Anthropogenic stress on the Pacific harbor seal (*Phoca vitulina*) based on behavior and fecal corticosterone analyses

Kristen M. Krick

Writer's Comment: *I created this manuscript for my research project at the Bodega Marine Laboratory through BIS 122P. Our small class had the chance to develop, complete, and write about a project of our choice for five weeks of the quarter out at the lab. I chose to research the Pacific harbor seal and whether anthropogenic issues are causing increased stress levels in the seals. The data brought through some significant data and pushed my curiosity to pursue this project towards a graduate thesis. The entire project was a very rigorous process; I only had a five week time period to complete and write the thesis. But the experience and knowledge I gained along the way was well worth the struggle. I cannot go without thanking Professors Steven Morgan and Ernest Chang at BML for guiding me through and seeing me to this point. I am forever grateful and encourage any UC Davis students interested in marine biology to participate in the BML program.*

Instructor's Comment: *Students relocate to Bodega Marine Laboratory (BML) from main campus to receive intensive instruction and hands-on experience in marine ecology and physiology as well as the tools of the biological trade, including hypothesis testing, experimental design, data analysis, oral presentation, scientific writing, and critiquing primary literature. The capstone of these courses is an original research project that is conducted during the last five weeks of the quarter, culminating in an oral presentation to the BML community and a publication-ready manuscript. Kristen was keen to study harbor seals, which are logistically challenging subjects. Even with permits, access is restricted during pupping season when her study was conducted. She overcame these obstacles by designing a feasible study on the impact of humans on seal stress using complementary approaches. Behavioral observations revealed that heavily visited seal populations are accustomed to visits and may be less stressed than infrequently visited populations, although her physiological assay could not confirm this finding. Kristen plans to continue her research in graduate school with the goal of better managing seal populations.*

—Steven Morgan, Bodega Marine Laboratory,
Department of Environmental Science and Policy

Abstract

The increased presence of human disturbance in the vicinity of the Pacific harbor seal (*Phoca vitulina*) appears to cause stressful situations, possibly leading to changes in individual behavior and corticosterone levels. A short-term field experiment was performed to determine the different vigilance behaviors of the seals at high, medium, and low human disturbance sites. Fecal samples were also collected from each site to determine the average corticosterone levels in each population. Observations were performed on the vigilance of the seals and their frequency of entrance into the water relative to the number of humans in the area. Seals appeared to be habituated to humans in highly disturbed areas, but they panicked in response to humans in rarely disturbed areas. Fecal corticosterone tests were not significantly related to human disturbance, although sample sizes were low. Overall, vigilance seems to be lower in seals where more humans are present, suggesting respectable regulations are in place for the conservation of the Pacific harbor seals, but more observations and samples are needed to determine if increased human numbers also increase the internal stress levels of the seals.

Key Words

Anthropogenic, corticosterone, disturbance, ELISA assay, flushing, habituation, Pacific harbor seal, pinnipeds, stress, vigilance

1. Introduction

Pacific harbor seals (*Phoca vitulina*) are considered one of the most timid species in the pinniped family (Haley, 1978). These mammals seem sensitive to disturbance, and any trace of terrestrial predator activity near the seals' haul-out locations causes them to flush into the water (Haley, 1978). This includes anthropogenic disturbances such as motorboats, hikers, canoes, beach goers, and tourists (Codde & Allen, 2013). The seals have been under the protection of the Marine Mammal Protection Act since 1972, and studies show their numbers increasing and returning to historical population levels (Allen, Mortenson, & Webb, 2011). But an increase in human disturbance also seems to be occurring. Increases in human and seal populations could potentially lead to more encounters. This could be unfavorable for the seals' numbers.

Pupping season is when human disturbance appears most offensive to the seals (Osborne, Calambokidis, & Dorsey, 1988). Any human disruptions could lead to the separation of the mother and pup and possibly pup abandonment. The direct interaction of humans towards the seals may be causing them additional stress, affecting their reproduction, and causing birth abnormalities (Allen et al., 2011). This could cause a decrease in population size if human disturbance were to greatly increase.

