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Olfactory Enrichment in California Sea Lions (*Zalophus californianus*)

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OLFACTORY ENRICHMENT IN CALIFORNIA
SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

by

Myстера Marie Samuelson

A Dissertation
Submitted to the Graduate School
and the Department of Psychology
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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December 2015

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ABSTRACT

OLFACTORY ENRICHMENT IN CALIFORNIA

SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

by Mystera Marie Samuelson

December 2015

In the wild, California sea lions (*Zalophus californianus*) are exposed to a wide array of sensory information at all times. However, it is impossible for captive environments to provide this level of complexity. Therefore, unique procedures and practices are necessary for the maintenance of physiological and psychological health in captive animals (Wells, 2009). This project aims to explore the behavioral effect of scent added to the environment, with the goal of improving the welfare of captive sea lions by introducing two scent types: 1.) Natural scents, found in their native environment, and 2.) Non-natural scents, not found in their native environment. The use of scent to add complexity to the captive environment has been utilized with big cats (Szokalski, Litchfield & Foster, 2012; Wells, 2009), canids (Steele & Steele, 2005), and other zoo-housed species (Wells, 2009), yet this method has not been explored in marine mammals. Nor has this approach been documented in the scientific literature for use with captive sea lions, despite caretaker reports that scents may be a fruitful approach for captive sea lion enrichment. Scent enrichment was found to significantly impact sea lion behavior, as demonstrated by a reduction in pattern swimming, increased habitat utilization, and reduction in stereotypical behavior; however, there appears to be no relationship between these variables and a preference between natural and non-natural scents.

DEDICATION

This dissertation is dedicated to my family. For my parents, who taught me to love and respect the natural world from a very young age; for my amazing husband, Derrick, who has supported every one of my wildest dreams; and for Tobe and Abbie, who have been my partners in nearly every adventure. I am blessed to have such an amazing family. This one is for you.

ACKNOWLEDGMENTS

This project would not have been possible without the support of my committee members: Drs. Heidi Lyn, Stan Kuczaj, David Echevarria, Alen Hajnal, and Susan Friedman. I would also like to thank Dr. Richard Mohn who consulted on the statistical analysis for this project. I have been very lucky to have had such wonderful mentors for my doctoral studies, and cannot thank them enough for their guidance. Special thanks, of course, go to Dr. Lyn: Thank you for welcoming me into the Comparative Cognition and Communication Lab family! I would also like to thank Dr. Moby Solangi, Kelly Pulis and the caretaking staff at The Institute for Marine Mammal Studies who provided amazing support for this project.

I have been extremely fortunate to be a member of a supportive lab and class of graduate students here at The University of Southern Mississippi who have all provided support, assistance and laughter for which I am very grateful. I would especially like to thank Lisa Lauderdale, Erin Smart, Stephanie Jett and Megan Broadway for their friendship and support throughout the duration of this project. Lisa: Thank you for coding hours of reliability data, editing endless manuscript drafts and making graduate school fun on a daily basis. Erin: Thank you for keeping a smile on my face no matter how far graduate school has taken me away home. Stephanie and Megan: Thank you for welcoming me into the lab and for creating a truly collaborative research environment where we all can grow and thrive as scientists.

Of course, this study would not have taken place without Gabby, Maya, Sage and Kaytee. Thank you for allowing me a glimpse into your world.

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CHAPTER I

INTRODUCTION

California sea lions (*Zalophus californianus*) are amphibious marine carnivores, and one of several species of pinnipeds native to the Pacific coastline of North America (Reeves, Stewart, & Leatherwood, 1992). Capable of dynamic behaviors and very responsive to training, this species was a common sight in zoos and aquariums (Clark, 2013). Past research had demonstrated that sea lions can successfully complete advanced cognitive tests such as delayed match-to-sample tasks with both auditory and visual stimuli (Schusterman, Reichmuth Kastak, & Kastak, 2002), as well as comprehend artificial symbols (Schusterman & Kastak, 2004). With this knowledge of the sea lions' cognitive capabilities, it is clear that the captive environment alone cannot satisfy the cognitive and psychological needs of these captive pinnipeds (Clark, 2013). This was especially evident when one considers that sea lions naturally exist in dynamic environments with infinite opportunities for varied sensory stimuli. Unfortunately, in terms of providing adequate stimulation for many species, captive environments are impoverished and simply cannot compete with this diversity. As a result, the environment must increase in complexity to maintain both the physiological and psychological wellbeing of captive sea lions (Carlstead, Seidensticker, & Baldwin, 1991).

Sea Lions in Captivity

Adding physical complexity to captive environments, or “environmental enrichment,” has been established as an important approach to reducing stereotypies that arise and for encouraging species-specific behaviors in a variety of species (Ginrod &

Cleaver, 2001; Shyne, 2006). Behaviors are considered to be stereotypic if they are invariant and repetitive (Franks, Lyn, Klein, & Reiss, 2010; Shyne, 2006). These behaviors are often considered to be the result of inadequacies within the captive environment such as stress-inducing stimuli (i.e., noise), a lack of critical stimuli, or particular brain abnormalities that are a result of prior trauma (Mason, Clubb, Latham, & Vickery, 2007). Still, adding stimuli to a previously sterile environment does not necessarily guarantee enriching benefits for captive wildlife. On the contrary, additional stimuli must be evaluated to ensure that it does not cause additional abnormal behaviors (Lyn, 2009).

Possibly as a result of psychological and physiological trauma involved in their initial stranding, many captive sea lions exhibit some form of stereotypical behavior when housed in captive environments (S. Sayre, personal communication). In addition, because many sea lions develop illnesses following stranding, while housed in rehabilitation centers, it is possible that many animals enter captive environments with pre-existing physiological abnormalities, disabilities, and cognitive deficits.

