The impact of contaminated pilings on nudibranchs at the docks of Friday Harbor

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BIOL 479

Spring 2014

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Keywords: nudibranch, sea slug, creosote, preference test, behavior, docks, pilings, *Triopha catalinae, Archidoris montereyensis*

Abstract

Docks in the Friday Harbor Island have been observed to have many nudibranchs present on them. One hypothesis for this is the creosote, a chemical used to help preserve the wood from wear, is attracting them to the pilings. Creosote has been shown to have negative effects on the environment, and because nudibranchs' rinophores are sensitive chemoreceptors that are used to help find food, navigate, and find mates, an interaction could be harmful to these animals. Our study looked at the preference of location in a tank with one side having creosote-treated wood and one side having non-treated wood samples. We used *Triopha catalinae* (Clown Dorid) and *Archidoris montereyensis* (Sea Lemon) as our two species. We found there was no significant preference to creosote. We also saw no preference to creosote during the day or evening, but saw a trend in *T. catalinae* to prefer the sides of the tank especially during the afternoon runs. There was also a trend to avoid the drain in the tank for both species. Nudibranchs therefore show no attraction to creosote in their environment.

Introduction

Marinas historically have used creosote to protect wood in marine environments, preventing the wood from breaking down. In the early 1800's the industry took off, using creosote oil to cover vacuumed dried wood (Railway Age Gazette 1910). Creosote is a group of chemicals that are used for wood treatment, food flavorings, and fuel. When creosote is disposed of correctly there seems to be minimal impacts on human health, but it is still unknown the full effects of long exposure it can have on a person (Mueller et al. 1989). One study has seen that bacteria growing in creosote contaminated soil has been shown to have no significant difference in diversity and abundance compared to uncontaminated soil and not be negatively effected due to the creosote (Derry et al. 1998).

The effects of creosote contamination has primarily been studied in sites near factories, and is "generally associated with surface soils, waters in treatment lagoons or evaporation areas, and contaminated groundwater" (Mueller et al. 1989). These factories have produced up to 1,500 metric tons each year, and many sources of contamination come from improper disposal, broken equipment, and freshly treated lumber (Mueller et al. 1989). Benthic creatures near creosote runoff have been shown to be at risk of being exposed to lethal dosages (Pastorok et al. 1994).

With marine environments being exposed to this treated wood, we are interested in the effects it might have on nudibranchs, benthic animals that are part of a subclass of Gastropods. It has been observed that nudibranchs have been found in great numbers on creosote pilings in marinas in the San Juan Islands, particularly by Friday Harbor fueling docks (Personal Observation Bosch 2014). A key feature of nudibranchs are their

rhinophores found at the dorsal part of the head region (Kozloff). The nerves found in rhinophores are connected to the cerebral ganglia (Behrens and Hermosillo 2005). These are thought to be chemosensors to chemicals in the water. They use these chemical cues to help them navigate, seek out food, and mates (Wyeth and Willows 2006). We hypothesize that the dock pilings that are covered with creosote are attracting the nudibranchs, who are sensitive to chemicals in the water. We will be using a preference test on *Triopha catalinae (Clown Dorid) and Archidoris montereyensis (Sea Lemon),* common species found in the San Juan Islands, to see if there is an attraction to creosote.

Methods

Materials and Specimens

We collected 26 nudibranchs of several species from Friday Harbor Labs (FHL) docks, public docks in Friday Harbor, and Argyle Creek. We used the species *Triopha catalinae* (Clown Dorid) and *Archidoris montereyensis* (Sea Lemon) to run preference tests between treated and untreated wood. There was a pool of 13 *A. montereyensis*, and 11 *T. catalinae* to choose from randomly for each trial. Ten tanks were set up for each trial, and with one nudibranch in each tank.