A seal's vigilance is a good indicator of the animal's stress. Several studies have demonstrated seals becoming more habituated to humans as human disturbance increases. This appears to have concurrently decreased the alertness of the seals towards the humans (Terhure & Brillant, 1996). On the other hand, more recent investigations have documented very chronic disturbance, leading to very high vigilance, the constant flushing of the seals, and sometimes abandonment of the haul-out area completely (Allen et al., 2011). Vigilance may serve as an accurate measurement of the anthropogenic effects upon the seals, allowing us to better understand modern-day stressors humans may be placing upon them.

More advanced techniques have recently emerged to quantify the stress levels of marine mammals. Glucocorticoids are hormones produced by mammals in response to a stressful situation, protecting the animal from possible harm done by the stressing agents (Sapolsky, Romero, & Munck, 2000). They prevent any dramatic decreases in blood volume and pressure by controlling the dilation of the smaller vessels in their arteries (Frye, 1967). Without glucocorticoids, blood pressure would drop in the presence of high stress, causing pools of blood to form in the tissues and soon leading to death (Frye, 1967). The least invasive way to determine glucocorticoid levels in a mammal is through the corticosterone – a type of glucocorticoid – that enters the bile and is secreted through feces (Petrauskas, Atkinson, Gulland, Mellish, & Horning, 2008). Human contact is not necessary to collect feces, making this a non-invasive method of measuring stress. The ratio of corticosterone to feces weight from these collections can be determined and compared with other samples, to determine high and low concentration levels, respectively.

The measurement of corticosterone may allow for the "real" stress levels of the seals to be calculated. Behavioral observations may underestimate the stress; a population of seals may appear habituated to humans, leading to an appearance of low stress. In reality, the animals could be under high stress levels. Only a physical measurement of glucocorticoids can result in a firm conclusion regarding the intensity of stress.

In this experiment, I investigated the stress levels of the Pacific harbor seals by studying their vigilance behaviors and reactions in different habitats and human abundances. I also tested the relative levels of corticosterone within each population's feces, to determine which populations may be more physically stressed. I hypothesized seals to be more vigilant and stressed in areas of greater human exposure and disturbance (Russian River) versus isolated and protected populations (Horseshoe Cove). I also expected corticosterone levels to be higher in the feces of the highly disturbed populations, since I expected them to be more disturbed and flush more frequently to escape human disturbance.

My objective was to compare the behavioral and physical stressor results and establish a relationship between human disturbance and the seals' immediate and long-term outcomes. This would allow me to evaluate if the protections placed on these seals are enough to allow the continued success of the population's breeding and survival.

2. Methods

2.1 Behavior Observation Methods

A vigilance scale was created to measure the different behaviors being performed by the seals, varying from sleeping (level 0) to escaping into the water (level 7; Table 1). Observations were recorded from May 14 to 24, 2014 for two seal populations in northern California: Horseshoe Cove near Bodega Bay and the mouth of the Russian River near Jenner. Five 1-h observations were conducted at each site, where the behaviors of up to 20 seals were recorded every 5 min. using the vigilance scale. I recorded the number of humans and dogs in the area, as well as the vigilance behaviors of the juveniles versus the adults and the number of seals going in and out of the water. Observations were conducted more than 50 m away on each population using a Bushnell spotting scope.

Data were compiled, prepared, and analyzed using JMP and Microsoft Excel software programs. Data were analyzed for the analysis of variance (ANOVA). A two factor ANOVA was conducted to determine the effects of the habitat type and seal type on the averaged vigilance levels and on the percent of seals at vigilance level seven (escaping into water). A one factor ANOVA was conducted to determine the effects of the habitat type on the number of humans nearby. I also conducted t-tests to determine the effects of the number of humans nearby, based on the habitat type, on the mean vigilance levels of the seals and the percentage of seals at the highest vigilance level.

2.2 Fecal Collection Methods

Feces were collected from the Horseshoe Cove (n = 4) and Russian River (n = 10) populations using the permits of Sarah Allen of the Point Reyes National Park and James Harvey of the Moss Landing Marine Laboratory. Samples were also donated from the staff at The Marine Mammal Center (n = 5) in Sausalito, California. I collected the samples at Horseshoe Cove and the Russian River 2 to 3 hours before high tide and after waiting for the seals to leave the location. Because my collections occurred during pupping season, I avoided flushing the animals. Fecal samples were placed into a 50 ml conical tube and transported on ice to the Bodega Marine Laboratory for storage in a freezer, where they were kept until further analysis. The sex and age of the animals and the time the samples were produced were not recorded. Fecal samples from four separate holding cages at the Marine Mammal Center were prepared using the same protocol. All samples from the Marine Mammal Center were from juvenile seals.

