

**Are marine isopods picking favorites with colors? The role of color sensing in food preference in *Pentidotea wosnesenskii***

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## **Abstract**

The marine isopod *Pentidotea wosnesenskii* is one of many species that can change its color throughout its life. The variety of colors it can take on allows it to match the species of algae it lives on and consumes. While this isopod can change its color when moved to a new substrate, this requires it to build a new cuticle and molt, a process that takes weeks. It seems likely that it would be more efficient for an isopod to seek out algae that it is already matched to. This species is highly likely to have color sensing abilities as a way of adjusting their chromatophores, so they may use those abilities to search for a substrate that they can match. I placed isopods in containers where they had access to three different colors of algae. I hypothesized that adult isopods would show a preference for algae that was similar in color to their cuticles. However, the results of this study do not suggest that isopods use color sensing to move to matching backgrounds. Instead, they may be choosing substrates based on its quality as a food source or how well they can attach themselves to it. Because they can adjust the shade of their body with their chromatophores, these other factors may be a higher priority than a well-matched color.

## **Introduction**

Marine isopods often exhibit a variety of color morphs within their species. The genera *Pentidotea* and *Idotea* contain many species which can match their color to the substrate they are found on. One example is *Pentidotea (Idotea) wosnesenskii*, which can range in color from bright green to duller greens and browns, to red and even black. (*P. wosnesenskii* has previously been classified as *Idotea*, and the species has been referred to by both names in many papers. Here, I refer to it as the genus *Pentidotea* in accordance with the World Register of Marine Species.) The mechanisms of color change in *P. wosnesenskii* are not fully understood. For

instance, analysis has indicated that pigments in their cuticle are not being directly sequestered from the algae they eat (Hultgren & Mittelstaedt, 2015). However, some predictions about their color changing behavior can be made based on studies of related species.

Some species are primarily dependent on pigments in their cuticle for color, such as *Idotea granulosa*, while the species *Idotea montereyensis* uses a combination of both pigments and chromatophores (Lee & Gilchrist, 1972). *I. montereyensis* is also capable of changing color when moved to a different substrate (Lee, 1966). Its overt color comes from pigments, while chromatophores are used to make minor adjustments on quicker timescales than molting. Isopods that use chromatophores can concentrate and disperse pigment according to light levels, and full concentration can occur in half an hour while dispersion is slower (Lee, 1972). This allows them to match different shades of algae that are otherwise the same color, for example dark versus light red algae. For species that are able to use chromatophores, the ability to sense color in their environment is likely to be necessary. Some isopods use melanophores that operate on a circadian rhythm, but even these species have been shown to use eyesight to adjust their color on short timescales (Oguro, 1962). *Idotea japonica* uses melanophores to adapt its color. Their diurnal rhythm which persists under constant darkness but is interfered with by illumination. When the individuals are placed on a white background, the melanophores begin to concentrate immediately, and they begin expanding when placed on a dark background (Oguro, 1959).

Because there is variation in color control between species of the same genus, I set out to further investigate color change behaviors in *P. wosnesenskii* through two methods. First, I conducted preference trials with red, green, and brown algae to see whether isopods would show a preference for substrate that matched their color. Second, I conducted color sensing trials with red, green, and brown backgrounds to determine whether isopods would seek out a matching

background regardless of the presence of food. I expected this might be the case if the isopod is dependent on its color to avoid predation. Potential predators of *P. wosnesenskii* include various species of fish, and potentially birds (Hultgren & Mittelstaedt, 2015). The ability of these fish to detect color is not fully understood, but some species have been identified to have retinal cones that could give them limited color vision (Hultgren & Mittelstaedt, 2015). In tropical waters where these fish are present, warming temperatures could increase their abundance, putting the isopods in these areas at higher risk of predation.

The ability of *P. wosnesenskii* to change color throughout its life presumably allows the isopod to utilize a wider variety of food sources and gives it the opportunity to shift habitats while avoiding predators. This color change serves an effective method as shown by Hultgren & Mittelstaedt (2015). Those authors tethered brown and red isopods to the brown alga *Fucus distichus*, and the better matched brown isopods experienced significantly lower rates of predation. Hultgren & Mittelstaedt (2015) also demonstrated that *P. wosnesenskii* must undergo a molt in order to change its color. Because they typically go more than a month between molts as adults, it seems that it would generally be beneficial for an isopod to remain on the same color substrate as long as possible. The time between moving substrates and molting to match the new substrate could make it vulnerable to predators. Because of this, I hypothesized that adult isopods would show a preference for algae that most closely matched their color. I also examined juveniles. Juveniles are much more difficult to find on any substrate due to their small size, and they have not had as much time to adapt their color to a specific substrate, so I hypothesized that they would not show a strong color preference.