Creosote wood samples came from a log found on Orcas Island, and was cut into 10 cm square pieces. The non-treated wood was from a log on FHL shore and was also cut into 10 cm square pieces. Rocks used as anchors for the wood were found on FHL shoreline, and were all picked to be smaller than the wood in diameter, and smooth surface.

Layout of Tank

Figure 1 shows the layout of a tank where a metal grid was laid on top to determine the location of the nudibranch. A string marked the appropriate increments where to lay the grid down, which can be seen in figure 2 on the right side of the tank. A wide pipe tool was used to determine which of the squares the nudibranch was in when it could not be observed directly overhead. The grid had 5 spaces across and three down resulting in a 17x5 grid in each tank.

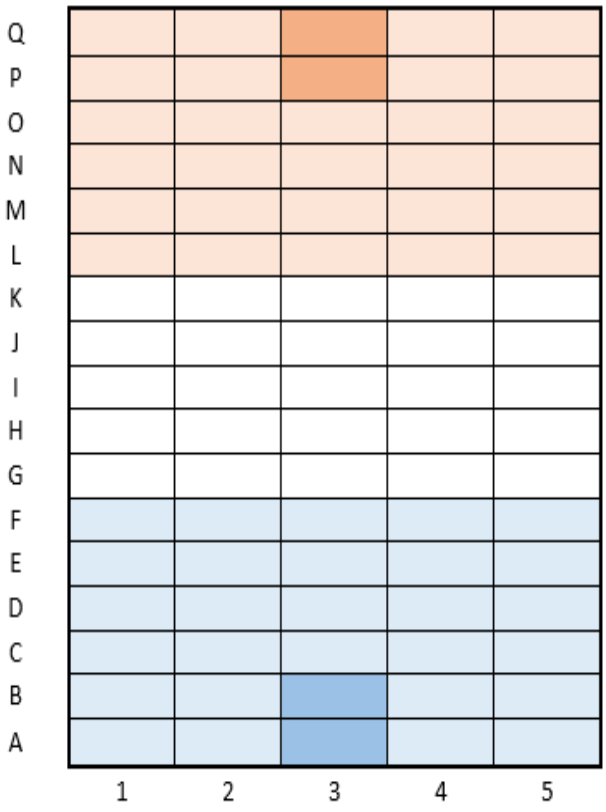

Figure 1: Layout of tank grid. A3 and B3 is location of the drain of the tank with the wood sample between it and the tank wall. Q3 and P3 is the location of other wood sample.

The trials

We ran 8 trials total, 4 for each species. We ran two trials with the creosote by the drain and non-treated wood at the far end, the AM trial was with a morning start time and PM trial was with an afternoon start time. There was an AM and PM trial for the trials with the non-treated wood by the drain and creosote at the far end of the tank. There were ten tanks running during the trial, with each tank having one nudibranch of the same species in the tank. When starting a trial we would document start time, species being tested, if it was morning (AM) or afternoon (PM), location of creosote (C), the drain (D) and the non-treated wood (NT). We would then once for every 2 h document the location of each individual for a total of 6 h.

