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Behavioral Criteria for the Diagnosis of Domoic Acid Toxicosis in Zalophus californianus

Christiana Wittmaack
Nova Southeastern University, christiana.wittmaack@ttu.edu

NOVA SOUTHEASTERN UNIVERSITY OCEANOGRAPHIC CENTER

Behavioral Criteria for the Diagnosis of Domoic Acid Toxicosis in *Zalophus californianus*

By

Christiana Wittmaack

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Approved:
Thesis Committee

Major Professor :______________________________
*** *********, Ph.D.

Committee Member :___________________________
*** ******, Ph.D.

Committee Member :___________________________
**** ******, Ph.D.

Abstract

Introduction

California sea lion (*Zalophus californianus*) health is severely compromised by domoic acid toxicosis, which occurs in high levels during harmful algal blooms of *Pseudonitzschia australis* along the coast of California. Current diagnostic protocols are often inconclusive due to a 2-48 hour window of detectability within the urinary, circulatory, and gastric systems (Cook, *et al.* 2011 and Monte, Pers Comm, 2012). Past studies suggest that *Z. californianus*, with domoic acid toxicosis, commonly display abnormal behaviors (Goldstein, *et al.* 2008). However, many of these abnormal behaviors are also associated with other diagnoses and are therefore unreliable as diagnostic indicators. This study fills in a knowledge gap relating to abnormal behavior types and their correlation to domoic acid toxicosis and helps solve the problem of current, inconclusive, diagnostic protocols. In this study, my objectives were to identify abnormal behaviors correlated to domoic acid toxicosis, create a diagnostic ethogram, determine the applicability of the method in the field, and determine the applicability of triage based on the relationship between abnormal behaviors and domoic acid levels.

Methods

I conducted focal animal continuous scans (continuous observation of a single animal at a time, for a set period) with continuous data entry, on animals admitted to the Marine Mammal Center (main study location during 2011-2013) and the Marine Mammal Care Center (comparison location, 2013). I conducted my observations from behind a blind to prevent both human habituation and behavioral influence of the observer. Observations lasted between 10-15 minutes (10 minutes per pen in 2011, 15 minutes per animal in 2012-2013). Subjects were selected based on an admit date no later than 7 days from the observation date.

I conducted focal animal continuous scans at Pier 39, a haul out location, in the San Francisco Bay. Animals included in the study had identifying marks or were isolated from other animals (making them easy to identify). I observed animals once per observation day with a total observation period not exceeding 15 minutes per animal.

I logged domoic acid levels in feces, urine, and serum (collected by veterinary staff and analyzed with liquid chromatography and bioassays for the presence of domoic acid). I then compared these results to the types and severity of abnormal behaviors displayed by the domoic acid toxicosis sample.

Results

Results from data collected at the Marine Mammal Center suggest that head weaving (Wilcoxon, $p<0.0001$), and muscle fasciculations (Wilcoxon, $p<0.01$), along with swift scanning, and dragging the hind flippers are suitable for use as domoic acid toxicosis diagnostic indicators. Of these four behaviors, dragging the hind flippers and swift scanning were sensitive to environmental conditions (e.g. noise levels and space limitations). Head weaving and muscle fasciculations occurred at both the Marine Mammal Center and the Marine Mammal Care Center. Additionally, I found that the inclusion of observations conducted by rescue crew - as a part of routine protocols -raises the precision of the diagnostic criteria. Within my sample, 88% of animals with domoic acid toxicosis displayed abnormal behaviors from the behavioral diagnostic criteria.

Results from the Pier 39 study suggest that behavioral criteria may be applicable for ruling out domoic acid toxicosis in groups of animals. However, I did not test the method during times of harmful algal blooms. Therefore, the applicability of the method for use as a diagnostic tool in the field is unknown and further research is required.

Results for the triage study were inconclusive. The number of animals that tested positive for domoic acid was small and not suitable for statistical analysis. I suggest further research into triage abilities.

Conclusion

Based on the results of these studies, I can conclude that behavioral analysis offers a reliable diagnostic tool for rescued *Z. californianus*. Practitioners can use behavioral diagnostic criteria with confidence for the diagnosis of domoic acid toxicosis in *Z. californianus*.

Dedication

I whole-heartedly dedicate this thesis to my mother, Jena Wittmaack. She has been the cornerstone of my academic career. Without her understanding, encouragement, and guidance, this thesis would not have been possible.

Additionally, I dedicate this thesis to my father, Steven Wittmaack (1948-2012). His goal for me was to gain a solid education. This thesis, in his eyes, would have been a conformation of his final dream.

Finally, I also dedicate this thesis to Dr. Edward Keith (1951-2012). Originally, the brainchild of Dr. Keith, this thesis has grown beyond the scope of an M.S. degree and into, quite possibly, a lifelong research theme. Dr. Keith began my career as a marine biologist and I am forever grateful.

Acknowledgments

I thank, first, Dr. Edward Keith for providing me with the encouragement needed to begin a large project and for sharing his expertise on pinniped physiology. I am indebted to Dr. Caryn Self-Sullivan for graciously picking me up after the untimely passing of Dr. Keith. I especially thank Dr. Self-Sullivan for her eye for detail as her fine toothed comb editing ability taught me more than she realizes.

I thank my committee members at both the Oceanographic Center and elsewhere. First, I thank Dr. Curtis Burney, who took the project on out of keen interest. Dr. Burney has provided solid feedback that, if lacking, would have left holes in the project. I thank him for his outstanding teaching ability in both the Marine Ecosystems and Marine Chemistry classes. I thank Dr. Garet Lahvis for eagerly taking on the project from afar. Dr. Lahvis' attention to minute detail has strengthened the project and helped it grow. I would like to take this opportunity to point out that, at the time of this writing, I have not met any of my committee members in person. I must applaud them for their dedication and perseverance throughout this thesis project that, as I understand it, is the second completed at the University via distance.

I thank my Mother, Jena Wittmaack for her constant patience during my research and her monetary help when times were tight. Without her help and encouragement, this project would never have grown. I thank my Father, Steven Wittmaack for providing me the funds, although in a tragic way, to continue. My education is indebted to both my parents. Furthermore, I thank the rest of my family, Ruth McDonald, Lex McDonald, and friends for their support and genuine interest in my work.

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Introduction

People have documented the presence of harmful, toxin producing algal blooms in the coastal marine environment for over two centuries. Known sources of these algal blooms are diatoms, dinoflagellates, and cyanobacteria (Glibert, *et al.* 2001). Some of the toxins produced by algae include domoic acid, okadaic acid, dinophysistoxins, brevetoxins, saxitoxins, and gambiertoxins.

In humans and animals, exposure to algal toxins is through ingestion of the algae, (via shellfish or finfish) trophic transfer, contact with contaminated water, or the inhalation of toxic aerosols. In humans, toxic levels of domoic acid (a neurotoxin) are 1-5mg/kg (Van Dolah, 2000). Symptoms of toxicity may include problems with the gastrointestinal tract (e.g. vomiting, diarrhea, rectal burning, and cramping), bradycardia, dilation of pupils, rash, hypotension, and neurological problems (e.g. headache, inability to speak, and short term memory loss) (Backer and Mcgilicuddy, *et al.* 2006).

Harmful algal blooms have been associated with fish die offs (Glibert, *et al.* 2001), marine mammal and sea bird stranding, human illness (Gulland, 2000), and economic decline (Hoaglan, *et al.* 2002). Large blooms of phytoplankton eventually die and decay, ultimately resulting in dead zones. Off the coast of California, domoic acid has become increasingly associated with harmful algal blooms and has been associated with marine mammal and sea bird die offs (Gulland, 2000).

The current protocols (blood, urine, serum, and feces) for the diagnosis of domoic acid toxicosis in *Z. californianus* are often inconclusive (Grieg, Pers Comm. 2011). Prior to this study, the use of standardized behavioral criteria for the diagnosis of domoic acid toxicosis was unknown. Although practitioners rarely use behavioral diagnostics, consideration is necessary to further both animal care and research.

Domoic Acid and Marine Mammals

Domoic acid production is associated with macro algae and pinnate diatoms of the genus *Chondria* (Takemoto and Daigo, 1958), *Amphora* (Lelong, *et al.* 2011), and *Nitzschia* (Kotaki, *et al.* 2000) along with the centric diatom *Pseudonitzschia*. Of the *Pseudonitzschia* genus, 14 species have been identified that are capable of producing domoic acid (Lelong, *et al.* 2011), making the genus the top producer worldwide. Blooms of domoic acid producing diatoms are increasing along the coast of California (Sun, *et al.* 2011). Anthropogenic stressors may be a contributing factor; however, the exact cause has not been determined.

Chemically, domoic acid is a water soluble, marine neurotoxic metabolite attracted to α-amino-5-hydroxy-3-methyl-4 isoxazole propionic acid and the neuronal glutamate ionotropic receptors in the kainate subclass (neurons containing immunoreactivity to kainite). Domoic acid that has bonded to glutamate receptors behave like an excitotoxin resulting in cell depolarization and possible cell death (Jeffery, *et al.* 2004). Long-term neurological impacts, including epilepsy and hippocampal sclerosis, can persist after exposure (Goldstein, *et al. 2008*).