Environmental enrichment, when utilized properly, has been demonstrated to have an anti-depressant effect (Brenes, Padilla, & Fornaguera, 2009; Carlstead et al., 1991), prevent dementia (Kolb & Wishaw, 1998), and mitigate the behavioral and physiological effects of stress in many species (Francis, Diorio, Plotsky, & Meaney, 2002; Wells, 2009). However rapid habituation to consistent stimuli can cause animals to develop stereotypical behaviors more rapidly than dynamic, variable enrichment (Kuczaj

et al., 2002). This is especially true long-lived animals with extensive memory capacities, such as sea lions (Kuczaj et al., 2002; Schusterman et al., 2002),

Numerous techniques for enriching the lives of captive pinnipeds have been documented over the years and have been found to be successful in reducing stereotypies and increasing species-specific behaviors. For example, novel objects such as toys (e.g., balls, Frisbees, etc.) have been shown to be effective in reducing these stereotypic behaviors (Hoy, Murray, & Tribe, 2010; Shepherdson, 1998). However, the fact that animals become habituated quickly requires these items to be presented in novel ways in order to maintain interest (Hoy, Murray, & Tribe, 2010; Kuczaj et al., 2002; Law & Reid, 2009; Shepherdson, 1998). Object-based stimuli, or environmental enrichment devices (EED's), have also been shown to reduce circle swimming in captive common seals (*Phoca vitulina*; Ginrod & Cleaver, 2001) and Australian sea lions (*Neophoca cinerea*; Smith & Litchfield, 2010). Similarly, species-specific foraging behaviors are often encouraged by hiding food throughout the enclosure or inside of EED's (Ginrod & Cleaver, 2001). These forms of naturalistic foraging opportunities have been shown to reduce circle swimming in captive walruses (*Odobenus rosmarus*; Kastelein & Wiepkema, 1989).

Training had also been shown to be a potential method of enrichment for captive marine mammals; this approach has been demonstrated to effectively reduce circle swimming (Kastelein & Wiepkema, 1988). However, the mechanism by which training influences behavior had yet to been identified, which was why further research was

necessary to elucidate the relationship between the enrichment qualities of these “social enrichments” (Clark, 2013).

An Evolutionary Approach

Sea lions are caniforms (a taxa including ursids, canines, pinnipeds, and mustelids). This taxon has been well known for being heavily reliant on scent in order to survive in the wild. Scent has been established as critical for effective foraging (Apfelbach, 1992; Gittleman, 1991; Hughes, Price & Banks, 2010; Schwartz, Miller, & Haroldson, 2003; Ylönen, Sundell, Tiilikainen, Eccard, & Horne, 2003), navigation (Rogers, 1988) and even many social behaviors (Beckoff, 1981; Rothman & Mech, 1979). As such, many enrichment approaches for these species tend to focus on scent (i.e., Andrews & Ha, 2014; Kitchener & Asa, 2010; Leonard, 2008; Nelson, 2009; Price, 2010; Rafacz & Santymire, 2014; Schneider, Nogge, & Kolter, 2014; Wells, 2004). However, the behavioral effects of scent have not been examined in pinnipeds to the same extent as other species in this taxon. Due to similarities in both physiology and behavior, it was likely that sea lions from the same taxonomic groupings may benefit from similar interventions (Agnarsson, Kuntner, & May-Collado, 2010).

As caniform predators, sea lions may have evolved to attend to natural scents through their biological preparedness. Therefore, the introduction of natural scents into the environment might have a greater impact on behavior than would non-natural scents. A preference for scents that would have been present in the natural environment should suggest that natural scents should have a higher impact on the animals’ behavior than non-natural scents. For example, South African fur seals (*Arctocephalus pusillus*), which

are otariids like sea lions, have been shown to possess the capability to distinguish between chemically similar odorants. Specifically, fur seals are capable of distinguishing numerous odorants that are naturally present in the marine environment (i.e. aliphatic odorants). These findings indicate that a species' environment may play a role in that animal's olfactory development, that environmental cues may have significant implications for an animal's behavior (Laska, Lord, Selin, & Amundin, 2010). This also suggests that sea lions would have similar capabilities, as they also belong to the otariid family.

Sea lions' biological preparedness for the use of olfaction comes as a result of the species' evolutionary history as amphibious tetrapods (four-limbed vertebrates; Kishida, Kubota, Shirayama, & Fukami, 2007) and caniform predators (a sub-order of carnivora), related to ursids (bears), canids (wolves, dogs, coyotes, etc.) and mustelids (weasels, otters, etc.; Eizirik et al., 2010; Martin, 1989; Zielinski, Spencer, & Barrett, 1983). Terrestrial tetrapods are capable of identifying and distinguishing between hundreds of odors, indicating that these species possess a vast olfactory repertoire. However, marine tetrapods such as minke whales (*Baleanoptera acutorostrata*), dwarf sperm whales (*Kogia sima*), and Dall's porpoise (*Phocoenoides dalli*) have a significantly reduced repertoire of olfactory receptor genes when compared with their terrestrial relatives. In contrast, amphibious tetrapods, such as the Steller's sea lion (*Eumetopias jubatus*) and loggerhead sea turtle (*Caretta caretta*), have maintained the majority of their olfactory receptors, not differing significantly from terrestrial species such as dogs (Kishida et al., 2007). According to Kishida et al. (2007), this indicates the importance of scent in

surviving in terrestrial environments, even temporarily, as amphibious species do. Still, aquatic mustelids' and pinnipeds' nasal turbinates (structures within the nose that maximize scent reception) possess significantly less surface area than terrestrial carnivores. This finding indicates that despite retaining scent capabilities, sea lions likely possess a less developed sense of smell, in comparison to their terrestrial relatives.

One hypothesis explaining why pinnipeds have developed reduced nasal turbinates suggests that pinnipeds' underwater foraging strategy does not require them to use olfactory cues (Van Valkenburgh et al., 2011). However, Catania (2006) describes an underwater sniffing method used by star-nosed moles (*Condylura cristata*) and water shrews (*Sorex palustris*) in which the animals exhaled bubbles onto a scent trail or object before taking the bubbles back in through the mouth and directing them into the nasal cavity. This technique was an alternative method of directing scents to the olfactory epithelium, in the nasal turbinates, without inhaling water. While it was unknown whether or not sea lions employ this technique, it was important to note that there are possible modalities of employing scent-based foraging in aquatic environments that have not been fully explored within the pinnipeds.

Wild sea lions have been documented to use olfaction as well as auditory cues to recognize kin (Pitcher, Harcourt, Schaal, & Charrier, 2011; Trimble & Insley, 2010). However, the role of scent in habitat utilization and navigation are unknown for pinnipeds. In addition, studies of caniform olfaction had typically only been studied in terrestrial species (e.g., Green et al., 2012), despite the pinnipeds' retention of olfactory capabilities after taking to the sea.

An Olfactory Approach

Olfaction presents one potential novel avenue of enhancing the captive environment for pinnipeds. This approach had been used as a successful method of reducing stereotypies and increasing species-typical behaviors in many species of captive wildlife such as felids (Bashaw, Bloomsmith, Marr, & Maple, 2003; Wells & Egli, 2004), ursids (Paccione, 2010; Schneider et al., 2014), and non-human primates (Boon, 2003; Wells, Hepper, Coleman, & Challis, 2007). In these studies, naturalistic scents, or scents found in an animal's natural environment, were often utilized. Natural, scent-based stimuli are often focused on the introduction of prey-related scents into a predator's enclosure (i.e., Bashaw et al., 2003). However, the functionality of a scent was also likely tied to the animals' natural environment. For example, African lions exhibit a stronger reaction to the scent of an African prey species, such as okapi, than do Asian felids such as tigers (L. Miller, personal communication).