## **Methods**

Isopods of the species *Pentidotea wosnesenskii* were collected from Eagle Cove on San Juan Island, Washington. They were kept and transported in plastic containers that had large sections of the sides and lids cut out and replaced with mesh. Algae were also collected from Eagle Cove. Algal species were selected based on abundance in the intertidal, and included the brown algae *Fucus distichus*, the red alga *Mazzaella splendens*, and the green alga *Ulva spp.* Additional algae of these species were collected from the beach and docks at Friday Harbor Labs.

For the algal preference experiment, isopods were sorted into three groups: juveniles (n=12), green adults (n=6), and brown adults (n=6). Juveniles were individuals 1 cm or less in length. Adults were longer than 2 cm. These isopods can exist across a range of colors, so I chose individuals that were bright green or very brown, as opposed to olive-green individuals that tend to be somewhere in between. Juveniles and adults were kept in separate containers. I could not locate enough red isopods for an additional adult group, but a red isopod was kept in the adult container for observation. *F. distichus*, *M. splendens* and *U. fenestra* were added to both containers in roughly equal amounts. Every morning and evening, the algae was removed and the number of isopods on each species was counted. Algae were returned to the container in a random order after each counting and switched out for fresh algae once per day. In addition to categories for each species of algae, a fourth category was created to count isopods that were sometimes found clinging to the mesh at the top of the container.

The color sensing trials were conducted using colored paper placed underneath a clear container. Strips of red, green, and brown paper were arranged in a random order and isopods were placed in the container. Every five minutes, the number of isopods present on each color was counted. At the end of the study, additional trials conducted with black and white paper were

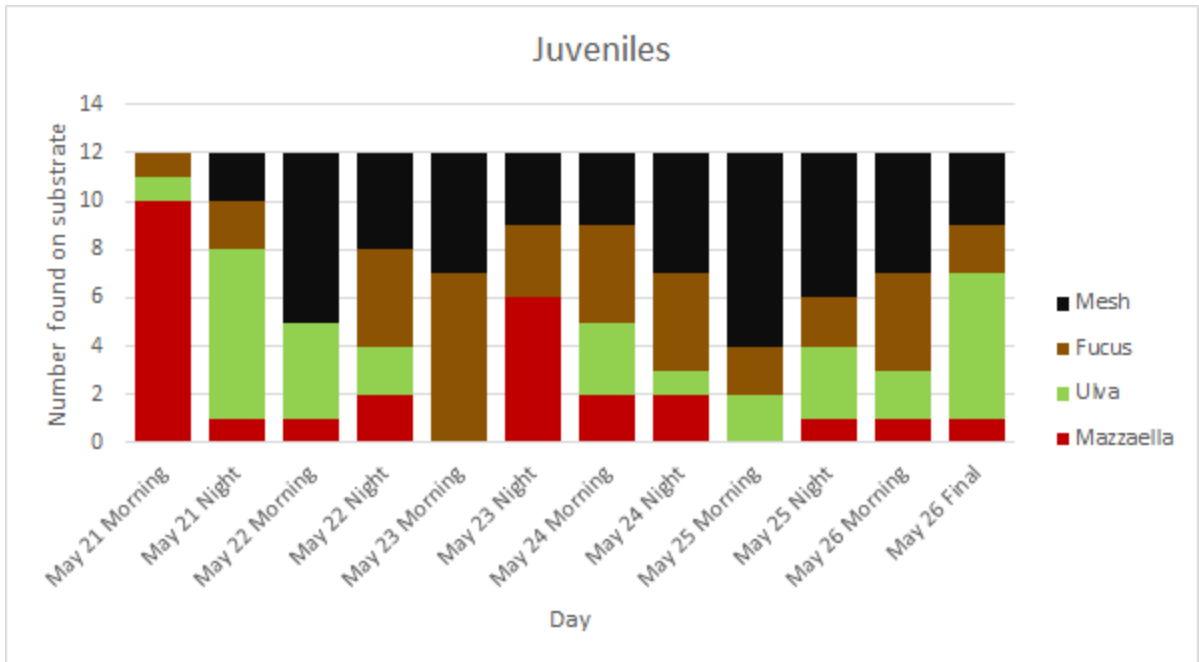
added. For the black and white trials, isopods were sorted into dark and light groups instead of red, green, brown, and juvenile.