Domoic acid accumulates in the soft tissues of primary consumers such as northern anchovies (*Engraulis mordax*), blue mussels (*Mytilus edulis*), and razor clams (*Siliqua patula*). The viscera of anchovies accumulate higher levels than other bodily tissues (Levebere, *et al.* 1999). Domoic acid biomagnifies at higher trophic levels within the food web. Secondary and tertiary consumers such as: finfish, some species of shellfish (Lelong, *et al.* 2011), cephalopods (Costa, *et al.* 2003), marine mammals, birds, and humans, are exposed to toxic concentrations via the ingestion of contaminated mollusks and finfish (e.g. anchovies) (Gulland, 2000). Novelli, *et al.* (1992) suggests that domoic acid is more toxic when ingested via shellfish than via phytoplankton alone. Therefore, the human population and populations of other animals that consume shellfish (e.g. marine mammals) are at an increased risk of poisoning from domoic acid.

Z. californianus poisoned with domoic acid show neuronal necrosis of the hippocampus along with necrosis of granule cells, the dentate gyres, and pyramidal cells.

Silvagni *et al.* (2005) identified lesions in the CA4, CA3, and CA1 zones of the cornu ammonis. Gliosis was also prevalent in these zones (Costa, *et al.* 2010). Clinical signs and symptoms include head weaving, ataxia, tetanic convulsions, muscular tremors, lethargy, and rubbing behavior (Gulland, 2000).

A range of abnormal behavior (e.g. scratching, tremors, seizing, head weaving) (Gulland, 2000) displayed allows for the possibility of behavioral diagnostic criteria for domoic acid toxicosis. To date, research does not exist regarding unique abnormal behaviors associated with domoic acid toxicosis versus other diagnoses seen in *Z. californianus* along the coast of California. This research fills in that knowledge gap.

<u>Objectives</u>

The primary objective of this study was to identify behaviors that will expedite the diagnosis of domoic acid toxicosis in *Z. californianus* in rehabilitative settings. The breakdown of that objective is as follows:

1. Identify abnormal behaviors correlated with domoic acid toxicosis in *Z. californianus*
2. Create a diagnostic ethogram of correlated abnormal behaviors that is applicable to multiple rehabilitative environments
3. Use behavioral diagnostic criteria to identify individual *Z. californianus* with domoic acid toxicosis and/or rule out unaffected individuals at haul out locations
4. Determine correlations between levels of domoic acid found in urine, blood, serum, or feces to types and severity of abnormal behaviors observed for triage purposes

I hypothesize that, *Z. californianus* with domoic acid toxicosis display unique abnormal behavior specific to the diagnosis. These abnormal behaviors are consistent throughout all rehabilitation facilities and are not affected by environmental conditions. Within the field, behavioral diagnostic criteria can help identify individual *Z. californianus* with domoic acid toxicosis. Finally, the type and severity of behavior is dependent on levels of domoic acid exposure in *Z. californianus*.

Justification: Problems Associated with Current Diagnostic Methods

Within rehabilitation settings, the diagnosis of domoic acid toxicosis has relied on the detection of domoic acid via liquid chromatography and bioassays for urine, serum, feces, milk, amniotic fluid, and blood (Goldstein *et al.* 2008; Maucher and Ramsdell, 2005; and Brodie *et al.* 2006). Additionally, diagnosis is possible with the detection of an atrophied hippocampus during necropsy or during a magnetic resonance imaging (MRI) scan of the brain (Gulland, 2000). (MRI scans are a reliable method of diagnostics; however, the sedation and transport involved pose hazards to the animal. Furthermore, the high cost of MRI technology makes the method impractical on a large scale.)

Recent research suggests that sub lethal levels of domoic acid are present in the water column in Monterrey Bay, California year around. Chronic exposure may contribute to the later development of abnormal neurological conditions (Bargu, *et al.* 2013). For example, blood and urine may test negative for domoic acid in an animal that presents with an atrophied hippocampus during necropsy, suggesting chronic or prior exposure.

The clearance rate (amount of time the toxin is detectible) of domoic acid from the urinary tract is between 2-48 hours post ingestion (Cook, *et al.* 2011 and Monte, Pers Comm, 2012). The clearance rate in the bloodstream is around 48 hours post ingestion (Truelove and Iverson, 1994). Feces is testable but is still restricted by the clearance rate. Serum profiling (detection of circulating antibodies) has shown some promise for diagnostics (Neeley, *et al.* 2012).

Because of the rapid clearance rate, domoic acid is rarely detected in the blood and urine of rescued animals (Goldstein, *et al*, 2008). If an animal tests negative but presents with seizing or abnormal behavior and veterinarians suspect domoic acid toxicosis, they may turn to the process of elimination of other conditions before diagnosing the animal with domoic acid toxicosis (Van Bonn, Pers Comm, 2012). Substantiation of the diagnosis in these cases is possible by the presence of domoic acid in local anchovies and the occurrence of blooms or other marine mammal strandings relating to domoic acid (Gulland, 2000).

Z. californianus suffering from domoic acid toxicosis display abnormal behavior (Goldstein, *et al.* 2008). Veterinarians sometimes use this abnormal behavior (e.g. head weaving, seizing activity) as an indicator of domoic acid toxicosis; however, the published symptomatology overlaps other common diagnoses. The similarity of domoic acid toxicosis to other diagnoses makes definitive diagnosis in the absence of positive laboratory results or MRI results challenging. For example, seizures are associated with domoic acid toxicosis (Gulland, 2000); seizing activity is also associated with epilepsy and blunt head trauma. Other diseases that present with abnormal neurological and behavioral signs include septicemia and hypoglycemia (Grieg, Pers Comm, 2011).

As a case in point, in October of 2011, a yearling *Z. californianus* - with gas bubble disease - stranded in Moro Bay California. The Marine Mammal Center in Sausalito California admitted the animal for rehabilitation (Van Bonn, *et al.* 2013). The animal displayed seizures and disorientation. Veterinarians suspected domoic acid toxicosis until further tests were completed (Personal Observation, 2011).

An antidote to domoic acid toxicosis does not exist. The current treatment protocol for animals suspected of domoic acid toxicosis consists of lactated ringers or 0.9%NaCl fluids administered subcutaneously to facilitate with rehydration as animals generally have not consumed prey for a prolonged period. Intramuscular injectable diazepam or intravenous injectable lorazepam controls seizures symptomatically. Intramuscular injection or oral phenobarbitone controls continuous seizing behavior. If seizing is not controllable or persists despite treatment, euthanasia is considered. If seizing activity has ceased, intramuscular dexamethasone can reduce cerebral edema (Gulland, 2000).

In this thesis, I investigate and present a novel set of behavioral criteria suitable for use for the diagnosis of domoic acid toxicosis in *Z. californianus*. Chapter 1 is an introduction to *Z. californianus* and the history of domoic acid. Chapter 1 also includes basic biology information relating to *Z. californianus* and diatoms capable of producing domoic acid to familiarize readers who are outside the field. Chapter 2 details the research at the Marine Mammal Center and presents the bulk of the diagnostic criteria. Chapter 3 discusses the impact of differing environmental conditions on behavioral criteria and provides advice to rehabilitative centers. Chapter 4 investigates the use of behavioral criteria in the field and is of interest to both veterinarians and marine mammal researchers. Chapter 5 discusses the applicability of triage potential and provides guidance for further studies. Chapter 6 concludes the study with a detailed description of the proposed diagnostic method, the guidelines necessary for correct usage, and future research recommendations. Finally, appendix V provides working datasheets, sample databases, and sample training overviews for use.

California Sea Lion Population and Biology

Zalophus californianus (Lesson, 1828) is an Otariid pinniped - an eared seal of the order Carnivora, with a dark brown to blond pelt and the ability to rotate its hips under the body, allowing for increased terrestrial maneuverability. Additionally, movements of the head and neck provide extra thrust for walking (English, 1976). Animals attain swimming propulsion through waving movements of the front flippers (Feldkamp, 1987). *Z. californianus* can reach swimming speeds of up to 10.8 km/h (Lowry and Carretta, 1999) and dive to depths of 274 m where submersion can last up to 9.9 minutes (Feldkamp, 1987). Similar to other otariids, *Z. californianus* are sexually dimorphic. Adult males (up to 2.4 m and a weight of 350 kg) are significantly larger than females (up to 1.8 m and a weight of 100 kg) (Heath and Perrin, 2008). During pubescence, males develop a large, sagittal crest on the upper frontal area of the skull, which, females lack (Lavigne and Harwood, 2001).

Z. californianus prey on fish and squid found within the water column or near the seabed at ranges of 100 (Lowry and Carretta, 1999) and 450 km from shore for females and males respectively (Weise, *et al.* 2006). Common predators include the killer whale (*Orcinus orca*) (Baird and Stacey, 1989) and the great white shark (*Carcharodon carcharias*) (Long, *et al.* 1995).

California sea lions (*Z. californianus*) have a wide distribution from the southern coast of Alaska to the west coast of Mexico. Five breeding stocks are currently recognized: U.S., Western Baja California, Southern Gulf of California, Central Gulf of California, and Northern Gulf of California (Schramm, *et al.* 2009). *Z. californianus* population levels are abundant and increasing. Currently, the IUCN listing is at the *level of least concern*. The entire population is around 355,000 animals. In the United States the species is protected under the regulations of the Marine Mammal Protection act of 1972 (Aurioles and Trillmich, 2009).

Domoic Acid Production and History

Domoic acid is a water-soluble phytotoxin with neural excitotoxin characteristics. Specifically, domoic acid is a heterocyclic amino acid and a kainic acid analog. Domoic acid has the following properties:

- Chemical formula: $C_{15}H_{21}NO_6$
- Molar mass: 311.33 g/mol
- Density: 1.27 g/cm^3

Domoic acid was discovered in 1958 when it was isolated from the red, macroalgae species *Chondria armator* found in Japanese waters (Addison and Stewart, 1989). At that time, the toxicity of domoic acid to mammals was unknown. Low doses were used as a medication to rid the human body of intestinal worms (Lelong, *et al.* 2011).