Research Question

Can scent be utilized to enhance the captive environment for captive California sea lions?

Hypothesis I

I hypothesized that scent enrichment would increase habitat utilization, indicated by a change in habitat usage patterns, and reduce stereotypic behavior patterns in captive California sea lions.

Hypothesis Ia

If hypothesis one is supported, I propose that these trends will continue to be evident at both the enclosure and individual level.

Hypothesis II

I hypothesized further that natural scents would have a greater effect on behavior than non-natural scents, as indicated by a stronger effect on habitat utilization patterns and a statistically significant difference in instances of stereotypical behavior.

Hypothesis IIa

If hypothesis two is supported, I propose that these trends will continue to be evident at both the enclosure and individual level.

CHAPTER II
METHODOLOGY
Subjects and Facility

Research was conducted at the Institute for Marine Mammal Studies (IMMS) in Gulfport, Mississippi. Subjects included four two-year-old female California sea lions (*Zalophus californianus*) that had been acquired by IMMS. After they stranded along the California coastline and were deemed unfit for re-release by veterinarians (see Table 1). The sea lions were housed in pairs, with each enclosure displaying differences in pattern swims and stereotypical behaviors.

Table 1

Summary of Subjects

| Animal | Age | Sex | Attempted Re-Release? | Enclosure |
|--------|-----|--------|-----------------------|-----------|
| Maya | 2 | Female | Yes | 1 |
| Gabby | 2 | Female | Yes | 1 |
| Sage | 2 | Female | Yes | 2 |
| Kaytee | 2 | Female | Yes | 2 |

Materials

Scent enrichment was of two types, natural scents and non-natural scents. Natural scents were those that are likely to be present in the animals' natural environments. Food items currently included in the sea lions' diet were excluded because these food scents are likely already present in the entire enclosure as a result of past feedings. For example,

sardine oil was used as a natural enrichment scent. While, sardines are a natural food source for wild sea lions (Mancia et al., 2012), IMMS does not presently use sardines as a regular direct food source (D. Shannon, personal communication), ensuring that the scent of sardine is not present outside of experimental settings. Similarly, the smells of non-natural scents are those that would only be introduced anthropogenically (see Table 2 for a list of scents).

Table 2

List of Scents

| Natural Scents | Non-Natural Scents |
|---|--|
| Sterilized potting soil (no fertilizer added) – 1 cup was rubbed on the wall in the haul-out area of enclosure. | Orange – One large orange was cut in half and rubbed on the wall in the enclosure. |
| Sterilized playground sand - 1 cup was rubbed on the wall in the haul-out area of the enclosure. | Banana – One large banana was cut in half and rubbed on the wall in the enclosure either in the haul out area. |
| Kelp – 100% human food grade dried kelp: approximately 1 cup of dried sea kelp was wet down with saltwater and then rubbed on the wall. | Vanilla Extract – 1 tsp. of 100% food grade vanilla extract was applied to a wall in the haul out area. |
| Sardine Oil – 100% biodegradable, water-soluble, sardine bait oil scent (1 tsp.) was applied to a wall in the haul out area. | Cinnamon – 1 tsp. of 100% dried food-grade cinnamon was applied to a wall in the haul out area. |

Due to the fact that the sea lions at IMMS were likely to have already been exposed to a wide range of scents, it was impossible to ensure novelty in this study.

Therefore, the purpose of this study was to examine whether natural scents hold the animals' interest for longer periods and/or are more effective at reducing stereotypes than non-natural scents. This level of analysis was deemed appropriate in light of the fact that the animals had been housed until very recently at other facilities (The Marine Mammal Center and Pacific Marine Mammal Center) and we were unable to account for exposure to scents both at those locations as well as the diverse enrichment opportunities afforded them at the IMMS. Additionally, all animals were born in the wild and may have had the opportunity to smell items such as kelp, sardines, and/or sand/dirt before entering captivity. Therefore, no one scent was considered truly novel.

General Procedure

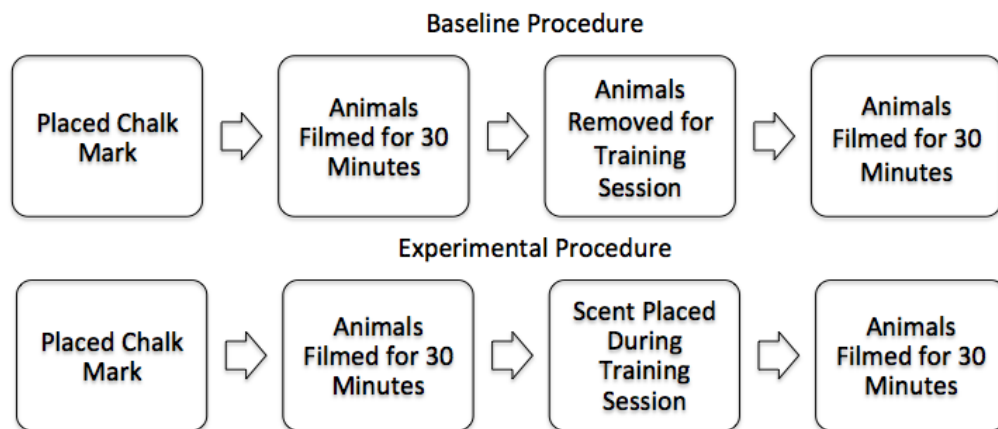


Figure 1. Study Design

Baseline Procedure

To begin this study the researcher collected baseline data for the span of one week (6 days) at the IMMS. During this time, researchers filmed one thirty-minute session in the morning and one in the afternoon (to parallel the experimental pre- and

post- sessions). To eliminate the confounding effect of a visual cue, this process included the introduction of a chalk mark on the wall (with no added scent) during baseline pre-sessions. No treatment was applied during the baseline post-session. In addition to allowing for the researcher to obtain baseline data, this also allowed for the animals to habituate to the researchers' presence.

Experimental Procedure: Natural and Non-Natural Scents

Each trial consisted of a pre- and post-session. The pre-session data collection period consisted of video data taken thirty minutes prior to the introduction of scent stimuli. Similarly, the post-session data collection period consisted of video data taken thirty minutes after the introduction of the scent stimuli. This approach allowed for the assessment of the animals' behavioral state prior to the introduction of scent enrichment, and allowed us to assess any changes potentially caused by this intervention.