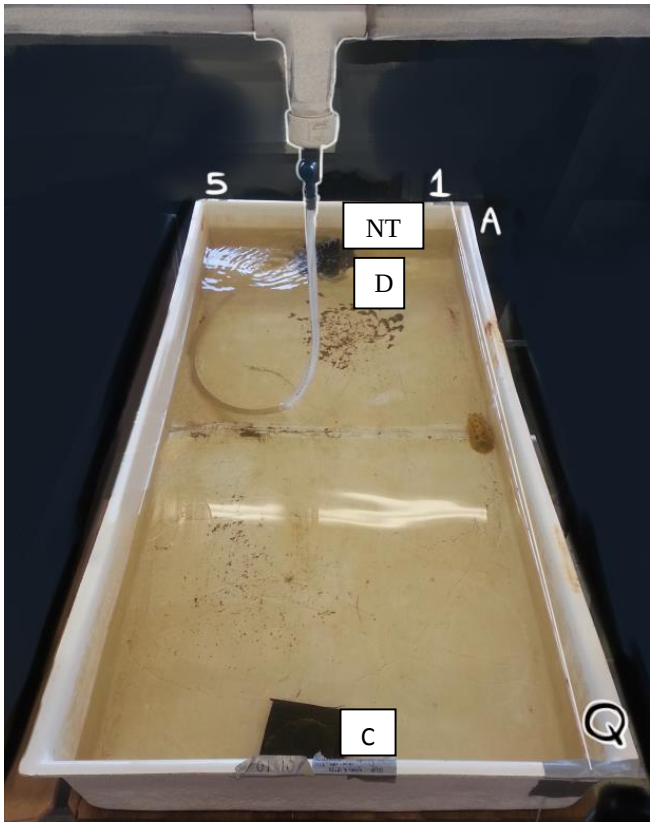

Figure 2: Photo of tank 10 with creosote piece in quadrant Q3. A nudibranch is located in center of the tank. C labels the creosote-treated wood, the Non-Treated (NT) sits behind the drain (D) in the tank. 1-5 label the horizontal quadrants, A-Q the vertical quadrants.

The drains of the tanks were all standardized to have the water at 10cm (±4.5cm). Tanks were cleaned once every two runs, or as needed. If a slime trail of the path the nudibranch was visible or there was a buildup of biofilm and algae particles in the tank it would be cleaned. Runs were done while alternating species when able.

Data Analysis

We grouped nudibranch location into three categories, A-F was category 1, being the closest to creosote treated wood. G-K was the middle of the tank, and L-Q was the side with non-treated wood. These were chosen to best split the tank into 1/3rd sections. The layout of the tank remained the same when wood samples were flipped, and were transposed into the correct categories. We also made similar categories for location of the drain that water flowed out of the tank from. Category 1 was the A-F which included the drain, 2 the middle of the tank, and 3 the furthest. To determine the preference to each category we ran one way and two way ANOVA tests. Data was analyzed using SPSS v. 19.

Results

Our first observation was the total number of times we observed the nudibranchs on the creosote itself during the trials (figure 3). We combined all observations (120 total for each species, 4 trials with 3 observations for each individual per trial) and found that there were only two instances where nudibranchs were on the creosote. *T. catalinae* was at no point seen on the creosote, while *A. montereyensis* was only seen twice on the creosote for all four trials.