I utilized a Cayman Chemical ACE Enzyme-linked Immunosorbent Assay (ELISA) for corticosterone analysis. I performed a practice round with 2 Horseshoe Cove samples and a second round with the remaining 17 samples. I extracted corticosterone from the samples with altered methods, using a consistent 0.28 g of the wet feces with 4.2 ml of ethanol, letting the extraction of the hormone continue for 40 min. The samples were centrifuged at 1200 rpm before removing 3.5 ml of the supernatant. The ethanol was evaporated using a Savant vacuum for 4.25 h. The sample was later reconstituted with 0.5 ml of the provided ELISA assay buffer and centrifuged at 12,000 rpm. The supernatant was then used as our sample in the plate.

A 1:10 dilution was created for each sample, using 20 µl of each sample with 180 µl of the CEIA buffer. The subsequent procedures followed were standard direction under the Cayman ELISA assay protocol. I devised an equation to determine the amount of corticosterone in an individual fecal sample:

$$\frac{21.4275\,X}{Y}$$

X = pg/ml of 1:10 dilution concentration

Y = percent of dry weight in the wet weight sample

The equation was developed as follows:

1. A 1:10 concentration was used from the 0.5 ml of reconstituted sample from the buffer. I had to adjust this to a 1:1 concentration.

$$10\,x\frac{pg}{0.5ml}$$

2. Because I only used 0.5 ml, I had to adjust to 1 ml to determine pg/1 ml.

$$\frac{10\,x}{2} = 5\,x\frac{pg}{1ml}$$

3. The ratio of grams actually used was then calculated, because I did not use the full amount of the original sample. I multiplied the original wet weight with the amount of extracted supernatant and divided the product with the amount of ethanol used.

$$\frac{(0.28g)(3.5ml)}{4.2ml}$$

4. The adjusted pg/ml of wet weight used was divided by the ratio of grams actually used.

$$\frac{5x\frac{pg}{1ml}}{\frac{(0.28g)(3.5ml)}{4.2ml}} = 21.4275x$$

5. Y was added to account for the percent of dry weight used in the wet weight sample.

$$\frac{21.4275x}{y}$$

I also determined the dry weight of each sample by weighing out ~0.15 g of the wet feces and letting each sample dry for 4 days. This determined the final concentrations of corticosterone per gram of feces. The ratio of wet to dry weight was used against the 0.28 g of original feces used. Data was compiled, prepared, and analyzed using JMP, SigmaPlot, and Microsoft Excel software. I ran a one factor ANOVA on the effects of the habitat type on the mean levels of corticosterone in the feces.

3. Results

3.1 Behavior Observation Results

Humans were most common at the Russia River on the weekends, less on weekdays, and least at Horseshoe Cove (Fig. 1, P = 0.008). Horseshoe Cove's low human abundance led to differentials in the seals' averaged vigilance levels (Fig. 2, P < 0.0001). The Russian River habitat during the week and weekend had no significant effect on the seal's vigilance levels (Fig. 2, P = 0.2682, P = 0.4628). All predicted habitat types had similar vigilance responses; the averaged vigilance levels (Fig. 3, P = 0.3276) as well as the averaged percent of seals escaping to the water (Fig. 4, P = 0.4672) had no significance. Both situations also had no significance with the different seal types (adult versus juvenile). The adult seals in the high human abundance location seemed to be less vigilant than the juveniles. The percentage of seals at the highest vigilance level was affected by the number of humans nearby at the low human habitats (Fig. 5, P = < 0.0001) but not for the medium and high human locations (Fig. 6 and 7, P = 0.6071, P = 0.3737).

3.2 Fecal ELISA Assay Results

Corticosterone levels of the seals at the locations (Horseshoe Cove, Russian River, and Marine Mammal Center) were not significant (Fig. 8, P = 0.8186). The concentrations were run through a SigmaStat test, and the power of the performed test was 0.049, below the desired power of 0.80.

4. Discussion

4.1 Behavior Observations

The protection put upon each population of seals clearly brought on different responses to disruptive human activities. Horseshoe Cove is located on a marine reserve, which keeps the general human population out of sight from the seals. The Russian River is a part of the Sonoma State Park system, which grants open access to the entire public. However, some protection for the Russian River population is provided by a rope boundary placed 50 m around the seals' sandy haul-out habitat. Even with such a boundary, there was still a clear difference in the number of humans in each area, as well as the amount of disturbance to the seals.

