Identifying changes in activity level in *Hemigrapsus nudus* under daylight and night conditions

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Abstract

Mobile predators in the intertidal zone play a key role in the abundances and distributions of their prey. *Hemigrapsus nudus* is an ecological generalist found in the intertidal that primarily feeds on green algae. With the constant fluctuation of conditions in their environment, *H. nudus* must adapt their behavior to better their chances of survival and reproductive success. This study aims to investigate the influence of different environmental conditions on the activity times of *H. nudus*, particularly comparing daylight and nighttime conditions and the presence and lack of cover. Behavior tracking experiments with no cover present were conducted in the daytime and nighttime, then repeated with cover present. There was no statistically significant difference between the times active in *H. nudus* in daytime and nighttime but one analysis test found that there may be a statistically significant difference in active times between conditions with cover and without cover present. While my findings stand in contrast with the published literature, there is evidence that crabs exhibit behavioral adaptations in response to their environmental conditions.

Introduction

Decapods play key predation roles in their ecosystems because predation is often the foundation of trophic structures (Boudreau & Worm 2012; Giraldes et al. 2017). For example, large benthic crabs are significant prey and predators alike and support food webs (Boudreau and Worm 2012). Depending on their environment, decapods may be generalists, specialists, scavengers, omnivores, or carnivores (Yamada & Boulding 1997; Boudreau & O'Connor 2003, Smallegange et al. 2008). These roles are important in the transfer of energy from lower trophic levels to higher levels. As such, predators apply top-down pressure on lower trophic levels. In New England the green crab, *Carcinus maenas,* has been found to influence algal abundances (Trussel et al. 2002). Top-down pressure influences the abundance of prey in the lower trophic levels.

Crabs are often found in the intertidal zone. The intertidal can be a location of intense competition for resources and predation is a strong influence on the distribution of prey and predators alike (Menge 1978). Predation, however, is not the only factor that influences the density and distribution of intertidal organisms (Trussel et al. 2002). Constantly changing conditions, prey competition, biotic and abiotic factors, and the risk of desiccation all impact the behavior and vertical distribution of organisms throughout intertidal zones. Defensive adaptations include appendages anchoring individuals to rock, living within congregations of tightly packed organisms, or, in the case of crabs, a tough carapace, claws for crushing, nocturnal activity, and the utilization of cover. All these adaptations allow them to better avoid predation and competition.

Hemigrapsus nudus is a shore crab found in the intertidal in the Pacific Northwest (Jacoby 1981). *H. nudus* is an ecological generalist, meaning they can survive in various conditions and have a varied diet, and are most frequently active at night (Yamada & Boulding 1997; Edwards 1982). I hypothesized that the active time of *H. nudus* would be greater under night time conditions than day time conditions. Additionally, I hypothesized that active time would be greater in conditions with cover available compared to no cover.

Methods and Materials

Data Collection

Hemigraspus nudus individuals were collected from Eagle Cove, Friday Harbor, WA. A total of 14 individuals were collected. Each was assigned a letter and was labeled using plastic flagging material super glued onto the carapace, labeled with the corresponding letter. *Ulva lactuca* was collected off the floats on the dock at Friday Harbor Labs, WA.

Trials were conducted in a designated experimental acrylic tank measuring 20.5 in by 30.5 in. For the treatment without cover, the tank was empty aside from the *U. lactuca*, the inflow pipe, the transport container, and the crab. When not actively in a trial, crabs were held in a holding tank below the experimental tank with overturned containers for elevation and shelter. Daylight treatment trials were conducted between the hours of 2:30 and 5:30 p.m. while the sun was still up, along with the fluorescent overhead lights remaining on. Night treatment trials began after sundown (in the summer, this is generally after 9 pm) with the blinds drawn to minimize ambient light. A red light headlamp was used to light the tank in order to reduce bright light.

Crabs were selected each day using a random number generator with values assigned to each letter (A=1, B=2, etc) with seven selected for the daytime trials and seven for the nighttime trials. Each trial began with a 10 minute timer being set and a crab being removed from the holding tank and placed in a plastic container with holes. *Ulva lactuca* was placed on the opposite end of the tank. The container was lowered into the water slowly to prevent water from rushing in and sweeping the crab up and out. A stopwatch was started when the crab began moving. Any time the crab was in motion, the stopwatch ran, and was paused anytime the crab stopped. If the individual began eating the *Ulva* the stopwatch timing active motion was paused and a second stopwatch tracking time at food was started. This was repeated in daytime treatments and nighttime treatments.

The second treatment applied was the addition of cover. Two clear glass jars and two red Nalgene bottles were placed on their side around the perimeter of the experimental tank. Trials were repeated as in the previous treatment.

Data Analysis

Once all trials were completed, each "time active" and "time at food" was transposed into seconds. Box and whisker plots were made to compare the results of daylight treatments for day 1, 2, and 3 of collection, then repeated for the night time treatments (Figure 1). The results showed that the means and the variances were similar enough that pooling the data was appropriate. The pooled data were graphed in histogram form to then begin visualizing the type of distribution exhibited, if any. Then, in R Studio, Wilcoxon rank sum tests were run between daylight and nighttime with each cover/no cover treatment. Finally, a two-way ANOVA was run for comparison with the Wilcoxon rank sum tests.