In 1987, a bloom of *Pseudonitzschia multiseries* was responsible for the contamination of the blue mussel (*Mytilus edulis*) along the Canadian west coast. Three deaths and 100 documented illnesses resulted from the consumption of the mussels post bloom (Bates, *et al.* 1989). Currently, at least 18 known species of algae are associated with the production of domoic acid (Lelong, *et al.* 2011). The most commonly studied species are within the genus *Pseudonitzschia*.

Table 1: Species of Pseudonitzschia that produce domoic acid.

Known species within the genus *Pseudonitzschia* capable of producing domoic acid			
*P. australis**	*P. cuspidate*	*P. galaxiae*	*P. multistriata*
P. brasiliana	*P. delicatissima*	*P. granii*	*P. pseudodelicatissima*
P. calliantha	*P. fraudulenta*	*P. multiseries**	*P. pungens*
		P. turgidula	*P. seriata*

* Predominate species responsible for the production of domoic acid along the coast of California

This study focuses exclusively on the species *P. australis* (*Bacillariales, Bacillariophyceae*), a pinnate, chain-forming diatom with a worldwide, coastal, distribution, especially along the west coasts of continents (see table two) (Lelong, *et al.* 2011).

Table 2: Known geographical locations of P australis.

Geographical Distribution of *P. australis*			
All Coasts		North Coast	West Coast
Peru	Spain	Russia	Canada
Chile	United Kingdome		North America
Argentina	New Zealand		Mexico
Uruguay	Tasmania		Australia
Brazil	Namibia		
Portugal			

Blooms of *P. australis* occur worldwide, making the species cosmopolitan (although it is absent along the east coast of North America). Domoic acid producing blooms of *P. australis* are more common along the west coast of continents due to the increased rates of upwelling (Lelong, *et al.* 2011) coupled with high levels of nutrients from river runoff (Schnetzer, *et al.* 2007).

This study focuses exclusively on the effects of blooms occurring along the coast of California. Within the Monterrey Bay area, nine species of *Pseudonitzschia* have been identified, however only two, *P. multiseries* and *P. australis*, are known to produce domoic acid. Off the coast of Southern California (Monterrey County to the boarder of Mexico), domoic acid producing blooms of *Pseudonitzschia* are more common during the late spring (Lelong, *et al.* 2011). Since 2000 - excluding 2004/2006 - *P. australis* has been the dominate diatom along the coast of California and the top producer of domoic acid (Jester, *et al.* 2009).

Within the water column, high levels of domoic acid occur between 10-20 meters depth in Monterey Bay, California (Ryan, *et al.* 2005). The toxin remains intact down to a depth of 800 meters off the coast of Southern California due to the sinking of dead diatom frustules (Lelong, *et al.* 2011). Trainer (2000) extracted domoic acid from cells found in sediment traps along the coast of California. Therefore, domoic acid is not restricted to a limited depth range, which allows it to have an impact on both neritic and benthic species.

Irradiance, specifically, UV-A (Lelong, *et al.* 2011) in concentrations of 115 µmoll photons m^{-2} s^{-1} has been shown to increase the production of domoic acid 24-130 times the normal range in *P. australis* compared to normal concentrations of 12 µmoll photons m^{-2} s^{-1} (Cusack, *et al.* 2002). The addition of nitrate also increased production of domoic acid. Ammonium had a similar effect with the production of domoic acid increasing to three times that produced in control samples (Howard, *et al.* 2007). Interestingly, when silica (a component of the frustule) is limiting, the production of domoic acid increases, however the exact cause is unknown (Lelong, *et al.* 2011).

Domoic acid accumulates only in animal flesh. The toxin does not accumulate in the water column due to dilution within the oceanic basins and sinking of cells to the benthos. (Lelong, *et al.* 2011). In shellfish and mollusks, domoic acid accumulates in the digestive glands and other bodily tissues (Lelong, *et al.* 2011). Copepods do not seem susceptible to the toxic effects of domoic acid. *Acartia clausi* is able to detoxify 63.6% of accumulated domoic acid every 24 hours. Like benthic species, copepods accumulate domoic acid in their tissues (Maneiro, *et al.* 2005). Although rarely considered a vector of exposure, octopi of the species *Octopus vulgaris* accumulate domoic acid in tissues, especially in the tissues of the digestive track and bronchial hearts (Costa, *et al*. 2004). Other common vectors include multiple species of finfish (Lelong, *et al.* 2011).

Between 1989 and 1991, necropsies of brown pelicans (*Pelecanus occidentalis*) along with Brandt's cormorants (*Phalacrocorax penicillatus*) stranded in Monterey Bay California, revealed that the birds had ingested anchovies contaminated with domoic acid (Work, *et al.* 1993). A similar event occurred in 1996 with seabirds, along the coasts of Mexico (Sierra-Beltrán, *et al.* 1997).

Fire *et al.* (2010) detected domoic acid in the feces, urine, and gastric fluid of a newly weaned minke whale (*Balaenoptera acustorostrata*) that stranded along the Southern California coast. Between the years 1997-2008, 24 pygmy and dwarf sperm whales (*Kogia spp*.) that stranded along the east coast of the United States, tested positive for domoic acid through fecal and urine samples (Fire, *et al.* 2009). Necropsy samples from North Atlantic right whales (*Eubalaena glacialis*) have also tested positive for domoic acid (Leandro, *et al.* 2010).

In 1998, over 400 *Z. californianus* displaying abnormal behavior (Lelong, *et al.* 2011) stranded along the California coastline. Veterinarians determined that domoic acid toxicosis caused the unusual mortality event (UME). Interestingly, similar reports from the years, 1978, 1986, 1988, and 1992 suggest that domoic acid related strandings of *Z. californianus* may have occurred previously (Scholin, *et al.* 2000), but remained unexplainable at the time. Since 1998, with the exception of 1999, domoic acid related strandings of Z. *californianus* have occurred annually (Bejarano, *et al.* 2008). On average, domoic acid toxicosis is responsible for 9% of *Z. californianus* strandings along the coast of California (Grieg, *et al.* 2005).

The Marine Mammal Center

The Marine Mammal Center is a wildlife rehabilitation hospital that began operation in 1975. The National Marine Fisheries Service has permitted the center to rescue, house, treat, release, place, and euthanize stranded pinnipeds and cetaceans (The Marine Mammal Center, 2013). The center also rescues sea turtles and sea otters but often transfers these animals to facilities that can provide specialized care (Personal Observation, 2012). The main hospital, located in Sausalito California, can house 200 pinnipeds at any given time. Smaller satellite facilities are located in Fort Bragg, Monterey, and San Luis Obispo Counties (The Marine Mammal Center, 2013).

The rescue range of the Marine Mammal Center spans 600 miles of California coastline from Mendocino County to the north to San Louis Obispo County to the south (see appendix IV) (The Marine Mammal Center, 2013). Additionally, the Santa Barbara Marine Mammal Center may send animals within their rescue range (county of Santa Barbara) to the Marine Mammal Center, (Frankfurter, Pers. Comm. 2013).

Table 3: Counties covered by the Marine Mammal Center. Bold counties represent acceptance of animals from another rescue organization.

Rescue Range Counties			
Mendocino	Sonoma	Marin	Solano
Yolo	Sacramento	San Joaquin	Contra Costa
Alameda	San Mateo	Santa Clara	Santa Cruz
Monterrey	San Luis Obispo	**Santa Barbara**	

The Marine Mammal Center has 28 animal pens with 25 of those pens containing above ground or in ground saltwater pools. Sea lions can occupy 24 of those pens with 21 having in ground or aboveground pools. The three additional pens are dry, with two used only for veterinary procedures or animal recovery from surgical procedures. A letter (A-H) and a number (1-3) identifies each pen with the exception of the largest enclosure that is termed the USDA pool. A public viewing deck and walkway allows visitors to view animals in the front pens only. Visitors have designated areas and cannot enter animal care facilities (Personal observation, 2011).

My study area included 17 pens, 16 having in ground or aboveground pools and one pen being dry (see appendix IV). Pen numbers in this study included the following: A 1-3, B 1-3, C 1-3, D 1-2, E 1-3, and F 1-3. Animals temporarily housed in corridors between pens were also included in the study.

Animals were provided with shelter (in the form of dog carriers), water bowls, and heating pads as deemed appropriate by veterinary staff (shade was also provided by solar panels over all pens). Veterinarians, student interns, and staff were responsible for the medical care of the animals, whereas volunteers were responsible for husbandry care including the administration of medication and fluids. Feeding times occurred thrice daily at 08:00, 14:00, and 20:00, although extremely young or emaciated animals might receive two additional feedings at 16:00 and 22:00. Pen and pool cleaning occurred once per day, in the morning or early afternoon (depending on admit load and volunteer availability). Volunteer and veterinary staff had limited contact with animals. Once the animal had received initial care, physical contact was ceased unless medically necessary (Personal observation, 2011).

Methods

I conducted focal animal scans (Altmann, 1974) using continuous methodology (single animal observations with continuous data entry) (hereinafter referred to as focal animal continuous scans) on *Z. californianus* in pens A-F. Animals were observed only once per admit to the Sausalito site. Criteria for the study included admittance to the Sausalito location no more than 7 days prior to the observation date. Either a trained volunteer or vet staff provided me with a written or verbal list of available animals. The observation list contained the name of the animal, date of admit, and the location of the animal's pen. I further identified animals via roto tags (small plastic tag, used for identification purposes, pierced into one of the front flippers) or grease pen markings on the head and back. Diagnostic data including generalized medical information such as age, weight, and gender, remained sealed throughout the observation to ensure single blinded survey methods.