Experimental trials were split into two scent types: natural and non-natural scents. Each of the 8 scents (Table 2) was presented three times, resulting in a total of twenty-four trials for each animal ($n = 4$). The order of presentation for these scents and placement within the enclosure were pseudo-randomized. Each scent was presented once per cycle, and excluded placement on the fourth wall (which was located on the same wall as the researcher, and was obscured from view due to the film angle; see Figure 3).

During the 30-minute pre-session data collection period, the researcher entered the enclosure with the animal caretaker to place a chalk mark on the wall, similar to the procedure for the baseline session. The chalk was matched in color to the scent item (e.g., dark brown for the dirt session), ensuring that the animals were being attracted to the

scent, rather than a visual cue in the experimental session. Chalk marks were placed at a minimum of three feet above the floor so that animals would need to actively stretch their necks to reach the scent and to avoid allowing the scent to mix with water on the ground thus diffusing the scent throughout the enclosure (Figure 2).



Figure 2. Maya sniffing the scent marker in enclosure one during a sardine oil session

The animal caretaking staff placed the enrichment scents in the enclosure by rubbing a pre-determined amount of the scent (see Table 2) onto the wall when animals were outside of the enclosure, while the researcher filmed from the viewing area. The viewing area allowed the researcher to see the entire enclosure. Data was collected on site using both a video camera as well as a data collection form. Additional notes were taken regarding other enrichment items given by the caretaking staff on that day, including special activities, unusual crowd/visitor dynamics, and any other potential confounding variables that could affect the results of the study. The scent location was cleaned with a bleach solution between each trial. Only one trial was executed each day to ensure that the scent had been eliminated prior to the subsequent scent trial.

Video data was then coded onto a Microsoft Excel spreadsheet in fifteen-second intervals to examine habitat usage, behavioral state, and stereotypic behaviors before and after the introduction of the enrichment (Table 3). To verify data coding, an independent coder was employed, reaching 92% agreement on over 20% of the video data.

Statistical Analysis

In order to assess the general impact of the study on individual animals, we initially examined overall habitat utilization and the exhibition of stereotypical behaviors in all four sea lions housed at IMMS, using a one-way ANOVA with Tukey’s Post-Hoc Test. This analysis was performed using IMB’s Statistical Package for the Social Sciences, SPSS 21 and Microsoft Excel. Independent-samples t-tests were utilized to examine changes in individual behaviors between the experimental pre-session and post-session conditions.

Table 3

Behavioral Ethogram

| Behavior | Description |
|---------------------------|--|
| Affiliative Interaction | Affiliative behaviors are non-aggressive interactions between two or more individuals. |
| Facial rubbing | Two or more individuals actively rubbing their snout against the others’ face and head. |
| Vibrissal rubbing | Two or more individuals actively rubbing their whiskers against the others’ face and head. |
| Snuffling | Sniffing that involves deep inhalations, often accompanied by the pressing of the nose into the fur of another individual, accompanied by auditory breathing sounds. |
| Affiliative – Other | Non-described affiliative behaviors. This code requires detailed descriptions in the notes for possible incorporation into the ethogram. |
| Agonistic Interaction | Agonistic interactions are aggressive interactions between two or more individuals. |
| Biting | Using the mouth to clamp down on any part of another animal’s body. |
| Chasing | Rapidly following another individual for more than 3 feet |
| Open Mouth | Opening of the mouth for a prolonged period, while facing another individual. |
| Display | |
| Pushing | Actively pressing the head and/or neck against the body of another animal displacing the other animal from its original position. |
| Agonistic – Other | Non-described agonistic behaviors. This code requires detailed descriptions in the notes for possible incorporation into the ethogram. |
| Above Water Vocalizations | Vocalizations used by the animals when above water (see Peterson & Bartholomew, 1969). |

Table 3 (continued).

| Behavior | Description |
|--------------------------|--|
| Bark | Loud, guttural sound similar to the sound of a dog barking associated with territoriality in male and female sea lions |
| Belch | Short “belch-like utterance” sounds akin to a human burp. |
| Growl | Long, deep sound, similar to the sound of a deep dog growl. |
| Locomotor Behaviors | Behaviors involving movement. |
| Circle Swimming | Swimming in a fixed pattern, which is the same at all points. |
| Pacing | Walking in a fixed pattern, which is the same at all points. |
| Resting | Lying on the ground in the haul-out area, or on the rocks along the side of the water. |
| Swimming | Swimming, without the inclusion of a fixed pattern. |
| Walking | Walking in the haul-out area, which is not the same at all points. |
| Self-Directed Behavior | Behaviors directed at an individual animals’ own body. |
| Grooming | Use of the hind flippers to clean the coat. |
| Self Directed – Other | Non-described self-directed behaviors. This code requires detailed descriptions in the notes for possible incorporation into the ethogram. |
| Scent Directed Behaviors | Behaviors directed at the exact location of the scent enrichment. |
| Rock Rubbing | Rubbing of the body/face against the rocks where the scent enrichment was placed. |
| Sniffing Rocks | Sniffing the rocks where the scent enrichment was placed. |
| Behaviors - Other | Behaviors not otherwise specified in the ethogram. |
| Feeding | Consumption of fish, squid, gelatin balls or other food items. |
| Interaction with Keeper | Direct interaction with a keeper in the enclosure attenuation to a keeper outside of the enclosure. |
| Interaction with Object | Direct interaction with an object in the enclosure. |

Habitat Usage and Pattern Swimming

The sea lions were housed in consistent dyads, with Maya and Gabby in enclosure one and Sage and Kaytee sharing enclosure two. The pools were kept at a consistent temperature (approximately 70 degrees Fahrenheit) throughout the course of the study and water quality was monitored daily by IMMS caretakers. Subjects had access to 25 ft. x 25 ft. enclosures complete with circular pools, each 20 ft. in diameter and 6 ft. deep.

To account for habitat utilization and swim patterns, the enclosure was divided visually into four equal zones and one central zone (zone 5; Figure 3). The amount of time spent in each segment of the enclosure was recorded for each individual, and compared between the pre-and post-sessions. To fully evaluate the hypotheses of this

study, data was analyzed both at the enclosure and individual level as the two enclosures differed greatly in their baseline habitat usage and pattern swimming. For example, diagonal swimming was observed only in enclosure one and was never observed in enclosure two. Similarly, circle swimming varied greatly in between the two enclosures. In enclosure one, circle swimming consisted solely of a counter-clockwise pattern, while enclosure two consists of a clockwise pattern. Stereotypical behaviors varied greatly by individuals with Sage and Gabby strong stereotypes. Gabby was the only animal to exhibit pool wall sucking, while Sage was the sole animal to engage in floor sucking, tail sucking, regurgitation and reingestion.