The seals performed different intensities of vigilance among the different habitat types. When a single human walked into the field of view for a Horseshoe Cove seal, on average, more seals responded and an increased number of seals escaped into the water. At the Russian River, a single human would cause some seals to flush. But when dozens of humans started to arrive, the average seal's vigilance was very low (vigilance level zero to two), suggesting habituation of their behavior towards increased human exposure (Terhure et al., 1996). In the high disturbance habitat of the Russian River, the adults seemed less vigilant than the juveniles, suggesting a learned habituation of human disturbance over the lifetime of the seal. The seals at Horseshoe Cove did not demonstrate an age-dependent behavior response; because these seals were exposed to such low amounts of human disturbance, perhaps the adults are just as uncomfortable around humans as the naïve juveniles, leading to equal flushing rates.

4.2 Fecal Collections

The results of my fecal ELISA assay readings vastly varied among the Horseshoe Cove, Russian River, and Marine Mammal Center seals, as well as between the populations. There were two samples from the Russian River that read concentrations three to five times larger than the other Russian River samples. I did not note what sample came from which individual seal, but I would think this seal was undergoing extreme physical stress, possibly being injured or even pregnant (Allen et al., 2011). These results greatly affected the corticosterone comparisons between the populations, leading the findings to be inconclusive.

The low power of my statistical test suggests the high P-value was due to a random sampling variability. Perhaps if more samples were collected, the variation would decrease and a relationship between the populations would be more obvious. Therefore, firm conclusions could be made regarding my hypothesis of higher corticosterone levels at higher human abundance.

4.3 Future Research

My expectation was to make a connection between the protections placed upon the Pacific harbor seals with the behaviors and corticosterone levels observed. I also wanted to determine whether the current safeguards are enough to allow a safe and continual growth pattern of the harbor seal populations. Due to the high variation in the corticosterone tests, it is not possible at this time to correctly relate high or low corticosterone levels to the Horseshoe Cove, Russian River, or Marine Mammal Center harbor seal populations.

It would be interesting to see if these outlying corticosterone levels from the Russian River population were normal for an injured or pregnant seal, or if these levels were elevated due to human interaction. None of this variation can be understood until dozens more samples are collected and analyzed. Continuing this endeavor when the seals are not pupping would be ideal; according to Osborne et al. (1988), disturbing the seals during one of their most critical interaction periods could be detrimental for their population numbers.

I would also like to collect fecal samples 24-48 h after a determined high or low stressor event. It has previously been determined this time frame is necessary for corticosterone levels to reach their peak from a given event (Petrauskas et al., 2008). If these were correlated, it could be possible to directly associate human disturbance with these corticosterone levels and see if humans are a major factor in sudden changes within a population.

Another intriguing future field experiment would be to introduce 15 to 20 humans at Horseshoe Cove to mimic a scenario similar to the Russian River to see if the seals' vigilance levels and corticosterone levels change. Horseshoe Cove does not naturally experience large numbers of humans in the area. It would be interesting to see what would occur if several humans suddenly appeared. I would want to see if these seals habituate like the seals at the Russian River. This could demonstrate if each population differs due to a lack of or overexposure to humans over a period of time or if they differ due to the actual number of humans at that specific moment.

Overall, vigilance seems to be lower in seals where more humans are present, suggesting respectable regulations are in place for the conservation of the Pacific harbor seals. More observations and samples are needed to determine if increased human numbers also increase the internal stress levels of the seals. Data collection and inspection of several more populations may help determine these results for seals along the entire coast of California. Many additional factors could be explored to explain the anthropogenic interactions towards harbor seals in relation to stress; examples, according to Codde et al. (2013), include construction, runoff, noise, and chemical spills. Knowing the overall relationship between harbor seals and humans could better help us comprehend our impact on these organisms and demonstrate how we should enhance their protection from the general public to care for future generations of seals.

5. Acknowledgements

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Thank you to Molly Engelbrecht for her resources, Jackie Sones for her approval of my research on the seals in the preserve, and the Stewards of the Coast and Redwoods for allowing me access to the Russian River population. The entire Spring Class of 2014 at the Bodega Marine Laboratory was also a huge help in all of my endeavors of collecting feces.