Veterinarians determined the diagnosis of each animal within the sample through epidemiology, microbiology, toxicology, radiology, and a basic workup of weight and length. Vet staff did not share diagnostic information with me, nor did I share observational data with them until my observation was complete and vet staff had assigned the animal a diagnosis. This insured that behavioral data did not influence veterinary staff during the diagnostic process. Furthermore, it upheld the blind survey requirements.

I conducted focal animal continuous scans (methodology approved by IACUC: control #147-398-13-0605) on a weekly basis between May 2011 and September 2013, weather and animal abundance permitting. Observations took place between 14:00-17:00, during periods of time that volunteer crews were absent from animal enclosures. I stood outside the pen, from behind a canvas blind at all times. The blind measured 185.42 cm x 77.47 cm, with two 30.48 cm x 22.86 cm wooden bases. A single hole measuring 22 mm in diameter permitted viewing. The blind consisted of .5-inch PVC pipe, canvas, and PVC and aluminum couplings. The blind reduced the likelihood of human habituation and lessened the influence of the presence of the observer on the animal’s behavior. If an animal in the pen approached the blind, stared at it, paced, began climbing the fence to reach the blind, or lunged at the blind for at least 20 seconds, the observation was terminated and either attempted at a later time during the day or abandoned.

Data recorded during the observation included the start and stop time of the observation, the number on the animal’s roto tag (if veterinarians had attached the tag), abnormal behaviors, and, beginning in 2012, normal behavioral states.

Criteria for abnormal behavior was any behavior that was indicative of distress or not seen in healthy wild populations (see table 4). For example, head weaving is an abnormal behavior because it is indicative of neurological stress and animals in healthy populations typically do not display head weaving. Because isolated abnormal events do occur that are unrelated to diagnosis, an animal had to repeat an abnormal behavior three times (within the observational period) before documentation began. For example, an animal might twitch to remove flies, which would be a normal behavior.

Table 4: Behaviors typically not observed in healthy populations

Abnormal Behaviors	
Open mouthed breathing	Drinking seawater
Waving flippers	Mouth chattering
Nursing off inanimate object	Erect vibrissa
Rump weaving	Seizures
Doughnut	Circling
Floating with head dunked	Constant, darting, swimming in confined space
Flapping flippers	Uncoordinated movements
Head weaving	Muscle fasciculations
Grimacing	Excessive scratching
Craning	Head shaking
Swift scanning	Twitching
Dragging hind flippers	

I used a Sport Line 240 stopwatch (EB Sport Group.; accuracy 1/100 of a second) to record all time increments in seconds. The entire observational period per animal did not exceed 15 minutes (In 2011, observations did not exceed 10 minutes per enclosure via agreement with Deb Wickham, Senior Monk Seal Health Coordinator who also oversaw sea lion care during that year. If multiple animals inhabited a single pen, I divided 10 by the number of animals in the pen and observed each animal for a total period based on the quotient. For example, if two animals on the observation list were in the same pen, I observed each for five minutes). I tallied and totaled the time increments for each abnormal behavior displayed at the end of the observational period. I also gave each abnormal behavior a score of severity that ranged from 1-3. The severity score was an indicator of consistency instead of duration. For example, a score of one indicated that the behavior occurred during a 0.1-3.32 minute period. A score of two indicated that the behavior occurred during a 3.3-6.2-minute period. A score of three indicated that the behavior occurred ≥6.3 minutes. Because the severity score was set to the original 10-minute timeframe, animals observed for 15 minutes received scores based on the first 10 minutes of the observation. This protected against methodological and statistical bias.

Upon the close of the observation day, I logged data from the subject's files. Data logged included the given name of the animal, the species, and the tag number and tag type (if different from the orange roto tag). Differing agencies and rescue organizations use differing types of tags (generally roto). I recorded the gender and age of each animal as determined by veterinary staff. Animal length was measured (cm) and recorded by vet staff during the admit examination. Animal weight was measured (kg) and recorded by volunteer crews on a weekly basis or as requested by veterinary staff. I copied medications prescribed by veterinary staff and included the dose, frequency of administration, and the method of administration. I separated and logged rescue locations according to the acronyms used by the Marine Mammal Center that included Sausalito (SAUS), San Luis Obispo (SLO), Fort Bragg (FBO), Monterey (MBO), and Santa Barbara (SBMMC).

Abnormal Behaviors

For this study, I defined abnormal behaviors as an act or bodily movement not seen in wild, healthy populations or a behavior that compromised health (e.g. the consumption of seawater in preference for fresh).

Table 5: Definitions of abnormal behaviors

Abnormal Behavior	Definition
Open Mouthed Breathing	Animal leaves mouth open and does not inhale or exhale via the nares or opens mouth with every breath. Labored breathing may be associated.
Nursing	Animal attempts to nurse off non-lactating pen mates or inanimate objects such as walls. Sucking sounds may be audible.
Waving Flippers	Animal holds flippers upright, waving them in a back and forth motion. May also include hind flippers.

Rump Weaving	Animal sways the rump from side to side. Sways are normally sporadic.
Drinking Sea Water	Animal ingests saltwater from the tank. (Healthy sea lions obtain fluids via the ingestion of fish. Freshwater may be consumed but is not a substantial hydration source).
Flapping Flippers	Animal spastically flaps the hind and or front flippers together.
Head Weaving	Animal sways head from side to side; front to back, or in a circular motion, often touching the torso with the back or side of the head. Neck may be loose or ridged. Sways may be prolonged or quick. Movements may be bobbing, jerking, or smooth. Head weaving can occur while animal is in any posture while on land.
Grimacing	Animal’s lips curl over both the incisors and canine teeth repeatedly (may occur on only one side or both sides of the mouth).
Craning	Head and neck repeatedly move straight out in a rigid fashion. Animal is normally non-mobile while craning.
Uncoordinated Movements	During locomotion, the front and/or back flippers move independently. Animal stumbles or has difficulty walking. Left to right coordination is often impaired.

Scratching	Animal continually scratches any area of the body with flippers, head, muzzle, or teeth. Animal may also scratch body against objects or pen mates. Scratching is excessive.
Muscle Fasciculations	Visible muscular ripples or large tremors occur along the entire torso or half of the torso. The head and neck may also be involved, which can involve the facial regions. In the instance of the head and neck, the movement must be smaller than head weaving and not involve side to side swaying.
Seizures	Animal has a grand maul seizure involving a suspected loss of consciousness and the contraction of muscles in the entire body or most of the body.
Head Shaking	Small continuous movements, generally from side to side, taking on a vibrating appearance. Flippers and eyes may also vibrate during bouts of head shaking.
Doughnut	Animal repeatedly and spastically arches the back flippers up and over the back while simultaneously arching the head back. The back flippers and the rostrum or back of the head often meet. Animal may also assume an S position between bouts of the doughnut.
Circling	Animal walks or swims in very tight circles, generally in only a single direction.
Swift Scanning	Animal turns head in all directions (left, front, right, back) in smooth, swift, single motion. Eyes are open. Intervals between scans are $\leq$ 90 seconds. Each scan lasts $\leq$5 seconds.

	(Surroundings must be void of excessive visual and auditory stimuli as scanning is a normal behavior observed in animals with increased levels of stimuli).
Twitching	Small, jerking movements of the limbs, eyes, vibrissa, pinna, tail, and muscles surrounding the stomach. Movements are too small and ridged for muscle fasciculations but are clearly visible.
Floating	Animal floats with head below water and back arched out of the water. Movement of the flippers is minimal and there is not a visible effort to swim. The animal tucks its rump below the surface, resulting in a U shape. Current (if present) pushes animal. Bubbles may be blown and seen at the surface.
Constant Swimming	Animal rapidly darts around pool without ceasing. Breaths are taken while animal is on the move (not observed in healthy animals held in small pools).
Dragging Hind Flippers	Animal uses only the front flippers for locomotion. Instead of tucking the back flippers under the body and using them to walk, the animal drags itself along with the front flippers, allowing both back flippers to point outward, and drag against the ground. (Often seen as a performance behavior by trained animals but never observed in healthy, wild populations).

Beginning in 2012, I documented not only all abnormal behaviors displayed but also normal behaviors.

Table 6: Definitions of normal behaviors.

Normal Behavior	Description
Resting	Animal lies on pen floor, corridor floor, pool edge, ramp, within or on top of animal crate, or on heating pad.
Sleeping	Animal remains motionless with eyes closed.
Thermoregulation	Animal turns on side and raises one front flipper in air.
Vocalizing	Animal emits roaring, barking, or snoring sounds.
Sitting	Animal is upright and often alert.
Restless	Animal continually shifts position.
Drinking Fresh Water	Animal drinks from water dish.
Flicking Flies Away	Animal jerks head, neck, flippers, or back to rid itself of flies.
Climbing	Animal climbs wall to reach ledge or climbs crate to reach top.
Aggression	Animal mouths or bites pen mate, often vocalizing, and animal may chase pen mate out of tank.
Walking	Animal maneuvers on land using all four flippers.
Physical Contact	Animal makes bodily contact with pen mate by either bumping, rolling against, or rolling over other animal.

Defecating	Animal defecates on pen floor or in tank.
Urinating	Animal urinates on pen floor or from elevated surface.
Alert	Animal observes activity in or around enclosure.
Socializing	Animal swims with or hauls out with pen mate.
Swimming	Animal swims in tank or sits in wading pool.

I assigned behavioral subtypes to head weaving and muscle fasciculations.

Table 7A: Classification system developed for abnormal behavioral subtypes.