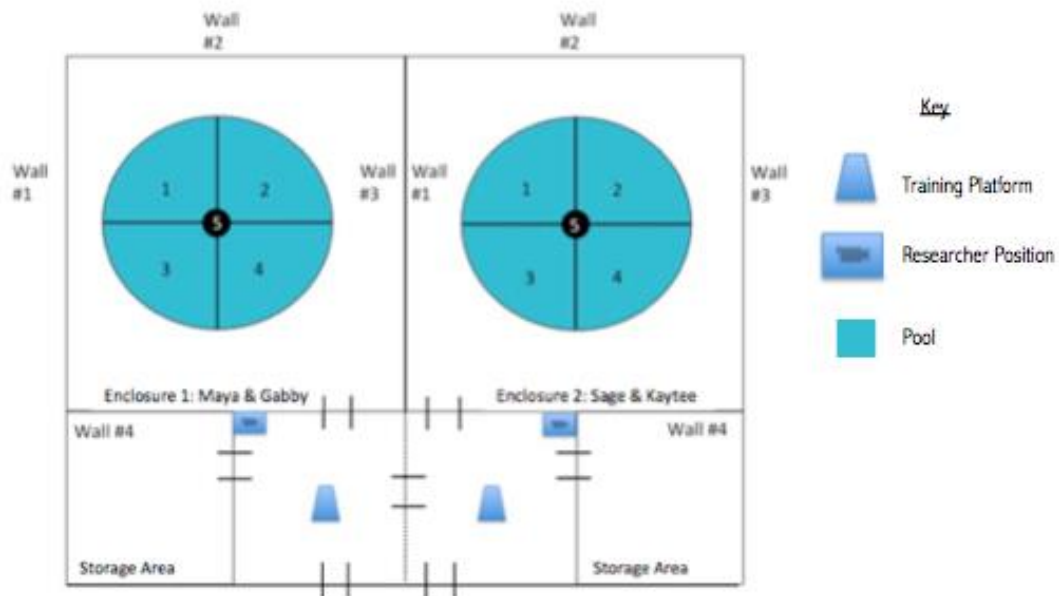


Figure 3. Enclosure layout. Walls 1, 2, and 3 received chalk and scent applications daily in the experimental procedure. The researcher filmed from outside the enclosure in the training area.

CHAPTER III

RESULTS

Hypothesis 1 Results

Hypothesis one was examined in order to determine if scent enrichment was effective in increasing habitat utilization, decreasing pattern swimming and reducing stereotypical behaviors, for these captive California sea lions.

Habitat Utilization

Changes in Habitat Utilization. Scent enrichment was found to have an effect on habitat utilization in sea lions. Animals were found to spend significantly more time out of the water (location 0, $t(190) = -5.276$, $p = .000$), and less time in locations 1 ($t(190) = 6.769$, $p = .000$), 2 ($t(190) = 4.435$, $p = .000$), 3 ($t(190) = 4.291$, $p = .000$), 4 ($t(190) = 5.456$, $p = .000$; Figure 4).

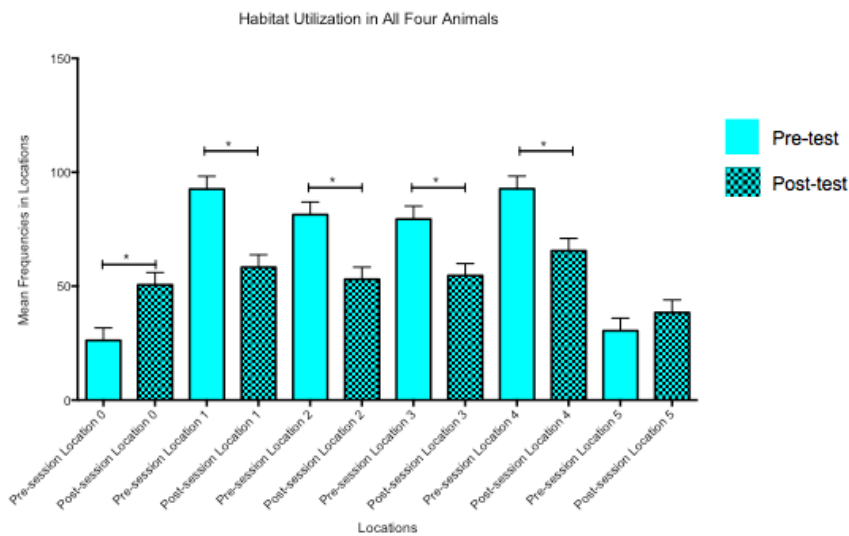


Figure 4. Overall mean location scores in pre-session and post-session experimental sessions for the effect of scent enrichment on habitat utilization

Habitat utilization by enclosure. Both enclosures showed effects of scent on habitat utilization. Sea lions in both enclosures spent more time out of the water (enclosure one $t(94)=-4.361$, $p = .000$; enclosure two $t(94) = -3.549$, $p = .001$) and less time at locations 1, 2, 3, and 4 (enclosure one location 1, $t(94) = 5.335$, $p = .000$; location 2 $t(94) = 2.506$, $p = .014$, location 3 $t(94) = 2.333$, $p = .022$, location 4 $t(94) = 3.875$, $p = .000$; enclosure two location 1 $t(94) = 4.386$, $p = .000$), 2 $t(94) = 3.846$, $p = .000$), 3 $t(94) = 3.854$, $p = .000$), and 4 $t(94) = 4.303$, $p = .000$). Time spent in the center of the pool (Location 5) did not differ significantly in either enclosure (see Figure 5).