I have to give a big thank you to my teacher assistants: Sarah Gravem, who helped me with all the crazy statistics on my behavior analysis and coached me mentally and emotionally, and Sukkrit Nimitkul for the endless hours in the lab helping me complete my ELISA assay and the nighttime feces investigations. Lastly, I cannot go without thanking my mentors Steven Morgan and Ernest Chang for directing me in the right path more than once. I will forever be grateful for your expertise.

6. References

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7. Tables

VIGILANCE SCALE USED	
Scale	**Description**
0	Sleeping
1	Twitching, movement of fins
2	Some rolling around
3	Looking up towards activity, movement, but not very suddenly
4	Entering water not scared
5	Looking up, very suddenly, long pauses
6	Start moving around the area more noticeably, making noise
7	Running away & going into the water

Table 1: the vigilance scale used during the behavioral observations on the Pacific harbor seals. I recorded the number of individual seals that performed at each scale level every five minutes, in concordance with the number of humans in the area.

8. Figures

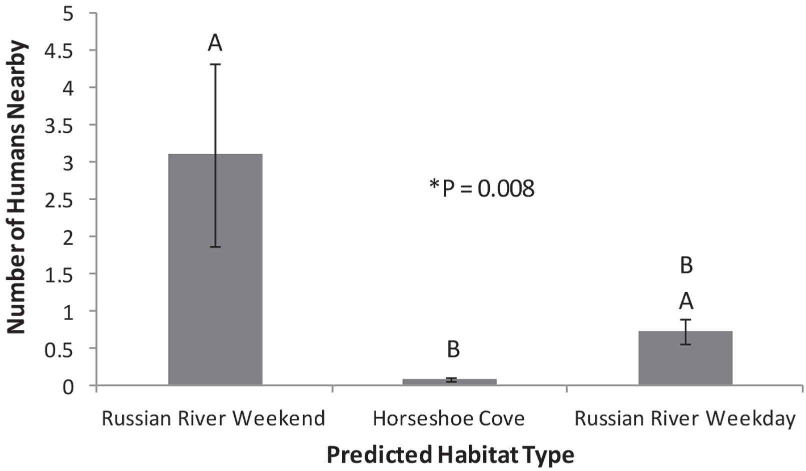

Figure 1: bar graph demonstrating the significance (P = 0.008) of the predicted habitat types on the number of humans in the nearby area.

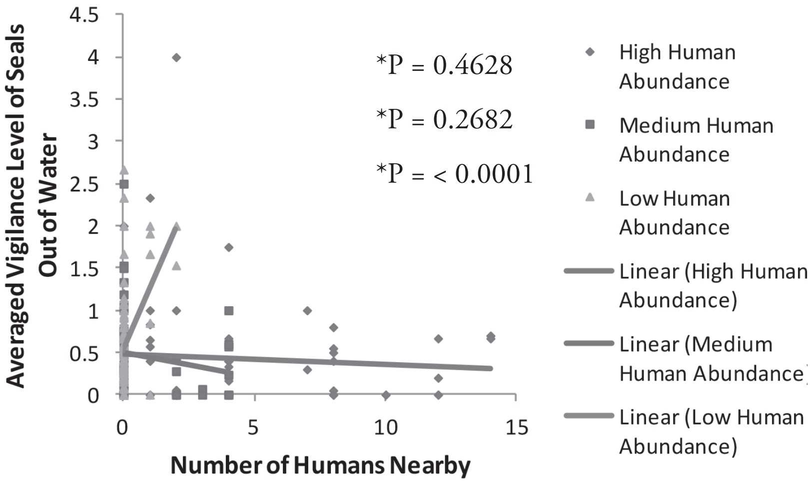

Figure 2: scatter plot comparing the effects of the three different human abundance scales (low = Horseshoe Cove, medium = Russian River on a weekday, high = Russian River on a weekend) on the average vigilance level of the seals out of the water. Low abundance has significance ($P < 0.0001$) for the low number of humans at Horseshoe Cove (0-2 humans) in the nearby area on the average vigilance; medium abundance has a lack of significance ($P = 0.2682$) for the medium number of humans at Russian River on a weekday (0-4 humans) in the nearby area on the average vigilance; high abundance has a lack of significance ($P = 0.4628$) for the high number of humans at Russian River on a weekend (0-14 humans) in the nearby area on the average vigilance.