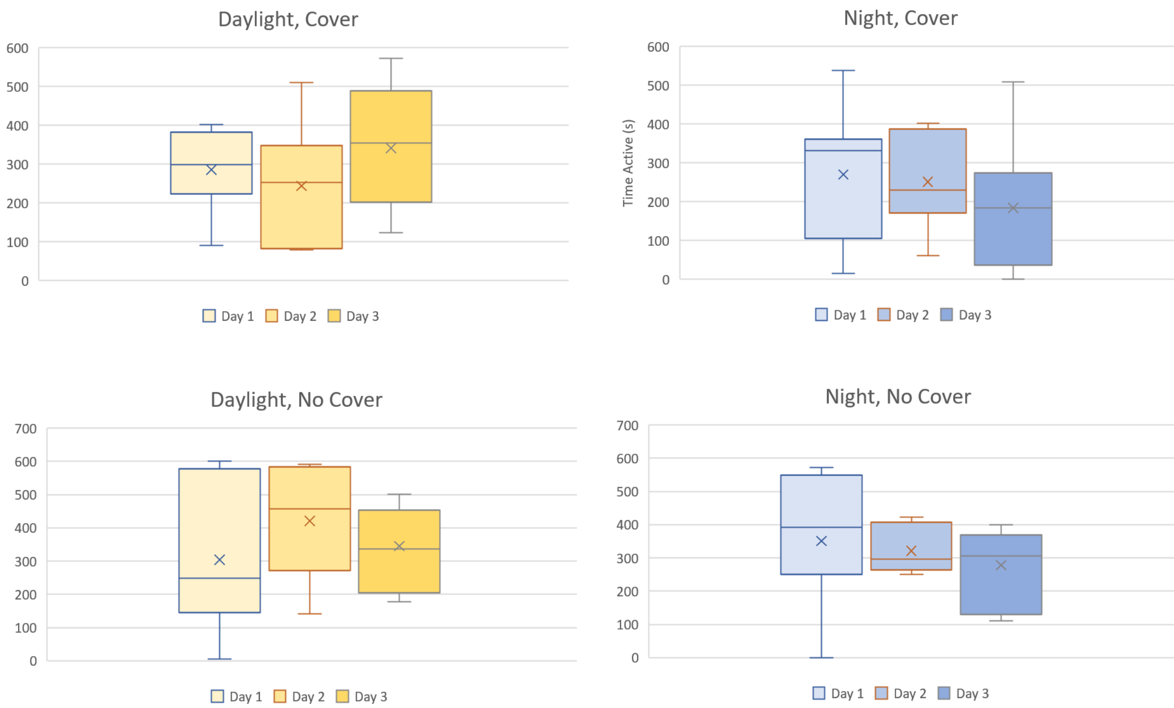

Figure 1: Box plots of each day in each treatment in order to identify variance to support pooling all data for each daylight and night treatment

Results

The histograms suggest that *H. nudus* active times are highly variable between each treatment (Figure 2). Data analyses performed were aligned with this conclusion. Few individuals (n=5 and n=14 for cover and no cover, respectively), spent time actively consuming food, the majority of most trials was spent in active movement or attempts to climb out of the tank.