Head Weaving Subtypes	Description
Craning	Animal lurches head forward and down.
Cannot Keep Head Still	Head constantly wobbles in all directions. (May occur between full head weaves).
Classic	Animal weaves head stiffly or loosely, from side-to-side or from front to back.
Slight	Head weaves but does not touch side or back of body.
Back	Head moves up and back.
Prolonged	Stiff movements in any direction where head makes contact with body and remains for a few seconds.
Circle	Head weaves in full circles.
Controlled	Animal halts head weaving upon the addition of stimuli.

Table 7B. Classification system developed for abnormal behavioral subtypes.

Muscle Fasciculations Subtypes	Description
Full Body	All muscles of the torso ripple or jerk.
Half Body	Muscles of only the upper or lower torso ripple or jerk.
Head	Muscles around the head and facial area ripple or jerk (may include the vibrissa and mouth).
One Front Flipper	Muscles within the front flipper pit jerk, causing the flipper to move upwards and/or outwards.
Both Front Flippers	Muscles within both front flipper pits tense, causing the flippers to move outwards.
Eye	The muscles around the eye socket jerk, causing the animal to squint spastically.

Statistical Analysis

Z. californianus of all age and gender groups were included in the study. Although the majority of animals seen with domoic acid toxicosis were adult female, we believed it was important to include other age and gender groups in the study in order to develop an accurate diagnostic method, as the possibility of symptomatological differences could exist between groups.

I categorized animals into either one of two samples within the dataset, a domoic acid toxicosis sample, and a non-domoic acid toxicosis sample. All animals placed in the domoic acid toxicosis sample received a diagnosis of domoic acid toxicosis by veterinary staff due to the presence of domoic acid in the blood, urine, milk, amniotic fluid, feces, or serum, or by the presence of an atrophied hippocampus (determined via MRI scan). In many cases, veterinarians used the process of elimination for diagnosis. All animals placed in the non-domoic acid toxicosis sample received diagnoses that did not include domoic acid toxicosis. The non-domoic acid toxicosis sample served as a comparison sample.

Using JMP 10 statistical software, I ran the Wilcoxon/Kruskal-Wallis test (Ranked sums) to determine whether abnormal behaviors correlated with the diagnosis of domoic acid toxicosis. I chose the Wilcoxon in place of the t test because the data were nonparametric.

Stranding crew volunteers and interns from the Marine Mammal Center documented head weaving, seizures, and muscle fasciculations that they observed before and during rescue on the stranding report. I analyzed these data with a Fishers Exact Test to determine whether these behaviors were more prevalent during rescue than at the center.

To test age and gender against time increments, I ran the Wilcoxon Signed Ranks and One-Way ANOVA tests. I then ran a Two Factor ANOVA to determine whether there was a correlation between ages crossed with gender.

I ran the Spearman to determine whether there was a correlation between severity scores and my continuous, timed data. I then ran the Fishers Exact Test to analyze whether the severity score had a correlation to diagnosis without considering time increments.

Finally, I ran the Wilcoxon Signed Ranks test to determine whether certain subtypes of a behavior were displayed significantly more often for animals with domoic acid toxicosis.

Results

I conducted 169 focal animal continuous scans between 5/22/2011-8/25/2013 for a total of 29 hours. Data collection began after the second feed of the day (generally around 14:00) and when animal care crew volunteers were absent from the pen area.

Observations fell between 14:01-17:43. Error rate was ±30--90 seconds per 15 minutes due to note taking. (Video was not clear enough to replace the human observer. Computerized notes were ill advised by staff due to often wet and windy conditions.) One hundred and sixty-nine animals were included in the data set with 50 having a confirmed diagnosis of domoic acid toxicosis and 119 having a range of confirmed diagnoses excluding domoic acid toxicosis.

Table 8: List of diagnoses of animals within the dataset.

Diagnoses of Sample from The Marine Mammal Center			
Corneal Ulcer	Abscess	Blind	Corneal Edema
Domoic Acid Toxicosis	Endocarditis	Dehydration	Cardiomyopathy
Entanglement	Head Trauma	Heart Murmur	Pneumonia
Malnutrition	Leptospirosis	Lice	Seizures
Shark Bite	Pox Virus	Osteomyelitis	Renal Failure
Septicemia	General Trauma	San Miguel Sea Lion Virus	Unknown
Azotemia	Oil/Tar		

The domoic acid toxicosis sample had 10 different diagnoses whereas the comparison sample contained 20 different diagnoses (see table 4). Six of the 30 diagnoses assigned to animals within the entire sample included both the domoic acid toxicosis and comparison samples. These six-shared diagnoses included abscess, malnutrition, cardiomyopathy, oil/tar, head trauma, and generalized trauma to the body (not including trauma not inflicted by a shark bite or blunt force to the head region). Domoic acid toxicosis was the sole diagnosis for the majority of the animals within the domoic acid toxicosis sample. The most common diagnoses for the comparison sample were malnutrition, leptospirosis, and pneumonia, with prevalence of 38%, 29%, and 12%, respectively. Interestingly, the occurrence of domoic acid toxicosis in the sample was unusually high at 30% compared to 9% reported by Grieg *et al*. (2005). This could be the result of increased occurrences of domoic acid toxicosis or an increase in the efficiency of rescue programs.

Figure 1: The range and occurrence of diagnoses seen in animals from the domoic acid toxicosis sample at The Marine Mammal Center. All 50 animals in the sample had a diagnosis of domoic acid toxicosis.

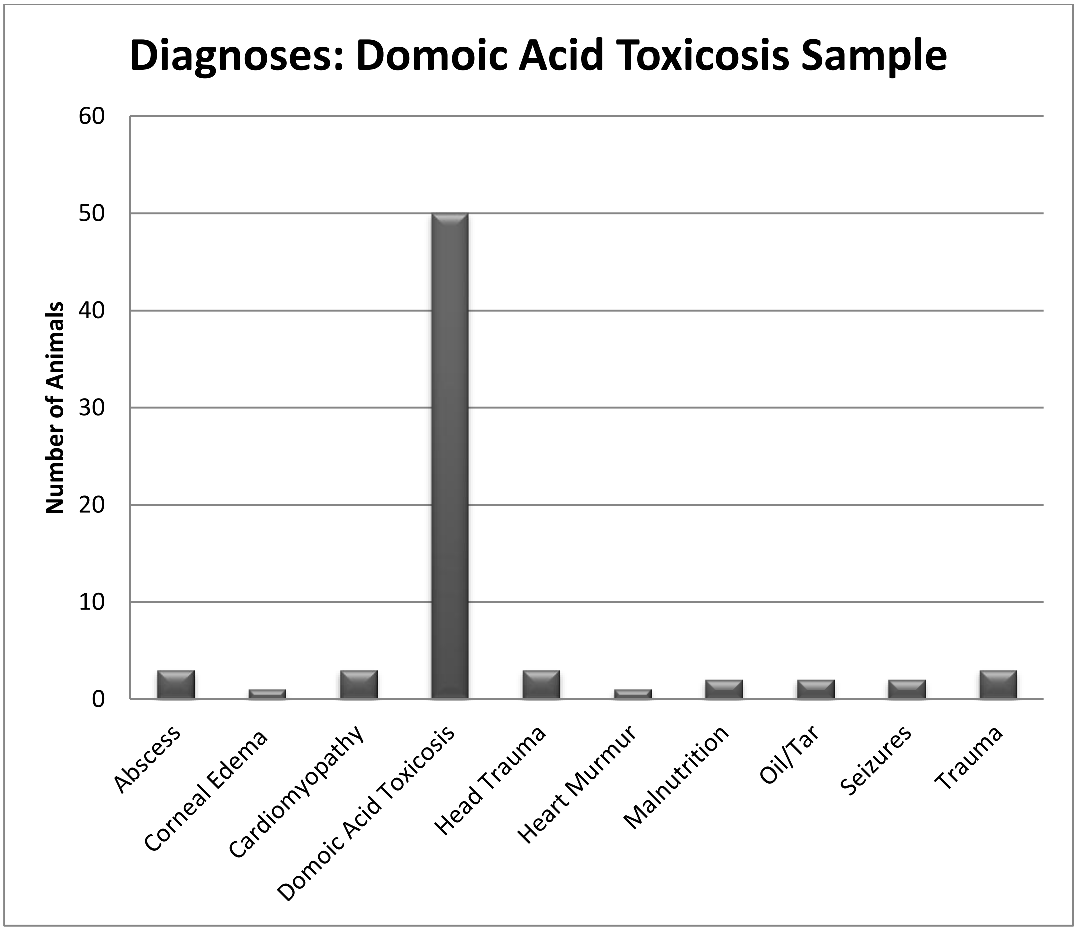


Figure 2: *The range and occurrence of diagnoses seen in animals from the comparison sample at The Marine Mammal Center.*