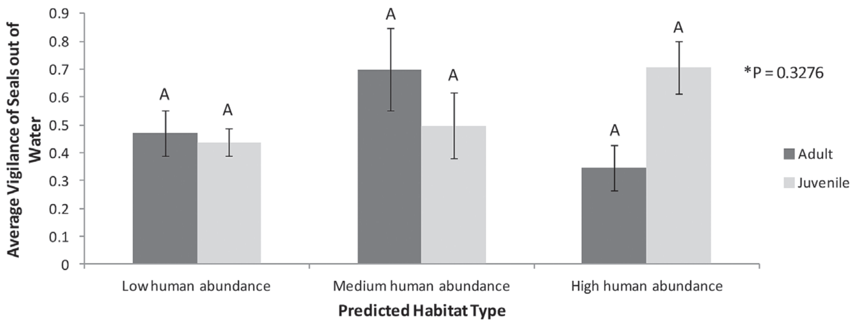

Figure 3: bar graph demonstrating no significance for the predicted human habitats (low = Horseshoe Cove, medium = Russian River on a weekday, high = Russian River on a weekend) on the average vigilance of the seals out of the water. There is a lack of differences between the seal types, with some differences for the high human abundance seals.

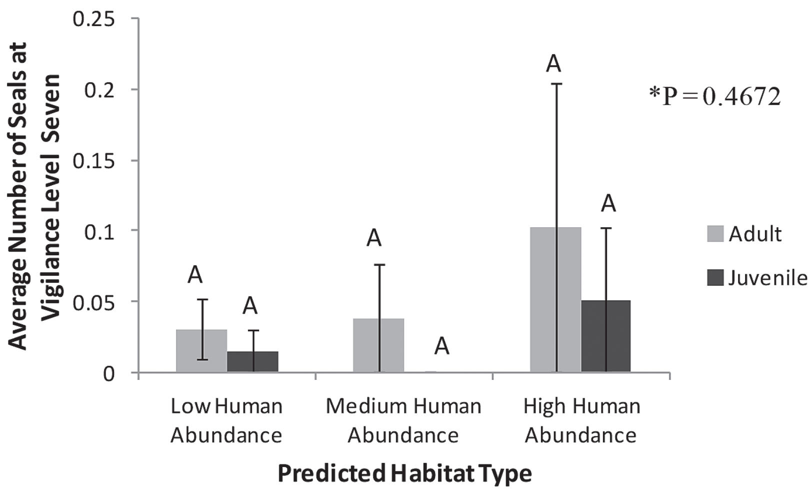

Figure 4: bar graph demonstrating no significance for the predicted human habitats (low = Horseshoe Cove, medium = Russian River on a weekday, high = Russian River on a weekend) on the average number of seals at vigilance level seven, or how often they flee into the water. This also shows the lack of differences between the seal types.

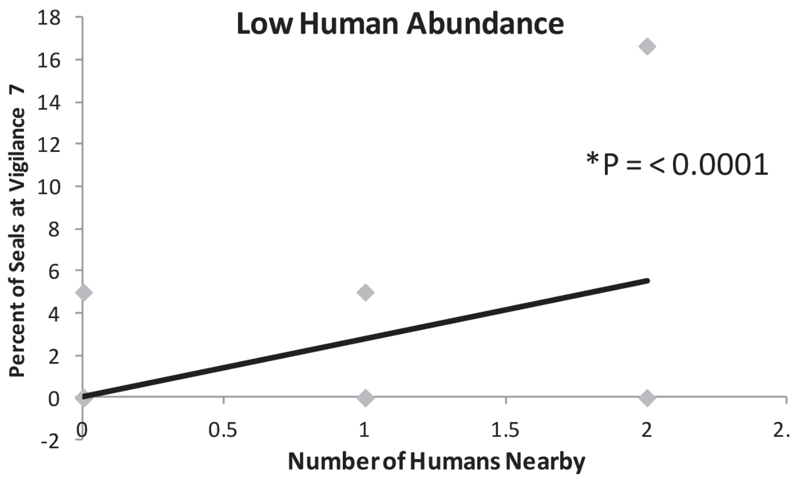

Figure 5: scatter plot demonstrating the significance (P < 0.0001) of the low number of humans at Horseshoe Cove (0-2 humans) in the nearby area on the percent of seals at vigilance level seven (seals fleeing into the water).