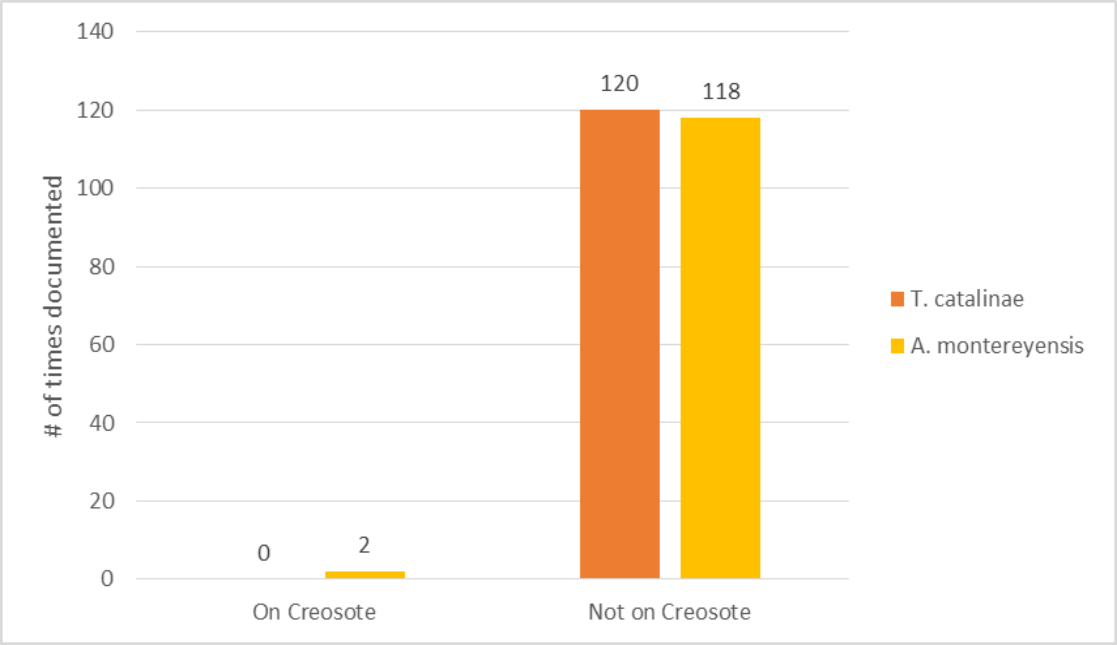

Figure 3: The number of times each species was observed to be on the Creosote sample during all trials (4 trials with 10 individuals in each trial. 6 hours for each trial with 3 observations per individual). T. catalinae were never seen on the creosote, and A. montereyensis was seen twice. Error bars were created using the standard deviation.

We then looked at the distribution inside the tanks in more detail to see if there is a preference in creosote for both species (Figure 4). We found no significant preference in *A. montereyensis* (One Way ANOVA, $F_{2, 33}$=2.840, P=0.073). We saw that *T. catalinae* significantly preferred the sides of the tank (category 1 and 3) to the middle of the tank (One Way ANOVA, $F_{2, 33}$=5.677, P=0.008). When comparing preference of creosote end to middle of the tank there is a preference to the creosote (Tukey's HSD, P=0.021) and when comparing the non-treated side to the middle of the tank there was a preference to the non-treated side (Tukey's HSD, P=0.014). When looking at the creosote to non-treated sides, there was no preference shown (Tukey's HSD, P=0.981).