To assess whether the frequency that isopods matched their substrate was statistically significant, an ANOVA test was used for the algal preference trials and the red/brown/green colors sensing trials. For the black and white color sensing trials, a T-test was conducted to assess statistical significance. At the end of the study, isopods were released at the beach by Friday Harbor Labs, and remaining algae was returned to the water.

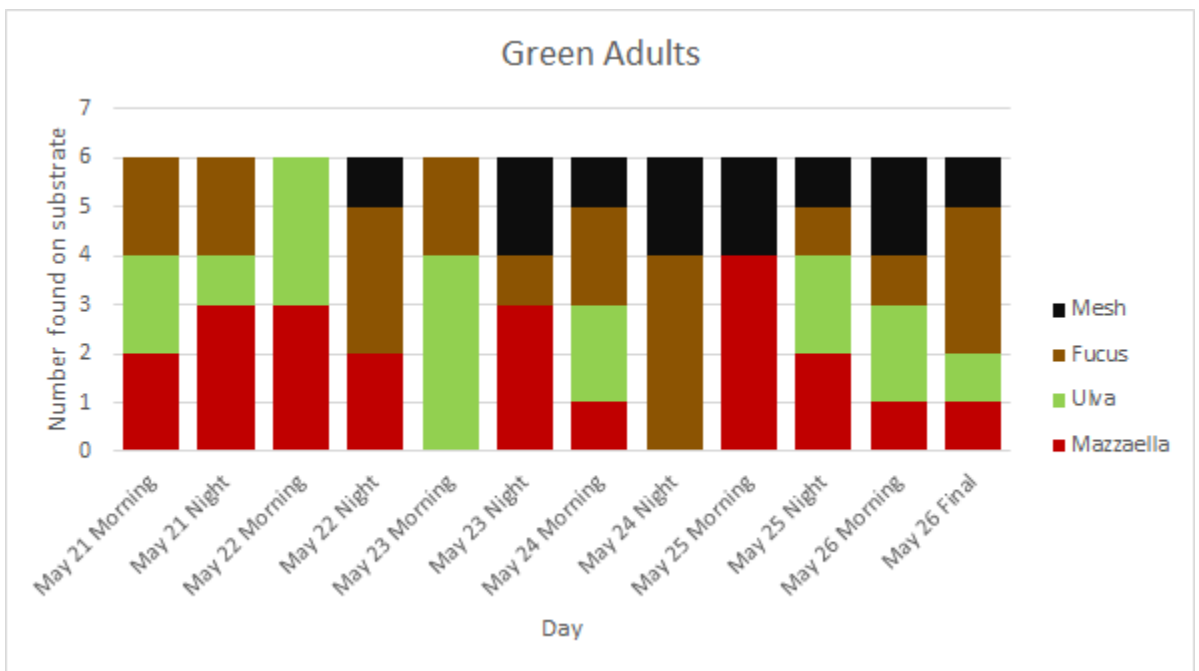
## **Results**

The ANOVA test for juveniles did not indicate a statistically significant difference between the frequency with which individuals were found on various substrates. The graph (Fig. 1) does not suggest any clear pattern over time for substrate preference. The juveniles appeared most frequently on the mesh, where they were counted a total of 57 times. They were counted 35 times on the *F. distichus*, 31 times on *U. fenestra*, and 27 times on *M. splendens*.