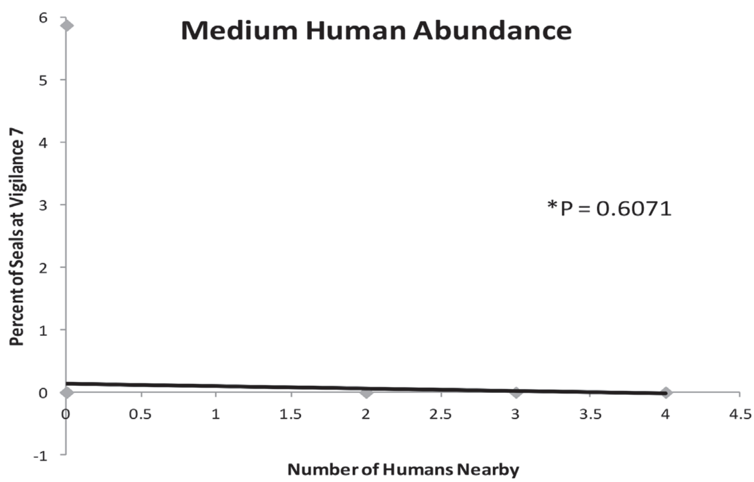

Figure 6: scatter plot demonstrating no (P = 0.6071) for the medium number of humans at the Russian River on a weekday (0-4 humans) in the nearby area on the percent of seals at vigilance level seven (seals fleeing into the water).

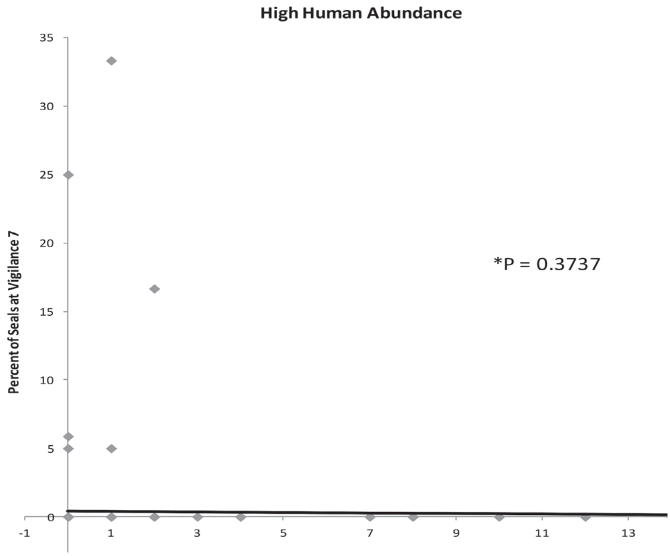

Figure 7: scatter plot demonstrating no significance (P = 0.3737) for the high number of humans at the Russian River on a weekend (0-14 humans) in the nearby area on the percent of seals at vigilance level seven (seals fleeing into the water).

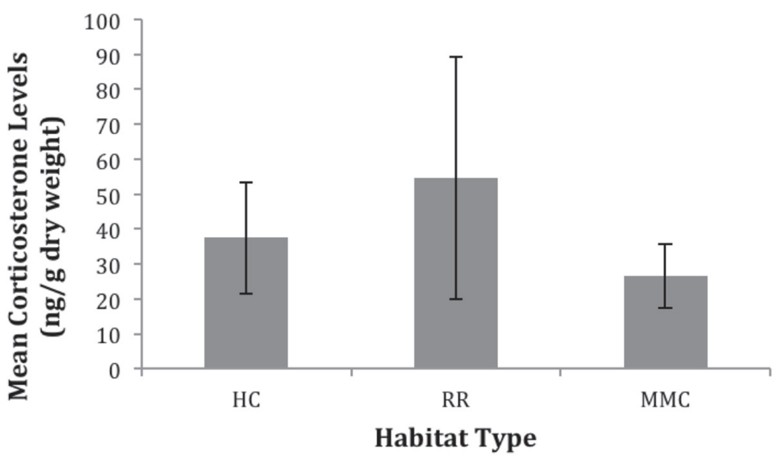

Figure 8: bar graph demonstrating no significance (P = 0.8186) for the habitat type (HC = Horseshoe Cove, RR = Russian River, MMC = Marine Mammal Center) on the mean corticosterone levels in the feces collected from seals at each location, based on dry weight.