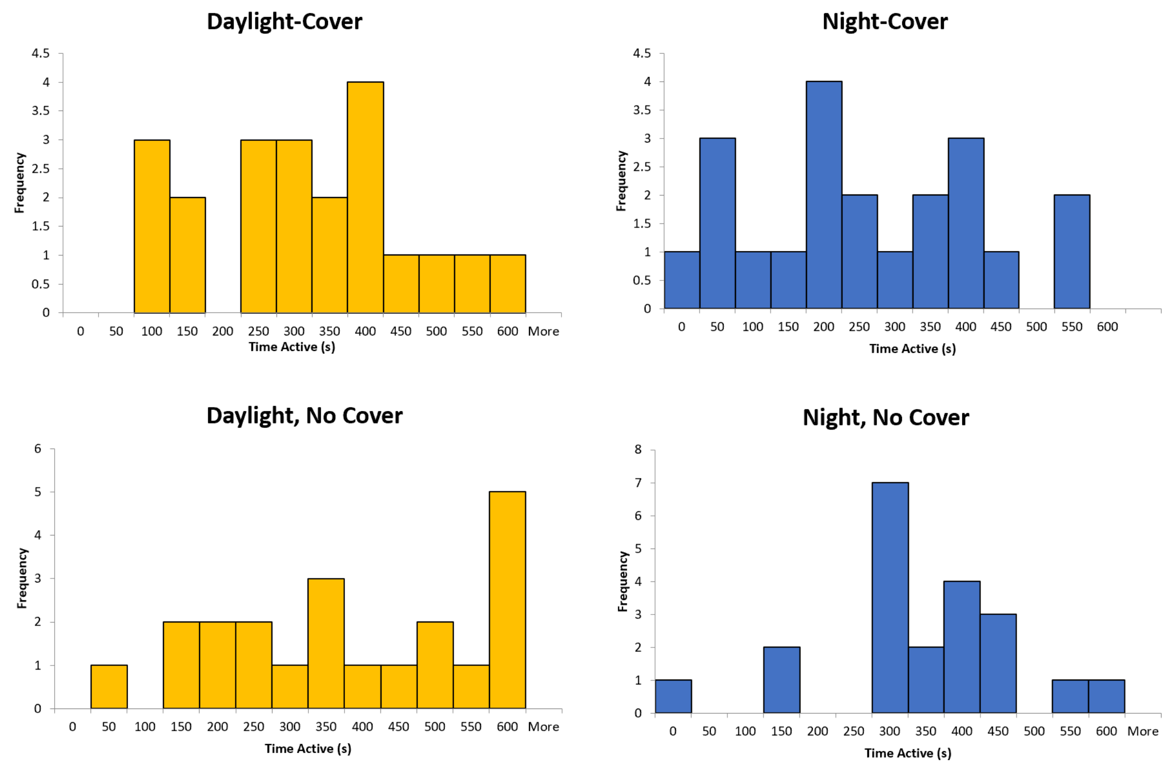

Figure 2: Histograms of activity levels in each applied condition and treatment

Both Wilcoxon rank sum tests for each treatment supported the conclusion that there is no statistically significant difference in activity levels between daylight and night (Figure 3).

Finally, a two-way ANOVA test (α=0.05) comparing the daylight/night treatments in both cover and no cover trials suggested that there is no significant difference between daylight and night, supporting the conclusions made by the Wilcoxon rank sum tests. However, the two-way ANOVA did suggest that there is some significant difference in activity level between covered and no cover treatment trials (Table 1).

```
        Wilcoxon rank sum exact test

data:  activity by treatment
W = 253, p-value = 0.4248
alternative hypothesis: true location shift is not equal to 0

        Wilcoxon rank sum exact test

data:  activity by treatment
W = 269, p-value = 0.2299
alternative hypothesis: true location shift is not equal to 0
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Figure 3: R output of Wilcoxon rank sum tests for no cover and covered trials respectively

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	116781.1	1	116781.1	4.86474	**0.030281**	3.960352
Columns	47610	1	47610	1.983286	0.162919	3.960352
Interaction	1318.126	1	1318.126	0.054909	0.81533	3.960352
Within	1920449	80	24005.62			
Total	2086159	83				

Table 1: Two-way ANOVA comparing cover vs no cover (sample), day vs night (columns), and between the factors (interaction)

Discussion

The hypothesis of crab activity being higher in night trials and in trials when cover was available was not supported by the data. Both tests suggested that day and night have no significant impact on the activity level of *H. nudus*. The ANOVA results are suggestive because of likely violations of the assumptions required for the type of analysis. Because the ANOVA requires data to be distributed in a normal distribution and the sample size used could not be definitively identified as being normally distributed, any ANOVA results were used for supporting the non-parametric analyses.

My findings stand in contrast to the published literature. Most studies on decapod foraging are performed at night when activity levels are generally higher (Edwards et al. 1982, Girlades et al. 2017). Additionally, studies show that crabs tend to utilize cover more in the day than at night, likely to avoid predation by hiding and reduce the effects of desiccation (Wieters et al. 2009). My study, on the other hand, found no significant use of cover in either daylight or night trials. This may be attributed to an insufficient amount of covers, short trial times, or no immediate threat of predation. Observations outside of trials found that crabs in the holding tank tended to spend more time in the center of the tank at night compared to daytime, when they would spend more time under covers or in the corners of the tank.

The decision to use *Ulva lactuca* came from a study by Birch 1979, which found that *H. nudus* gut contents were primarily made up of green algae, *Ulva rigida* (Birch 1979). The rest of the gut contents were brown algae, red algae, and animal tissue. The intent with providing food was to give the individuals a motivation for movement and see if the risk-taking behavior was different in varying conditions. There is a possibility that food preference may vary slightly based on location of collection and what algae are available, thus there is a chance that a lack of variety in diet influenced results.

Mobile predators like *H. nudus* are uniquely suited in their ability to have a various vertical distribution in response to avoiding predation or competition at the lower distribution limits and avoiding abiotic stress at the upper limits (Gravem & Morgan 2017). While my study found no significant differences in activity between each treatment, the literature reports that crabs' distributions are highly influenced by the predators present and the time of day (Dudas et al. 2005; Boudreau and Worm 2012).

Laboratory experiments almost always lack certain conditions that cannot be translated from the field and, as such, will have variables that may influence the outcomes. Because trials were conducted twice daily for six days, the test subjects experienced handling stress, possible hunger if they did not consume *Ulva* during their trial, and artificial light skewing their perception of day and night. These factors may have some influence on the recorded activity level.

Understanding the role that generalists like *H. nudus* play in complex ecosystems like the intertidal is critical as we continue to observe the impact that changes in predation can have on the food web as a whole.

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