating to shaded areas. This result is very interesting compared with the fact that 100% of periwinkles favored shade over blue light. It can be concluded from these results that the photoreceptors in Littorina can detect not only light and dark but also certain colors. It is possible that the photoreceptors can detect a blue light but not a green light, which explains why there was movement into the green area but not the blue. The green light was not very high in wattage, and if periwinkles do not have receptors for green light then it is possible that they could migrate into the green light without even realizing it is there, but just realizing it is less watts than the white light in the room. However, the blue light had low wattage as well, but all periwinkles migrated away from it, showing that they could sense a certain color of light being shown on the plate and they knew to move away from the color; which supports the hypothesis that the eye spots in gastropods have photoreceptors that can detect only certain colors. These data refute the hypothesis that the periwinkle will be similar to a majority of marine organisms in the fact that its eye spots can detect only light and dark areas. Although the hypothesis is refuted these data can lead to much more interesting research on the function of gastropod eye spots.

The third and last aspect of the research was to determine which factor influences Littorina feeding more, either the color of light or the abundance of food. Will Littorina graze under the shade for a smaller abundance of algae or will it graze under an unfavorable light for a larger abundance of algae? It was found that 100% of periwinkles chose to graze under a blue light, which was previously identified as an area of no periwinkle migration, in an area of higher algae concentration rather than a shaded area, which was found to be an area of high periwinkle migration compared to white light, with a lower algae concentration. This is interesting because it clearly suggests that the motivating factor when feeding (with a choice between light and abundance) is abundance of algae. This supports the hypothesis that periwinkles will migrate to an area of higher concentration of algae regardless of light color.

The controls for these experiments show that the tray, filter set up, and experimental set ups were not influencing factors in periwinkle migration. One trial was run with the same plate with no shaded area (all ambient cold room light) and there was the same amount of migration to the right side as the left side, 50% each. This shows that there was no experimental factor within the filter encouraging snails to move to one side over another. Another control, covering the entire tray with a garbage bag or shaded area, was performed and it was found that 57.2% of snails migrated to the left side of the tray and 42.8% migrated to the right side of the tray. These numbers show, again, that the apparatus with the shaded area was not an influential factor in migration.

One source of error in these experiments would be the fact that the ambient light in the cold room was on while the experiment was running with experimental lights that were faced on the filter. This was because other experiments were running in the cold room and turning off the lights would compromise the data for other experiments. If these experiments were to be repeated, the tray and entire experimental apparatus would be in a dark room, and the only light being shone onto the filter surface would be the colored light. Another possible source of error is that the entire tray was colored a dark green, which was used because it did not allow light to come through the sides where a garbage bag was creating a shaded area. Perhaps if a tray could be fabricated with one half allowing the penetration of light and the other half stopping the penetration of light more precise data could be attained. Also, instead of using a garbage bag to cover the top half of the tray perhaps a black lid could be made. This would make the amount of light that accidentally seeps into the shaded area minimized as well as making a perfectly straight boundary line between light/dark sections. Lastly, again this tray was utilized for each trial which made the vertical boundary line harder to distinguish after more and more trials. If a new tray could be used for each trial it would eliminate the variability between boundary lines in each new trial.

Future experiments should be done with the results from the light wavelength migration experiment. The data from this experiment would be useful as a starting point for finding the exact function of periwinkle eye spots. Other different colors could be tested under a more controlled environment to determine if periwinkles have photoreceptors that can detect other colors besides tentatively blue. If it is discovered that periwinkles have photoreceptors that can detect various wavelengths of light, it can be helpful to understanding that evolutionary adaptation.
One refinement that could be done to this experiment would be to keep the snails in a calm environment before testing. The periwinkles used in testing were kept in a white tub along with a lobster before experimenting was done, and perhaps this accounted for the large amount of snails that were excluded from testing due to no movement. Another refinement could be to have more concise protocols for movement, into high/low concentrations or a certain light color. Perhaps instead of the entire body having to be in a certain area only the anterior end of the animal needs to be out of the boundary line because that is the part of the animal used for feeding. This would give less exclusion as well as a more precise way of scoring movement. More studies need to also be done to get lower standard deviations, as this is only a preliminary study.

The collaborative data that most relates to this study is from Serena Strydom, who tested the effects of light intensity on sea anemone orientation. She found that light intensity did not affect the orientation of sea anenome's, which is different from this study's data set because snail orientation definitely changed due to different light colors. This shows that different marine invertebrates can act differently towards similar experimental factors. The sea anemones have adapted to not show a difference in movement to light colors, while another marine species, periwinkles, definitely show a change in movement. Maris Madeira's results also correlate to this experiment. In testing crab preferences to light and dark areas she found that most crabs preferred to be kept sheltered or hidden. This is the same as the experiments' results in "Conducting Periwinkle Migration to Normal Light vs. Shaded Areas" and that is that periwinkles prefer to be kept in the shade as opposed to a well lit area. This is an important adaptation because both of these marine species prefer to be in a more hidden/shaded area, presumably because of the fear of predators.

**Bibliography:**

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