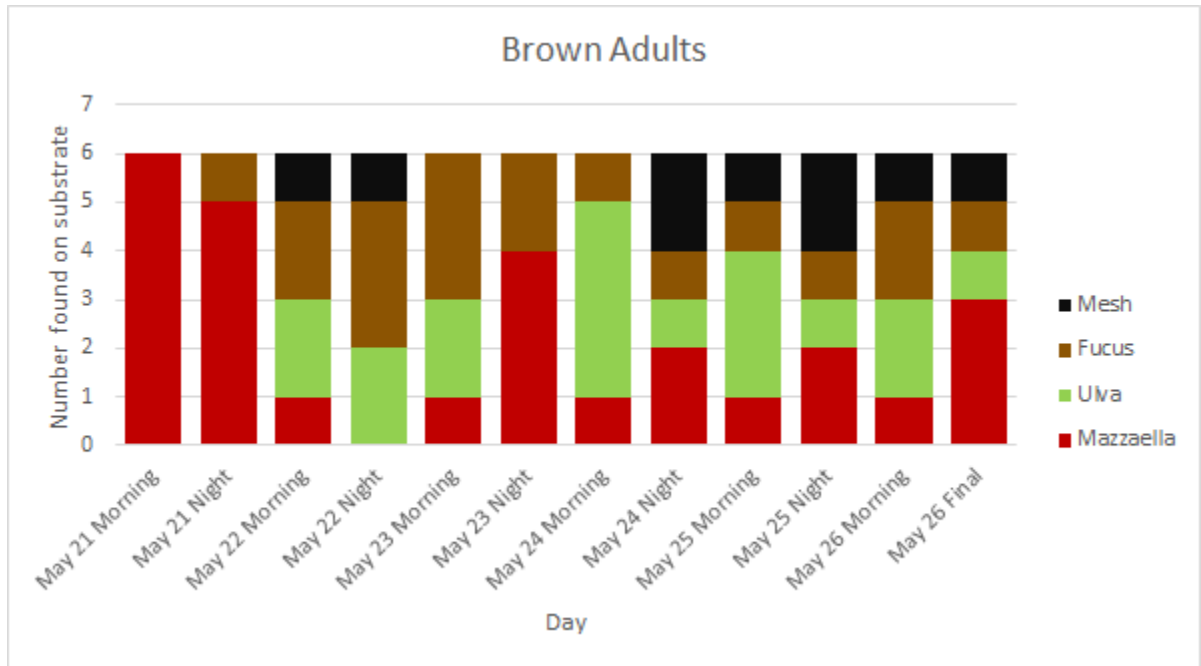
Among green adults, there was also no significant substrate preference. They were counted 22 times on *M. splendens*, 21 times on *F. distichus*, 16 times on *U. fenestra*, and 12 times on the mesh. The ANOVA for the brown adults was statistically significant. Brown adults showed significant differences in substrate preference ( $p = 0.05$ ). They were counted on *M. splendens* 27 times, 18 times on both *U. fenestra* and *F. distichus*, and 9 times on the mesh. Statistical analysis could not be performed with the red isopod, but it was counted 5 times on *U. fenestra*, 4 times on the mesh, 2 times on *M. splendens* and 1 time on *F. distichus*. It was never counted more than twice in a row on the same substrate.



**Fig. 1** The number of juveniles found on the substrates at each measurement time. There was not a statistically significant difference in the frequency with which they were counted on each substrate.



**Fig. 2** The number of green adults found on the substrates at each measurement time. There was not a statistically significant difference in the frequency with which they were counted on each substrate.



**Fig 3.** The number of brown adults found on the substrates at each measurement time. They appeared most frequently on *M. splendens* and least frequently on the mesh.

In the first color sensing trial, juveniles showed significant preferences for color, with individuals appearing most frequently on brown (47 times) and least frequently on red (15 times). They were counted 22 times on green. They also showed significant preferences in the final trial. In this trial, they also appeared least frequently on red (20 times) but appeared most frequently on green (36 times). They were counted 28 times on brown.

Among the adults, both color groups showed a significant preference for the brown background in the initial trial. Within the first ten minutes of the trial, the isopods had gathered together in a pile in one section of the container. By splitting the isopods into smaller groups for



the final trials, this issue was somewhat mitigated. In the final trial, the green adults were counted on the three backgrounds with near equal frequency (15 on both red and green, and 12 on brown) In contrast to the green adults, the final color sensing trial for brown adults was also statistically significant ( $p = 0.01$ ). They were mostly counted on green (23 times). They were counted 12 times on brown and 7 times on red. As with the algal trials, there was no statistical analysis conducted with the red isopod, but it was also tracked in the final color sensing trial. It was counted 2 times on the brown, once on green, 3 times on red, and then counted again on brown at the last time mark.

In the black and white color sensing trials, the dark-colored isopods were equally split between the black and white backgrounds. Light-colored isopods showed a significant preference for the white background ( $p = 0.05$ ). The dark-colored isopods did not show a statistically significant preference for either the white or black background.

In general, many of the color observations are hard to quantify, limited by personal perceptions of color and the varying light levels in the lab. The juveniles were mostly a pale brown, but some took on a more reddish or pale green appearance by the end of the trial. The red isopod observed throughout the algal preference trial sometimes appeared browner, and it was occasionally much darker in color. Several green and brown isopods were also observed to be very dark, almost black, when removed for counting. Later, they would be found to be their original color again.