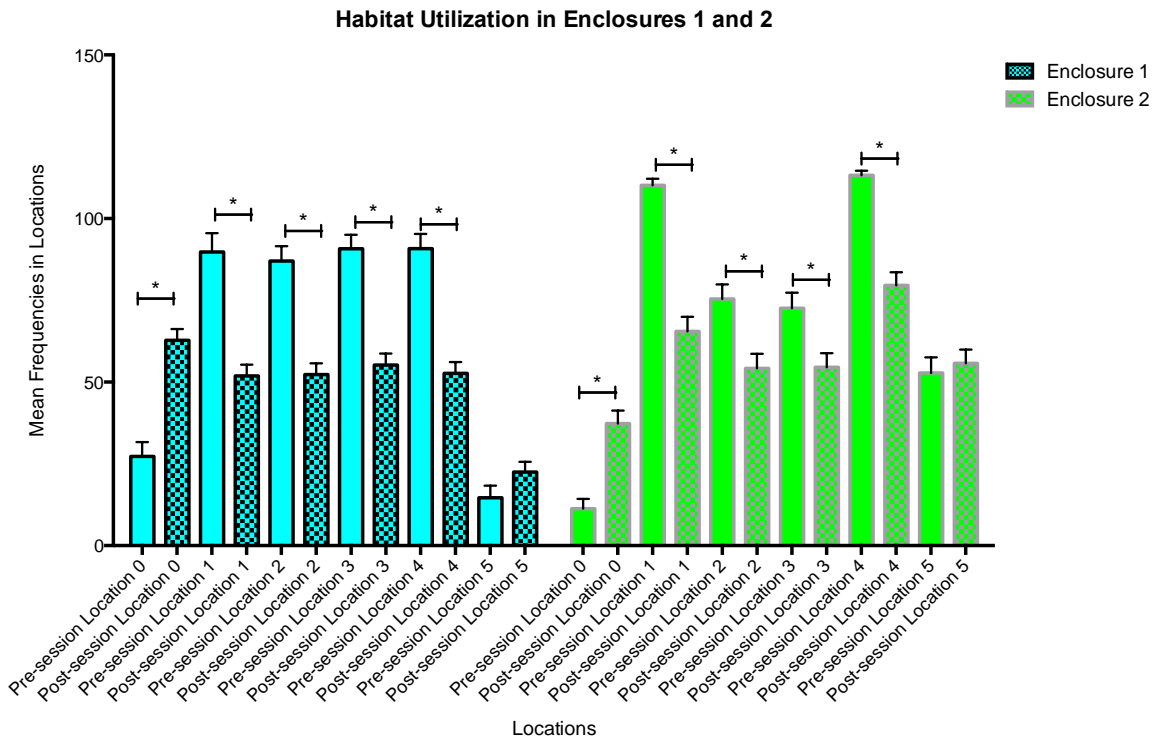


Figure 5. Changes in location between pre-session and post-session by enclosure

Habitat utilization by animal. These general findings held true for the individual sea lions as well. Gabby, Sage, and Kaytee all spent significantly more time out of the water and less time in locations 1-4. Maya instead decreased time spent in location one

($t(46) = 2.729, p = .009$), evenly dispersing that time spent across the other areas of the enclosure. However, none of the animals significantly altered the amount of time spent in the center of the pool (location 5; Figure 6).

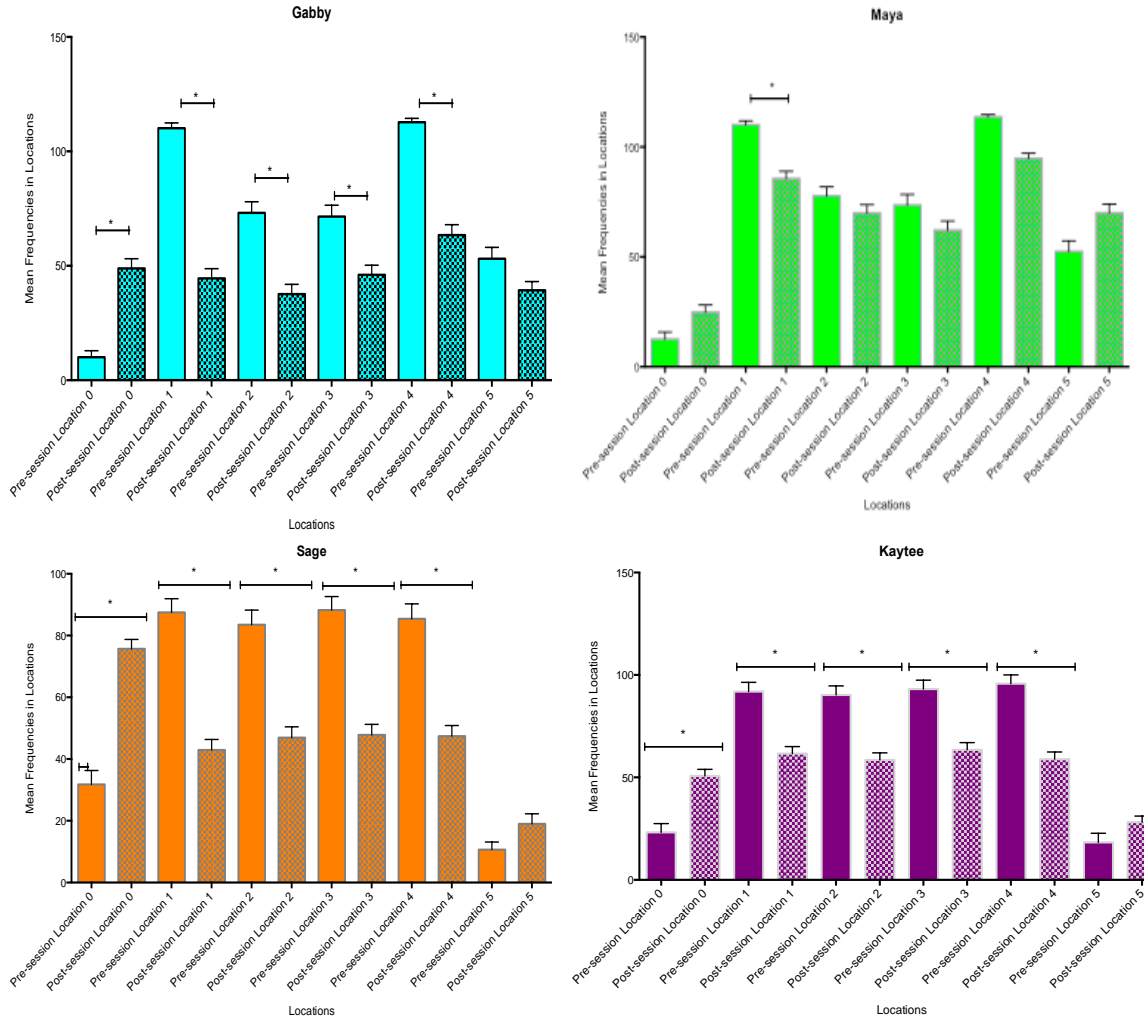


Figure 6. Pre-session and post-session changes in habitat utilization by animal Pattern Swimming

Overall, when examining data from all four animals, there was a reduction in both circle swimming ($t(189) = 6.728, p = .000$) and diagonal swimming ($t(189) = 2.338, p = .020$) when scents were present. In both enclosures one and two, this pattern was also

demonstrated, with a significant reduction in both circle ($t(94) = 4.548, p = .000$) and diagonal swimming ($t(94) = 2.647, p = .010$) in enclosure one and sharp reduction in circle swimming in enclosure two ($t(94) = 5.214, p = .000$).