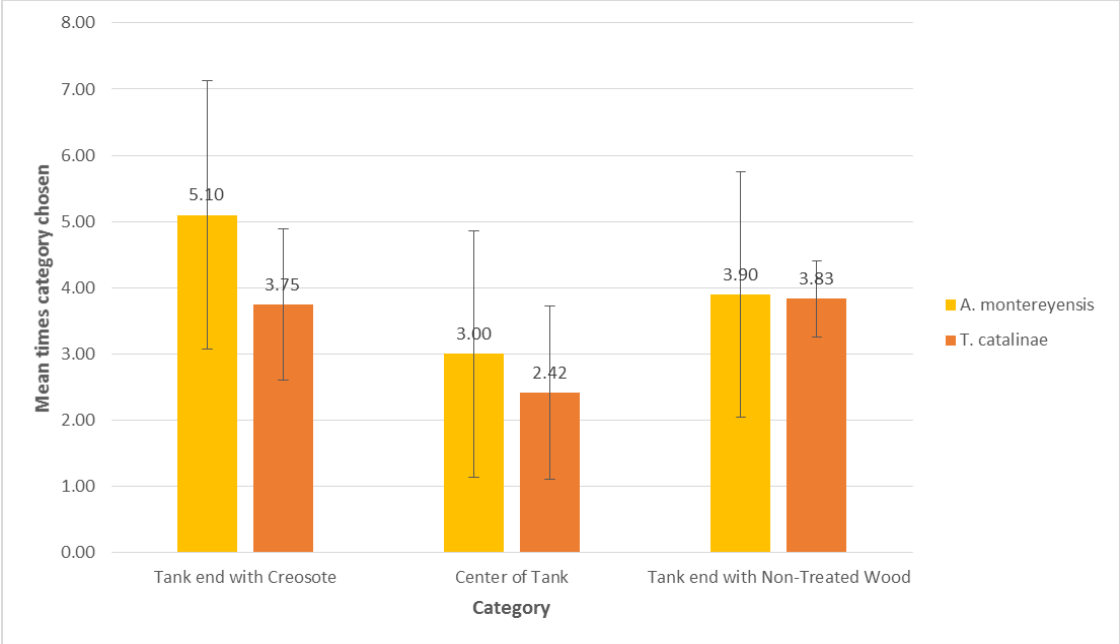

Figure 4: Mean preference in each category of creosote in different nudibranch species. There was no significant preference for A. montereyensis. There was significant preference for tank ends and avoidance of the center of the tank for T. catalinae. Error bars were created using the standard deviation.

Creosote preference did not change with the time of day the trail began. *A. montereyensis* showed no significant preference during morning or evening (Two Way ANOVA, $F_{5, 30}$=1.388, P=0.257). *T. catalinae* (Figure 5) showed a significant interaction of preference and time of day, with a preference to stay on the ends of the tank and avoid the middle of the tank during the afternoon trials (*Two Way* ANOVA, $F_{5, 30}$=12.132, P=0.000).

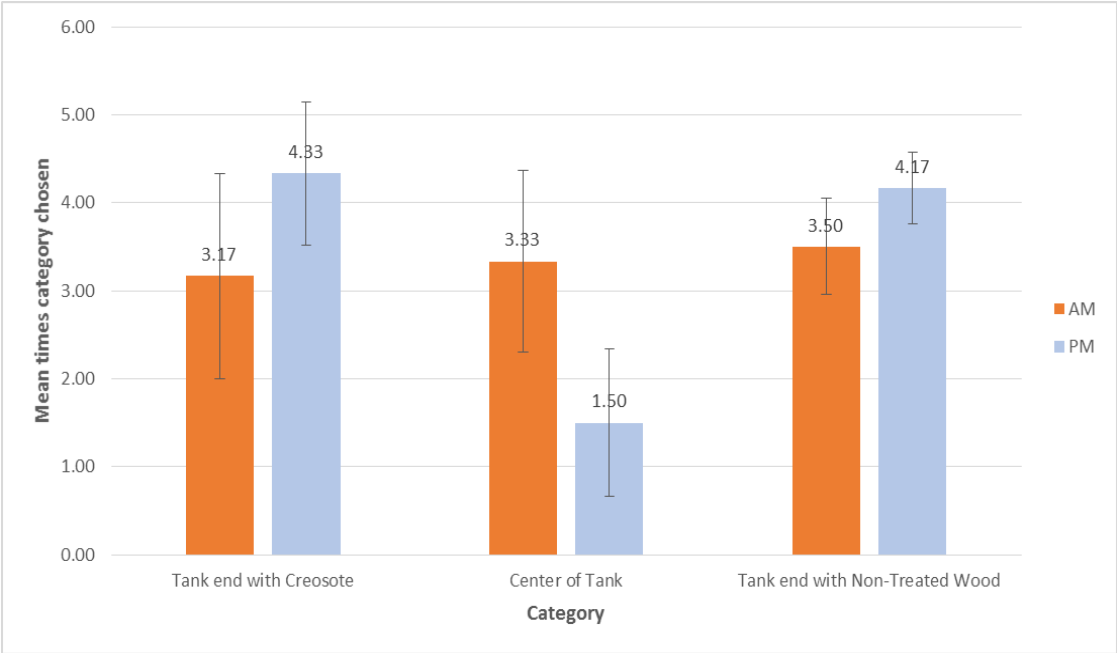

Figure 5: Mean T. catalinae preference to creosote during different times of the day trial is started. There was no significant preference. Error bars were created using the standard deviation.