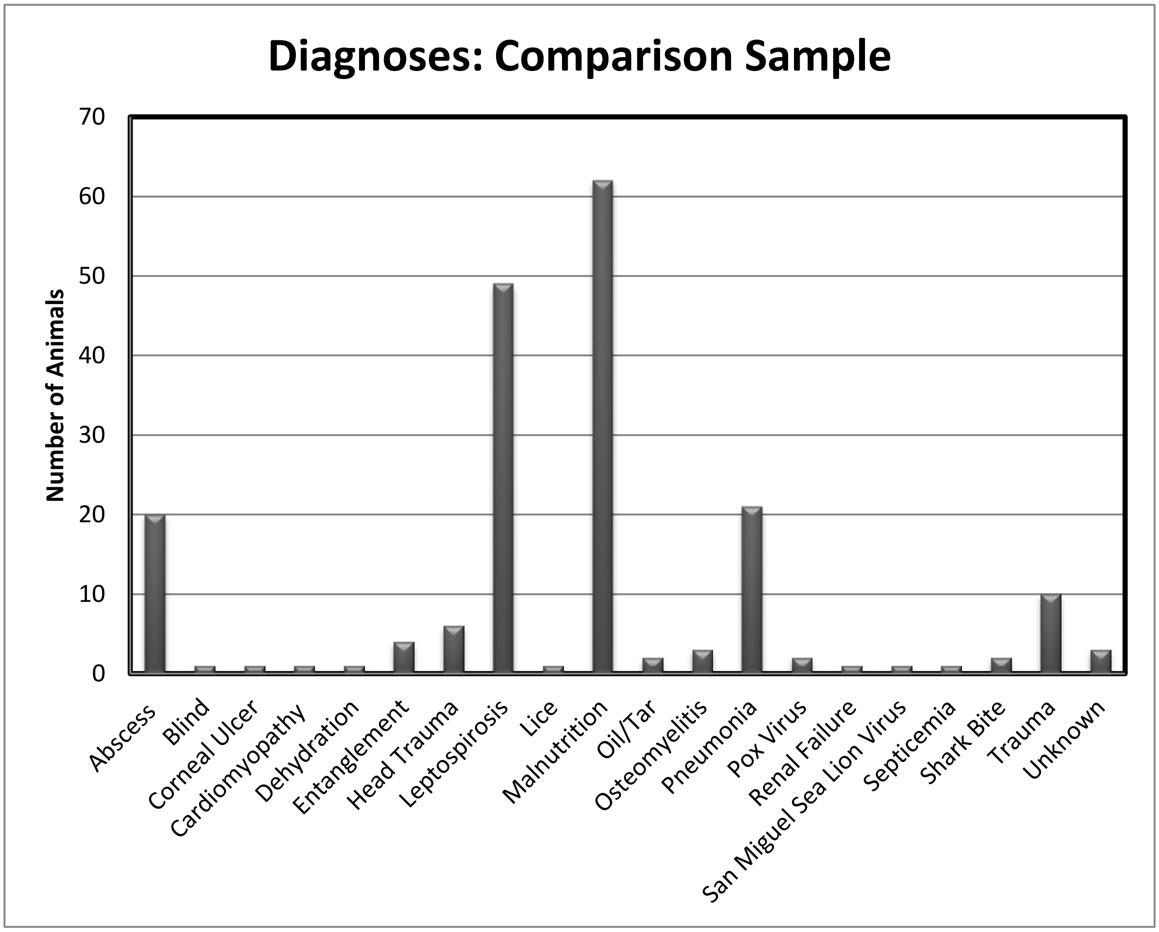


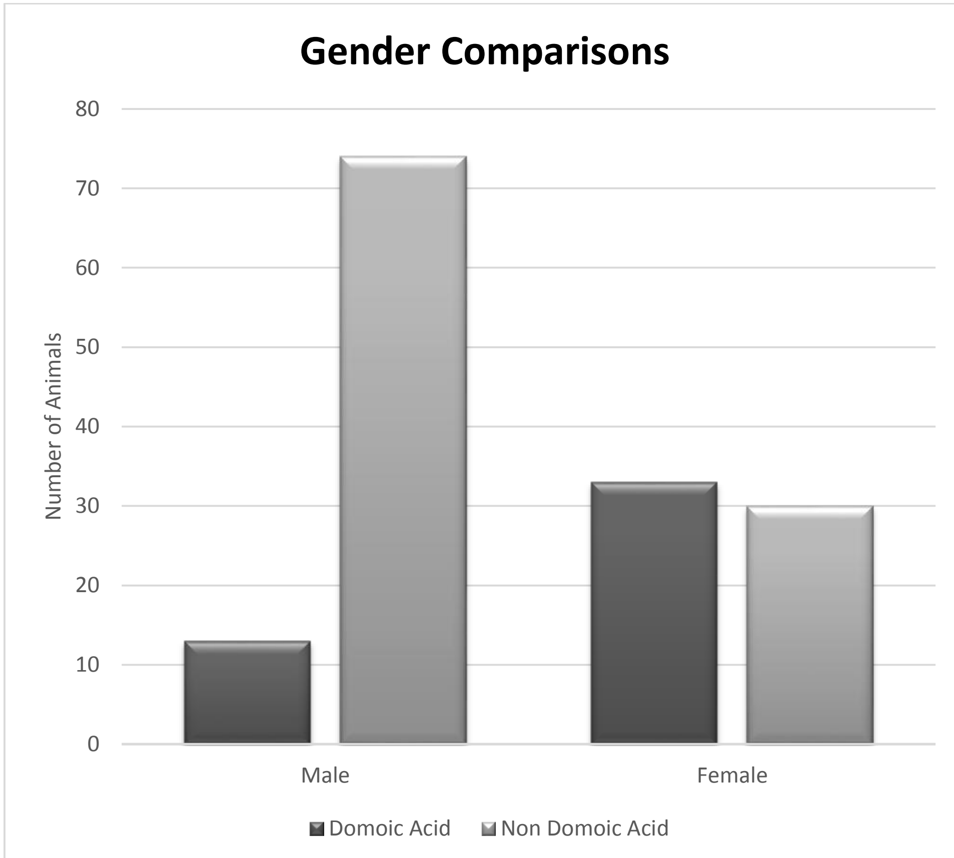
Table 9: Methods used by veterinary staff for the diagnosis of domoic acid toxicosis

Tag #	Diagnostic Method Used
LFF 27167	Necropsy
RFF 27152	Feces
RFF 27054	Urine
LFF 27196	Feces
RFF 27162	Feces
RFF 27013	Process of elimination
RFF 27070	Feces
RFF 27072	Process of elimination
RFF 27025	Process of elimination
LFF 25914	Process of elimination
LFF 25996	Process of elimination
RFF 25998	Process of elimination
RFF 25969	Process of elimination
RFF 27093	Process of elimination
RFF 27011	Process of elimination
RFF 25923	Process of elimination
RFF 25988	Process of elimination
LFF 25980	Process of elimination
RFF 27065	Serum
RFF 25976	Feces
RFF 25976	Feces
RFF 25938	Process of elimination
RFF 25938	Electroencephalography
RFF 27084	Necropsy
RFF 25952	Feces
LFF 25971	Process of elimination
LFF 25982	Feces
RFF27135	Feces
RFF 27132	Necropsy
LFF 27268	Feces
LFF 27284	Process of elimination
RFF 27301	Necropsy
LFF 27490	Process of elimination
LFF 27538	Process of elimination
RFF 27525	Necropsy
RFF 27508	Feces
LFF 27522	Feces
LFF 27360	Feces
RFF 27546	Process of elimination

LFF 27644	Functional magnetic resonance imaging
RFF 27667	Process of elimination
RFF 23837	Necropsy
RFF 27652	Process of elimination
RFF 23802	Necropsy
RFF 23958	Unknown
RFF 23545	Process of elimination
RFF 23823	Process of elimination
LFF 23633	Process of elimination
Unknown	Necropsy
RFF 23623	Process of elimination

The entire sample consisted of 103 males and 66 females, or 1.5 males to every female. The ratio of males to females was higher for animals in the comparison sample with 2.83 males for every female whereas the domoic acid toxicosis sample ratio was lower at 0.42 males for every female. Higher numbers of female animals admitted with domoic acid toxicosis were in line with the observation made by Gulland (2000).

Figure 3: Gender ratio between male and female animals within the domoic acid toxicosis and comparison samples.



The age of all animals within the sample included: pup, yearling, juvenile, sub adult, and adult. Veterinary staff determined age group based upon length, tooth eruption, and the presence or absence of a sagittal crest in males. The predominant age group for the entire sample was juvenile. The comparison sample followed this trend of juveniles making up the majority. Similar to the findings of Gulland, (2000), predominate age group in the domoic acid toxicosis sample was adult.

Figure 4: Age group ratio in comparison sample.