Pattern Swimming by Enclosure. Within enclosure one, the predominant swim pattern was diagonal swimming, which decreased when scents are present ($t(94) = 2.676, p = .009$). Circle swimming, although rarely observed, did reduce when scents are present ($t(94) = 4.619, p = .000$). Enclosure two pattern swimming, which was predominantly circle swimming, also decreased significantly in the experimental sessions ($t(94) = 5.214, p = .000$). Diagonal swimming was not observed in enclosure two throughout the duration of this study, and was therefore not investigated at the individual level.

Pattern Swimming by Individual. In enclosure one, where diagonal swimming was the predominant swim pattern, both Gabby and Maya showed a reduction in circle swimming (Gabby ($t(44) = 4.048, p = .000$), Maya ($t(44) = 2.829, p = .007$)). However, only Gabby showed a significant reduction in diagonal swimming (Gabby ($t(44) = 2.657, p = .011$), Maya ($t(44) = 1.143, p = .260, d = .345$)). When scents were present, in enclosure two, both sea lions exhibited reduced circle swimming (Sage ($t(44) = 3.098, p = .003$); Kaytee ($t(44) = 3.875, p = .000$)). This was the only pattern swim to show a reduction at the enclosure level as diagonal swims were simply not observed in this enclosure.

Stereotypical Behaviors

Both Gabby and Sage exhibited unique stereotypies, as well as individualized reactions to scent. While Gabby exhibited an increase in pool wall sucking ($t(44) = -$

2.733, $p = .012$) in the presence of scent, Sage exhibited slight increase in floor sucking ($t(45) = -.719$, $p = .476$, $d = .212$), while reducing tail sucking ($t(45) = .130$, $p = .898$, $d = .038$), regurgitation ($t(45) = 1.026$, $p = .310$, $d = .302$), and reingestion ($t(45) = .864$, $p = .392$, $d = .254$); for operational definitions of these behaviors refer to Table 3).

Hypothesis II Results

We then evaluated hypothesis two in order to determine if natural scents were more effective enrichment options than non-natural scents, increasing habitat utilization and reducing stereotypical behaviors for these captive California sea lions.

Habitat Utilization

The analysis demonstrated that the sea lions' habitat usage did not significantly differ between natural scents and non-natural scents. In addition, independent sample t-tests showed no differences for the enclosures, or for the individual animals.

Pattern Swimming

In the natural scent sessions, sea lions exhibited a reduction in both circle (natural scent session $t(94) = 5.047$, $p = .000$; non-natural scent session $t(94) = 4.528$, $p = .000$) and diagonal swimming (natural scent sessions $t(94) = 1.986$, $p = .051$, $d = .41$; non-natural scent conditions, $t(94) = 1.251$, $p = .215$, $d = .26$). Within enclosure one, animals were found to alter their habitat usage patterns in the presence of a natural scent, although not significantly. Similarly, in enclosure two, a non-significant reduction in circle swimming was found in both Sage and Kaytee when natural scents were present in the enclosure. Although not statistically significant, it was noteworthy that diagonal swimming did decrease in both sessions and was very near significance in the natural

scent sessions. This lack of significance may simply be due to the fact that it was only observed in one enclosure.

Pattern swimming in animals within enclosure one were not found to differ significantly between natural and non-natural scent sessions [circle swimming, $t(46) = .499$, $p = .621$, $d = .147$) and diagonal swimming, $t(46) = -.089$, $p = .929$, $d = .026$]. In enclosure two, animals were not found to differ significantly between natural and non-natural scent sessions ($t(46) = -.500$, $p = .617$, $d = .147$). However, at the individual level slight changes in behavior are evident.

Although not significant, all Gabby, Sage and Kaytee demonstrated a reduction in circle swimming when natural scents were present [Gabby ($t(22) = .834$, $p = .419$, $d = .356$), Sage ($t(22) = -.276$, $p = .785$, $d = .117$) and Kaytee ($t(22) = -.134$, $p = .895$, $d = .057$)]. Maya, alternately, spent more time circle swimming in the natural sessions ($t(22) = -.502$, $p = .621$, $d = .214$). Similarly, Gabby and Maya both spent less time in diagonal swimming in the natural sessions, although these results were not significant [Gabby ($t(22) = -.398$, $p = .694$, $d = .169$), Maya ($t(22) = .327$, $p = .747$, $d = .139$)]. Neither Sage nor Kaytee engaged in diagonal pattern swimming during the course of the experimental session, therefore statistical analysis for this behavior cannot be provided.

Stereotypical Behaviors

Individual variation was evident when stereotypical behaviors were observed in both Gabby and Sage, who exhibited non-significant decreases in individualized stereotypies in the presence of natural scent enrichment. Specifically, Gabby's pool wall sucking behavior decreased, albeit not significantly, in the natural scent sessions when

compared with the non-natural scent sessions ($t(21) = -.334, p = .742, d = .142$).

Similarly, Sage's exhibited non-significant decreases in floor sucking behavior ($t(22) = -.593, p = .559, d = .252$), tail sucking ($t(22) = -.250, p = .806, d = .106$), regurgitation ($t(22) = -1.517, p = .147, d = .646$) and reingestion ($t(22) = .928, p = .373, d = .396$).

Alternative Explanations

Pre-Existing Behavioral Repertoires. To account for the possibility that the sea lions may simply use their habitat differently at different times of day, I examined their pre-existing patterns in the baseline sessions. The sea lions used their habitat differently between the baseline pre-session, baseline post-session, and experimental pre-sessions (Wilk's Lambda = .290, $F(5,106) = 51.812, p = .000$). Post-hoc tests showed that habitat usage was different between baseline pre-session and baseline post-session as well as baseline pre-session and experimental pre-session (Tukey's HSD, $p = .02$). Sea lions spent more time out of the water in post-sessions than in pre-session during the baseline period. The baseline post-session habitat usage was not significantly different from the experimental pre-session (Tukey's HSD, $p = .21$). Importantly, I found a significant difference between baseline post-session and experimental post-session, which suggests that the sea lions spent more time out of water (location 0) and less time in the water (locations 1-5) in the experimental post-session, when compared to the baseline post-session (Tukey's HSD, $p = .000$). Therefore, the experimental condition changed the sea lions' habitat usage above and beyond the typical daily shift.