## **Discussion**

The findings from the algal trials only suggest that brown adults seem to prefer red-colored algae. This finding could have been influenced by the small number of times they were counted on mesh. In the color sensing trials, discounting the initial adult trials where isopods

tended to group together, significant preferences were shown by juveniles and brown adults. The juveniles showed a preference for brown background in the first trial and the green background in the second. In the final trial, brown adults showed a preference for the green background. For the light versus dark trials, it is reasonable that isopods may not seek out a darker or lighter background when they can adjust their chromatophores relatively quickly. This is supported by the isopods that occasionally appeared much darker when counted.

Because isopods of *Pentidotea vosnesenskii* can change color, they may prioritize other factors over color when choosing a substrate. Additionally, even when their color is not perfectly matched to their background, they can adjust chromatophores to mimic the shade, which may be sufficient for avoiding detection by predators whose color vision is not as developed as ours. This is somewhat at odds with the study by Hultgren & Mittelstaedt (2015), but it is possible that there are other factors that affected the survivorship of the red isopods that were moved onto *Fucus distichus* by the researchers. Isopods of several colors tended to be difficult to detect on the *Fucus*, because *Fucus* itself has color variation that allows a range of isopods to match it.

Instead of color, isopods may be choosing a substrate based on how securely they can attach themselves to it. Isopods attach themselves to substrate using the hooked claws at the end of their walking appendages, and their attachment strength changes with different substrates (Starko et al., 2016). This suggests that in a highly exposed environment, isopods would be limited to stronger and thicker seaweeds that they can cling to more tightly. In the study by Starko et al. (2016), *Fucus sp.* was one of the best seaweeds for strong attachment, while *Ulva sp.* was poor for attachment. *Mazzaella sp.* was not used in those trials, but based on morphology likely would exist somewhere between these two, or potentially be better than fucus. The sea tables where the isopods were kept did not have waves or high flow, which could lower the

likelihood that attachment strength influenced their choice of substrate. However, the isopods were frequently pulled off the algae for counting, which could have caused them to choose substrate they could cling better. This idea is potentially supported by the number found on the mesh, which was the most difficult surface to remove them from. There was not a statistically significant preference for mesh, but it could be a contributing factor, especially for the juveniles who were counted more on the mesh than on any one algal species.

Another possibility is that isopods were choosing algae based on its quality as a food source rather than a habitat. Feeding trials have previously been conducted with *P. wosnesenskii* and related species, and some of these trials are at odds with algae's potential for attachment strength and color matching. For instance, *Ulva spp.* was preferred over *Fucus gardeneri* in some feeding trials (Van Alstyne et al., 2006). This is presumably a result of phlorotannins produced by *Fucus sp.* as a chemical defense. Despite this, isopods in the wild during collection were mostly found on *Fucus distichus*, consistent with observations by Starko et al. (2016). Additionally, Jormalainen et al. (2005) found that phlorotannins in *Fucus vesiculosus* did not deter *Idotea baltica*, although they did negatively impact the isopod's assimilation efficiency.

Other feeding trials have also suggested *P. wosnesenskii* is generally less deterred by chemical defenses than other herbivores. Van Alstyne et al. (2001) found that *P. wosnesenskii* was not deterred by acrylic acid in concentrations as high as 8%. This is partially attributed to size, as other herbivores in a similar size range also seem to resist chemical defenses (Van Alstyne et al., 1999). There may also be a genetic component, as in *Idotea baltica* diet appears to regulate a group of genes involved in detoxification (de Wit et al., 2018). Whatever the reason, isopods in the genus *Idotea* and *Pentidotea* generally have a heightened ability to tolerate

chemical defenses of algae they feed on, including *Ulva sp.* and *Fucus sp.*, however these defenses may still have negative impacts on the isopod's ability to digest the algae.

Isopods of the species *P. vosnesenskii* are likely facing tradeoffs between algae that they can attach themselves to effectively in exposed environments, algae that matches their color closely enough to provide sufficient camouflage, and algae that fulfills nutritional requirements efficiently. Because *P. vosnesenskii* is capable of changing color, it may place color as a lower priority when making choices between algae. It may be more beneficial to seek out algae that will better fit the other two requirements and risk being a more obvious target for a brief period of time while building a new cuticle. Even when not perfectly matched to a substrate, they can make minor adjustments with their chromatophores that can help them blend into their environment and avoid predation.

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