There was no interaction between preference for or against creosote and drain placement (Two Way ANOVA, F_2=0.556, P=0.580). Interestingly, we found a trend to avoid the drain for both and *A. montereyensis.* In *T. catalinae*. For *T. catalinae* there was no significant preference for the end of the tank with the drain vs the middle of the tank (t-test, p=0.419) There was a significant preference for the end of the tank with no drain over end of the tank with the drain (t-test, p=0.003) and also a significant preference for the end of the tank with no drain vs the middle of the tank (t-test, p=0.006). *A. montereyensis* also showed no preference between the end of the tank vs the center of the tank (t-test, p=0.540), while showing a preference for the end of the tank with no drain vs the end of the tank with the drain (t-test, p= 3.273 E-11), and a preference to the end of the tank with no drain vs the middle of the tank (t-test, p= 2.282 E-10).

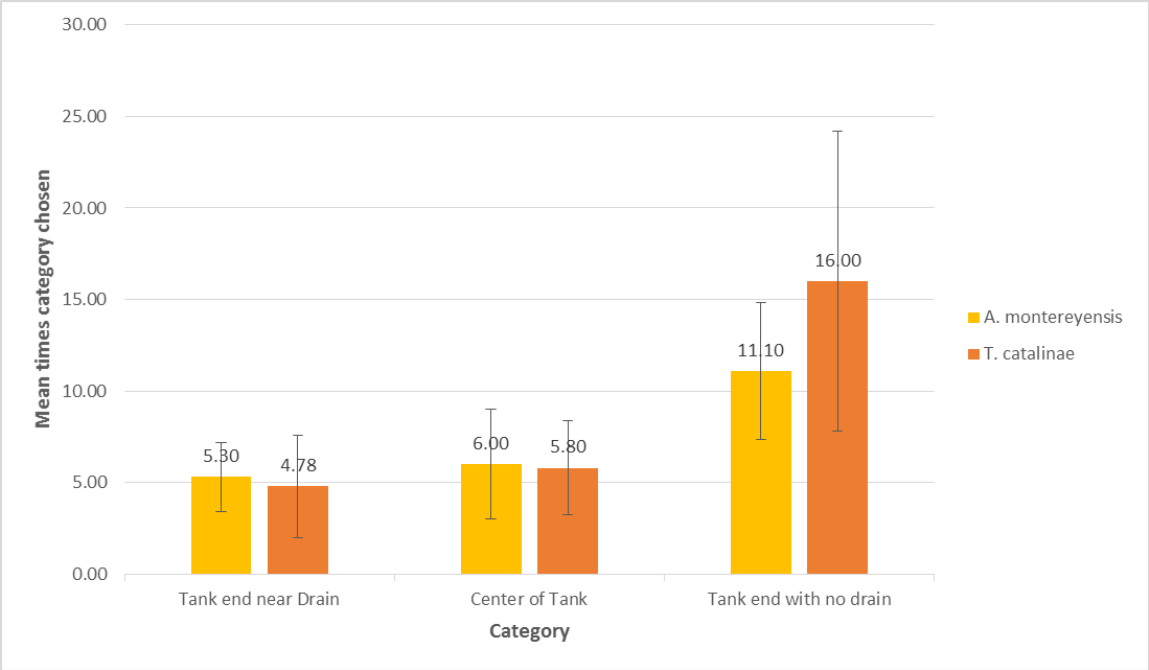

Figure 6: Preference of nudibranch species to drain in tank. No significant preference, but a trend to avoid drain was seen in both A. montereyensis and T. catalinae. Error bars were created using the standard deviation.

Discussion

We found no support for our hypothesis that nudibranchs are attracted to creosote. There were only 2 out of 240 points where Nudibranchs were seen on the actual creosote-treated surface. Furthermore, when we looked at a generalized area, still no preference was seen for the creosote end of the tank. This suggests that creosote is not what is attracting nudibranchs to Friday Harbor dock pilings in high densities.

Triopha catalinae preferred to stick to the sides of the tank rather the middle, particularly during the PM test runs. *T. catalinae* preference to select a side of the tank during the evening hours may be some kind of defense response to climb up away from predators that may be more active during the evening. By moving up on a horizontal

surface they are no longer on the bottom of the seafloor which may have an advantage over nudibranchs in areas not disturbed by humans that do not have pilings to climb on.