CHAPTER IV

DISCUSSION

Overall scent was found to be enriching as it caused an increase in habitat utilization and a reduction both pattern swimming and the majority of stereotypical behaviors both across and within individuals. These findings suggest that scent enrichment is a viable method for enriching the lives of captive pinnipeds without the introduction of EED's or human interaction. This approach increases the versatility of enrichment allowing for its use in facilities, which aim to keep human-animal interactions at a minimum, such as in rehabilitation facilities. While the second hypothesis, that sea lions would react more strongly to natural scents than non-natural scents, was not supported, it was the case that sea lions responded to scents in their environment in a positive manner by exhibiting a reduction in stereotypical behavior (e.g., pattern swimming, oral stereotypies, and voluntary emesis). Still, although individual stereotypies such as regurgitation, were not found to be statistically significant, the large effect sizes found in this analysis indicate that there was indeed a practical reduction in these problematic behaviors.

Sea lions were observed to explore old scent markers immediately upon discovering new scent markers, indicating that they recalled where old scents were placed from previous days. This unique observation supports the idea that sea lions are responding cognitively, although perhaps not on the basis of their own evolutionary backgrounds, to scents through the formation of cognitive maps as well as possessing a long-term memory for individual scents and scent placement.

These findings support what many zookeepers had suggested regarding scent enrichment for captive California sea lions (Pulis, personal communication) and suggest additional methods for enhancing the captive environment for these species. In addition, these findings reaffirm assertions made by Lyn (2009), indicating the need for individual evaluations upon the introduction of novel stimuli. Most notably, increase in Gabby's pool wall sucking may indicate that she does not benefit from scent enrichment. However, because her slight, and statistically insignificant, increase in stereotypical behavior was accompanied with a significant increase in habitat utilization and reduction in pattern swimming, this increase likely lacks any practical significance.

Alternative Explanations for Pattern Swimming Behaviors

Franks et al. (2010), observed the effects of training-based feeds on stereotypies in captive walruses. While their findings indicated that stereotypic swimming patterns decreased after a feed, oral stereotypies were found to have significantly increased. However, it was likely that both of these behaviors have explanations relevant to the animals' natural behavioral repertoire. For example, while swimming decreased immediately after feeds, the walruses were documented to spend approximately 70% of their time swimming. This percentage was very similar to the activity budget of a wild walrus. Findings such as these demonstrate that further consideration to a species' natural environment and behaviors should be taken into account when introducing enrichment items as well as evaluating behavioral data.

Due to the fact that subjects were observed to engage in pattern swimming at a higher frequency during pre-sessions than post-sessions, including the baseline, natural,

and non-natural scent sessions, it was likely that this was an adaptation of the animals' natural behavioral repertoire and considered an alternative method of utilizing a small enclosure. The animals' swimming pattern and preference for locations in the enclosure that allow them to see out of the sea lion building, strongly suggests that this behavior was also associated with patrolling behaviors in addition to habitat utilization.

Limitations

In the wild, sea lions are presented with innumerable scent stimuli, which are not accounted for in this experiment. As a result, it is possible that natural scents could be more enriching than non-natural scents but unimportant scents were used during this study. Additionally, because it remains unknown which scents animals were exposed to in the past, it was nearly impossible to state that any of these scents were truly novel. It was possible that these scents would have been enriching if it were indeed the animals' first exposure to that scent.

Implications for Future Research

Future studies may include the use of olfactory enrichment in captive pinnipeds species such as the elephant and harbor seal, as these are species that also commonly strand along North American coastlines (Colegrove, Greig, & Gulland, 2005) and therefore, may be more likely to be placed in captivity after being deemed unfit (i.e. unable to survive independently in the wild) for re-release. Examining the scent capabilities of the true seals in addition to otariids (fur seals and sea lions) would also increase our understanding of the evolution of olfaction in aquatic caniform predators, demonstrating another avenue of inquiry for future research.

Despite the individual variation found in reducing stereotypic behavior and increasing habitat usage, it is likely that animals at other facilities could benefit from exposure to olfactory enrichment. In addition to zoos and aquaria, rehabilitation facilities may benefit from this research. Pinniped rehabilitation centers are often comprised of sterile environments that do not allow for naturalistic enclosures and extensive enrichment procedures. This may be due, in part, to the staff's attempts to reduce human-animal interactions in order to discourage habituation, facilitate disease control, and reduce cost. However, scent enrichment may still encourage species-typical behaviors (through increased habitat utilization) at little or no cost to the center and may be used to assist rehabilitators in keeping the animals cognitively engaged during their stay at the rehabilitation center. Environmental enrichment has been demonstrated to assist in rehabilitation of animals with brain lesions (De Bartolo et al., 2008; Paban, Chambon, Malafosse & Alescio-Lautier, 2004), lead poisoning (Guilarte, Toscano, McGlothan & Weaver, 2002), domoic acid toxicosis (Cook, Bernard, & Reichmuth, 2011), malnutrition (Rose, 1988) and simply to increase exploration (Fay & Miller, 2015). Although currently classified as a species of least concern by the IUCN Redlist, sea lion strandings are highly influenced by the effects of climate change (Greig, Gulland, & Kreuder, 2005; Hui, 2011; Keledjian & Mesnick, 2013). As these effects continue to escalate, sea lion rehabilitation programs will likely continue to be a necessity for the maintenance of a healthy California sea lion population. Thus, information relating to low-cost enrichment that may impact post-release survival rates will only assist rehabilitators in these endeavors.

Additional applications of this research would be to further examine the sea lions' scent capabilities at the molecular level using both an olfactometer and molecularly similar odorants (e.g., aliphatic odorants). Using this method, one could more reliably quantify the extent to which the sea lions utilize olfaction in nature.

APPENDIX A



THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
118 College Drive #5116 | Hattiesburg, MS 39406-0001
Phone: 601.266.4063 | Fax: 601.266.4377 | iacuc@usm.edu | www.usm.edu/iacuc

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed August 14, 2014, by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. **USM has received the signed Appendix K-Owner Informed Consent Form. The project is approved by the Committee.** The Committee understands that the Audubon Zoo Research Committee which must sanction your research, will not act on your request without University IACUC approval of your protocol application. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

| | |
|----------------------------|--|
| PROTOCOL NUMBER: | 14081403 |
| PROJECT TITLE: | *Olfactory Enrichment for Captive California Sea Lions (Zalophus californianus) |
| PROPOSED PROJECT DATES: | 9/2014-9/2016 |
| PROJECT TYPE: | New |
| PRINCIPAL INVESTIGATOR(S): | Dr. Heidi Lyn |
| DEPARTMENT: | Psychology |
| FUNDING AGENCY/SPONSOR: | |
| IACUC COMMITTEE ACTION: | Full Committee Approval |
| PROTOCOL EXPIRATION DATE: | September 30, 2016 |

Frank Moore, Ph.D.
IACUC Chair

10/01/2014
Date

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