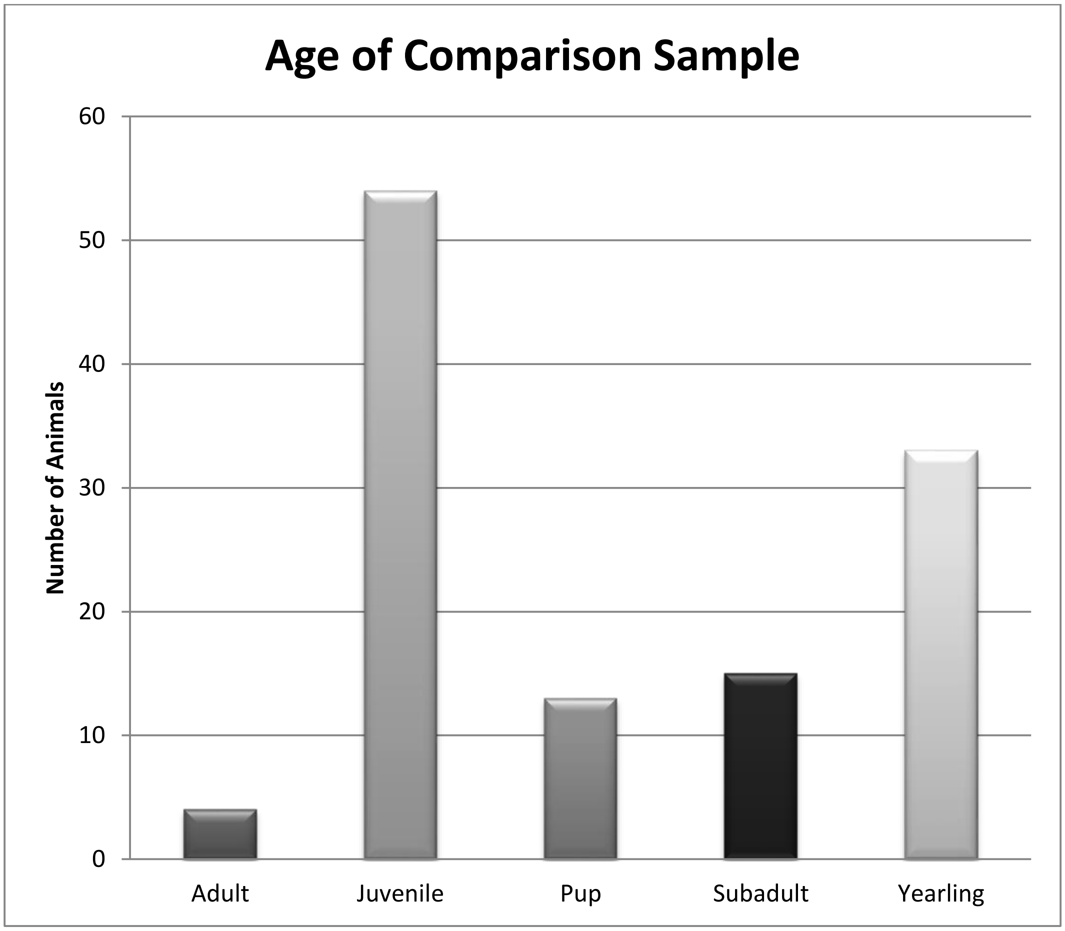
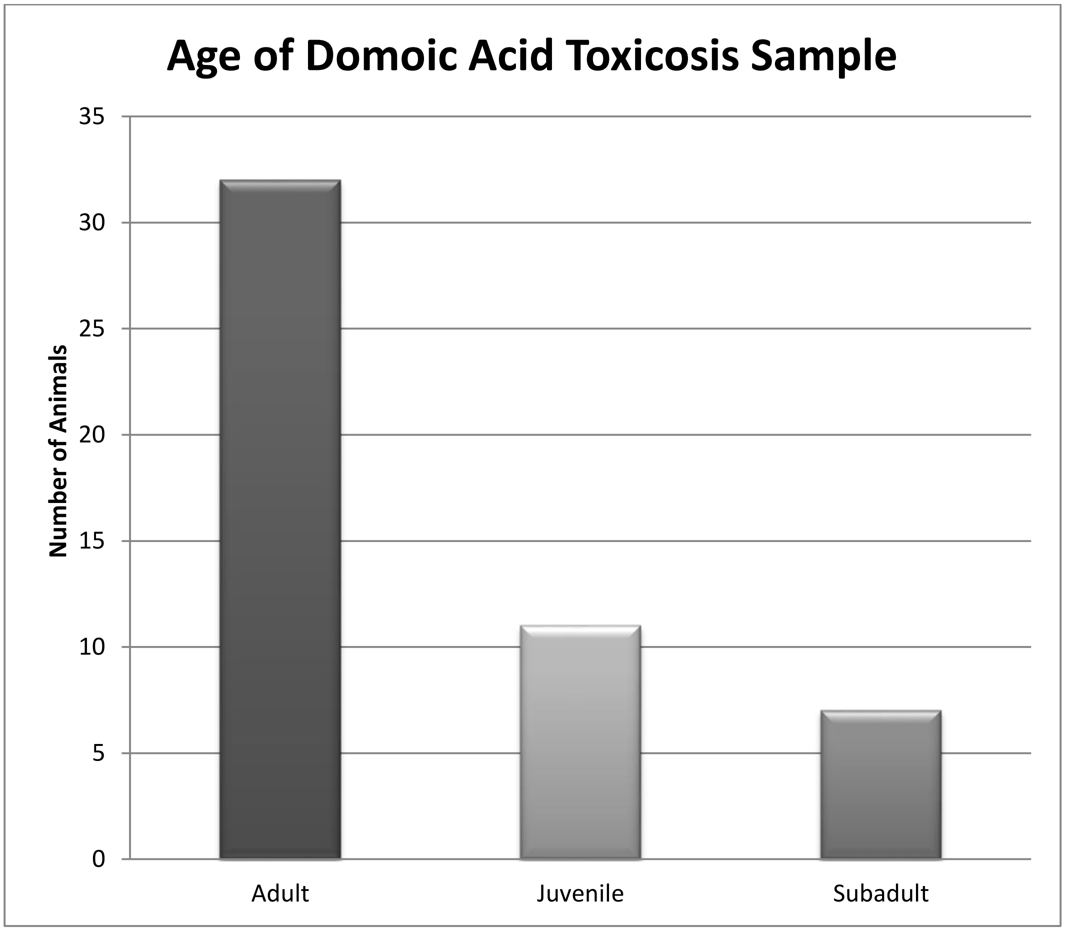
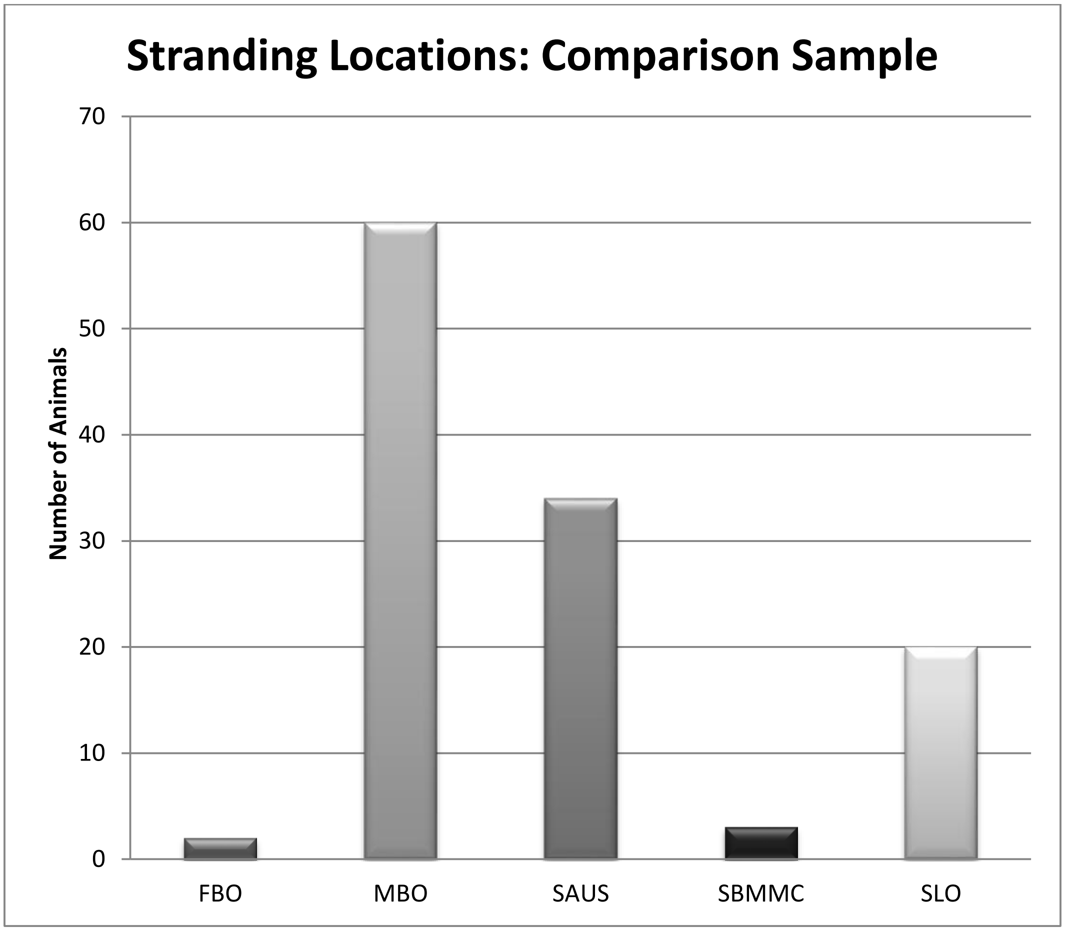


Figure 5: Age group ratio in domoic acid toxicosis sample.



Stranding locations consisted of MBO (Monterey Bay), SAUS (Sausalito), SLO (San Luis Obispo), FBO (Fort Bragg), and SBMMC (Santa Barbara) with the majority of strandings occurring at the MBO and SLO locations. After the closing of the Santa Barbara Marine Mammal Center in 2013, The Marine Mammal Center picked up some, but not all, of the rehabilitation work for Santa Barbara (Frankfurter, Pers. Comm. 2013).

Figure 6: Stranding locations of comparison sample.



FBO represents Fort Bragg Operations.