There was also a trend for both species to avoid the tank drain. Reasons for this, and may explain the behavior of the nudibranchs both in lab and in the wild, is first the drain might have stronger currents that could make the nudibranchs think they would be pulled off, or that the current moving isn't allowing chemicals to settle as well on that end of the tank. Nudibranchs may prefer pilings due to the vertical surface having different patterns of flow around them, and might be why we saw similar behavior in the lab.

Studies on creosote have focused on freshly treated lumber (Mueller et al. 1989), while our study used wood from a log that was washed up on the shore and showed signs of age. Our results may be due to the aging of the wood, and a study on comparing old vs newly treated wood on attracting nudibranchs might be done. The amount of creosote in new logs, may be necessary to have any kind of attracting power for Nudibranchs.

With the drain being an influencing factor on preference of location in tank, we recommend future trials in tanks with nudibranchs to have two drains on each end and inflow of water current be more standard so currents in tank were more unified. We also saw a large amount of egg case laying but do not know if this was due to large selection of mates available or stress being a factor.

Future studies may look at nudibranch preference to location on pilings in harbors at different times of the day to see if certain species prefer different pilings at different times of day over seasons. While collecting specimens, it seemed that *A. montereyensis*

were easier to find on docks in earlier spring, and then only *T. catalinae* could be found later in the season (Personal Observation Wachter 2014). This change in what species may lead to different results as seasonal changes in the environment may be playing a factor to when certain nudibranchs are abundant. Other factors to why Nudibranchs are attracted to the docks would be looking at substrate preference of sea floor vs piling, looking at growth of food sources on vs off docks, and possibly other types of chemicals found in marinas might help explain the abundance of nudibranchs. With creosote not being what is attracting nudibranchs to the docks of Friday Harbor, suggested studies are encouraged to better understand why there are such high densities.

Acknowledgements

Scholarship support received from the Mary Gates Endowment. Special thanks to Hilary Hayford for help with the setup of the project and stats analysis. Thank you to Friday Harbor Labs for use of research space. BIOL 430 students for help with collection of nudibranch specimens.

References

Behrens, D. W., and A. Hermosillo. Eastern Pacific Nudibranchs: A Guide of the Opisthobranchs from Alaska to Central America. Monterey, CA: Sea Challengers, 2005. Print.

Derry, A. M., W. J. Staddon, and J. T. Trevors. 1998. Functional diversity and community structure of microorganisms in uncontaminated and creosote-contaminated soils as determined by sole-carbon-source-utilization. World Journal of Microbiology & Biotechnology 14:571-578.

Kozloff, Eugene N. Invertebrates. Philadelphia: Saunders College Pub., 1990. Print.

Mueller, J. G., P. J. Chapman, and P. H. Pritchard. 1989. Creosote-Contaminated Sites - Their Potential for Bioremediation. Environmental Science & Technology 23:1197-1201.

Pastorok, R. A., D. C. Peek, J. R. Sampson, and M. A. Jacobson. 1994. Ecological Risk Assessment for River Sediments Contaminated By Creosote. Environmental Toxicology and Chemistry 13:1929-1941.

"Seasoning and Preservative Treatment of Wooden Ties." Railway Age Gazette. 8th ed. Vol. 48., 1910. 408. Print.

Seavy, B. E., and G. Muller-Parker. 2002. Chemosensory and feeding responses of the nudibranch Aeolidia papillosa to the symbiotic sea anemone Anthopleura elegantissima. Invertebrate Biology 121:115-125.

Wyeth, R. C., and A. O. D. Willows. 2006. Odours detected by rhinophores mediate orientation to flow in the nudibranch mollusc, Tritonia diomedea. Journal of Experimental Biology 209:1441-1453.