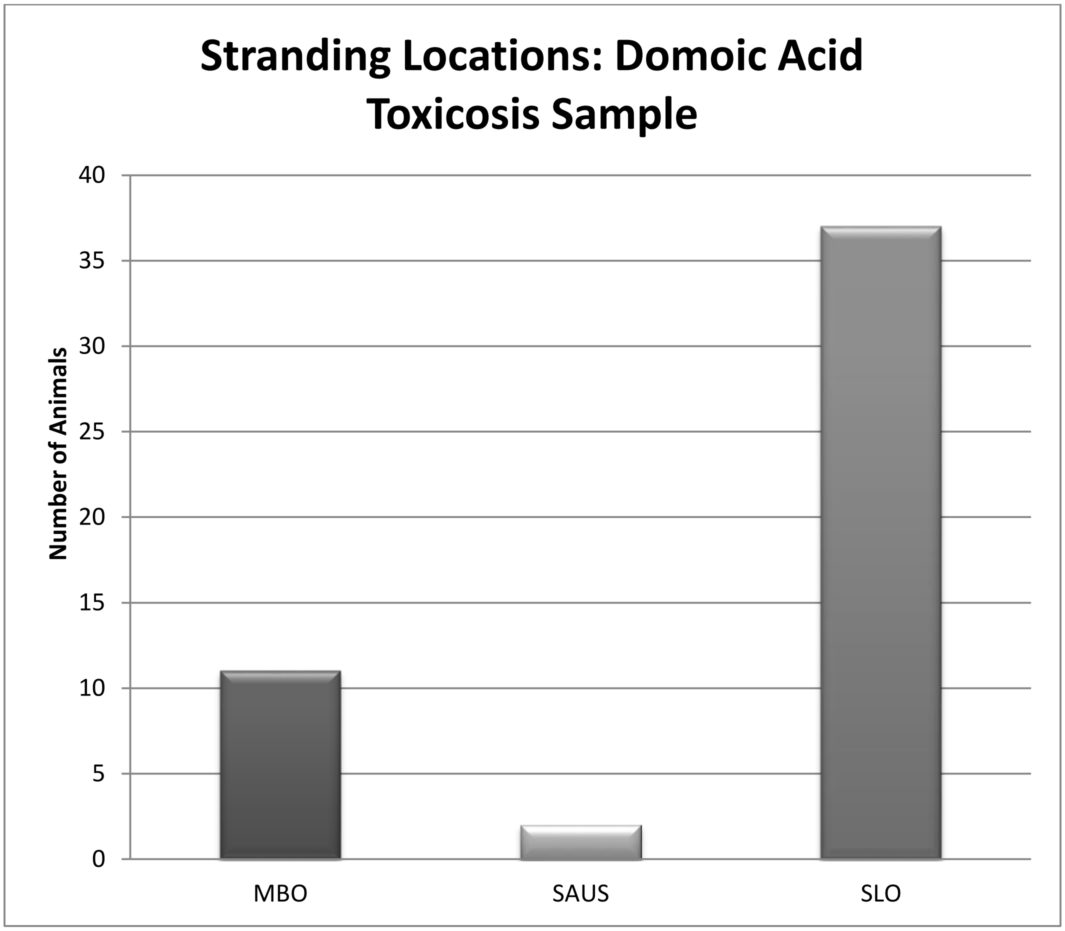
MBO represents Monterrey Bay Operations.

SAUS represents the main hospital in Sausalito.

SBMMC represents the Santa Barbara Marine Mammal Center rescue organization.

SLO represents San Luis Obispo operations.

Figure 7: Stranding locations of domoic acid toxicosis sample.



MBO represents Monterrey Bay Operations.

SAUS represents the main hospital in Sausalito.

SLO represents San Luis Obispo operations.

The mean animal length in the domoic acid toxicosis sample was 163.24cm with the min 132cm and the max 193cm. The mean weight was 73.83kg with the min 38kg and the max 148.5 kg. The mean animal length in the comparison sample was 131.2cm with the min 77cm and the max 216cm. The mean weight was 39.58kg with the min 10kg and the max 191kg.

Abnormal Behaviors

During focal animal continuous scans, I documented 23 types of abnormal behaviors. Of the 23 abnormal behaviors observed, 15 occurred within the domoic acid toxicosis sample.

Table 10: Abnormal behaviors observed during the study at The Marine Mammal Center.

Observed Abnormal Behaviors	
Comparison Group Only	Domoic Acid Toxicosis Group Included
Open Mouthed Breathing	Flapping Flippers
Nursing	Head Weaving
Waving Flippers	Grimacing
Rump Weaving	Craning
Drinking Seawater	Uncoordinated Movements
Mouth Chattering	Scratching
Erect Vibrissa	Muscle Fascinations
Seizures	Head Shaking
	Doughnut
	Circling
	Swift Scanning
	Twitching
	Floating
	Constant Swimming
	Dragging Hind Flippers

Grand maul seizing, which is typically associated with domoic acid toxicosis (Silvagni, *et al.* 2005), did not occur during my observations. Rescue volunteers documented grand maul seizures before or during rescue for only two animals within the domoic acid toxicosis sample. The lack of grand maul seizures could be the result of supportive care or anticonvulsants. It is also possible, yet not probable, that animals within the sample ceased all grand maul seizing activity once transported to the center and that veterinary intervention did not play a role in cessation. Many of the abnormal behaviors I observed involved myoclonic (brief periods of jerking muscle movements), clonic (repeated jerking of limbs), and clonic tonic (jerking of muscles preceded by stiffening) seizing. For example, flapping flippers, head weaving, grimacing, craning, muscle fasciculations, head shaking, doughnut, and twitching are forms of myoclonic and clonic seizures with flapping flippers and head weaving sometimes involving clonic tonic characteristics.

Four of the fifteen abnormal behaviors observed correlated to domoic acid toxicosis with two being exclusive to the diagnosis. Animals from the domoic acid toxicosis sample displayed head weaving (Wilcoxon signed rank, $Z= 6.5$, $S=5525$, $p<.0001$) and muscle fasciculations (Wilcoxon signed rank, $Z=3.77$, $S=4532.5$, $p<.001$) significantly more often than animals from the comparison sample. Swift scanning and dragging the hind flippers were exclusive to the domoic acid toxicosis sample.

Six of the fifteen abnormal behaviors were so rare (a single animal displaying the behavior) within the domoic acid toxicosis sample, that statistical testing was not possible. These behaviors included doughnut, circling, floating, head shaking, craning, and uncoordinated movements. Of these, doughnut, circling, and uncoordinated movements were exclusive to the domoic acid toxicosis sample. Further research, with a larger sample size, is required to test the significance of these abnormal behaviors.

Figure 8. Mean timeframe that animals displayed head weaving. Error bars represent one standard error from the mean. n=169.

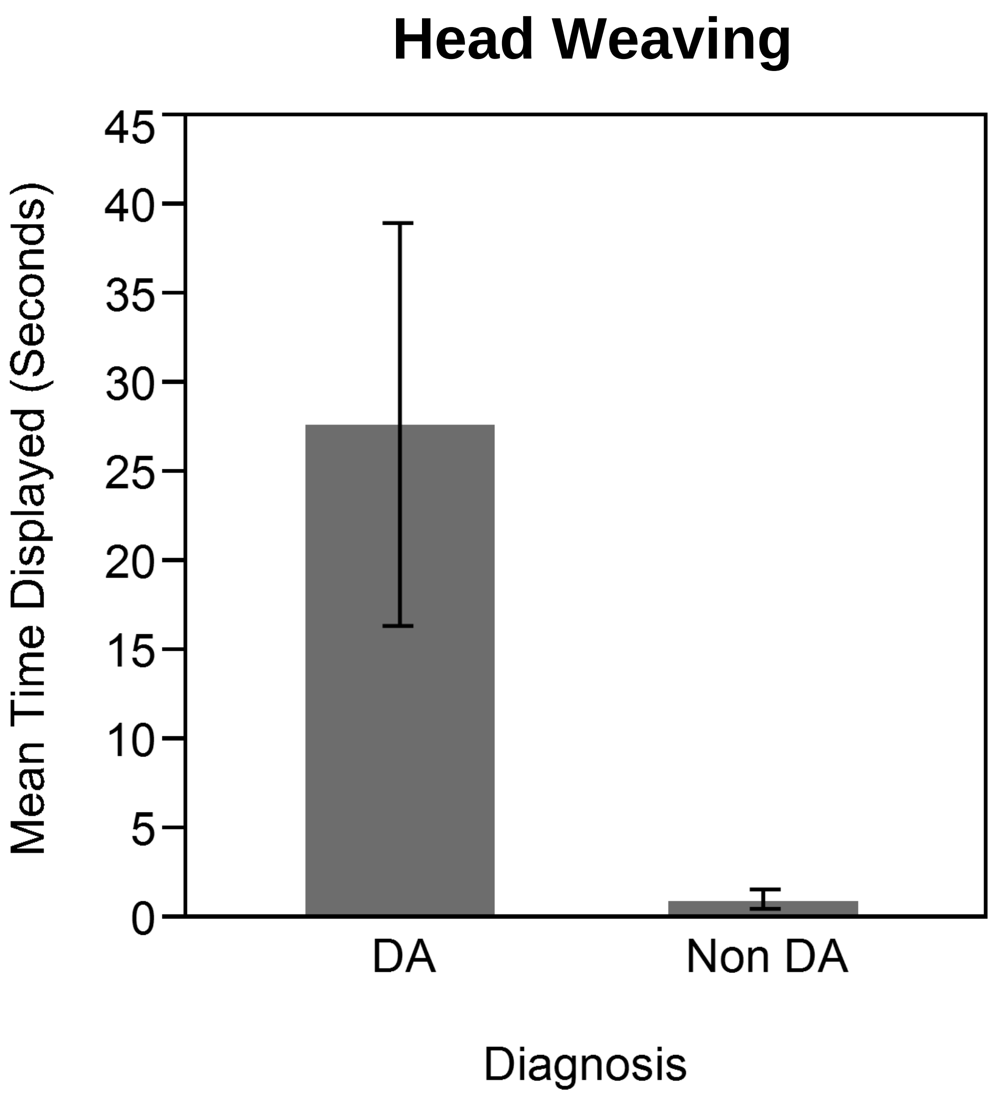


Figure 9. *Mean timeframe that animals displayed swift scanning. Error bars represent one standard error from the mean. Circles represent a lack of display by a sample. n=169.*

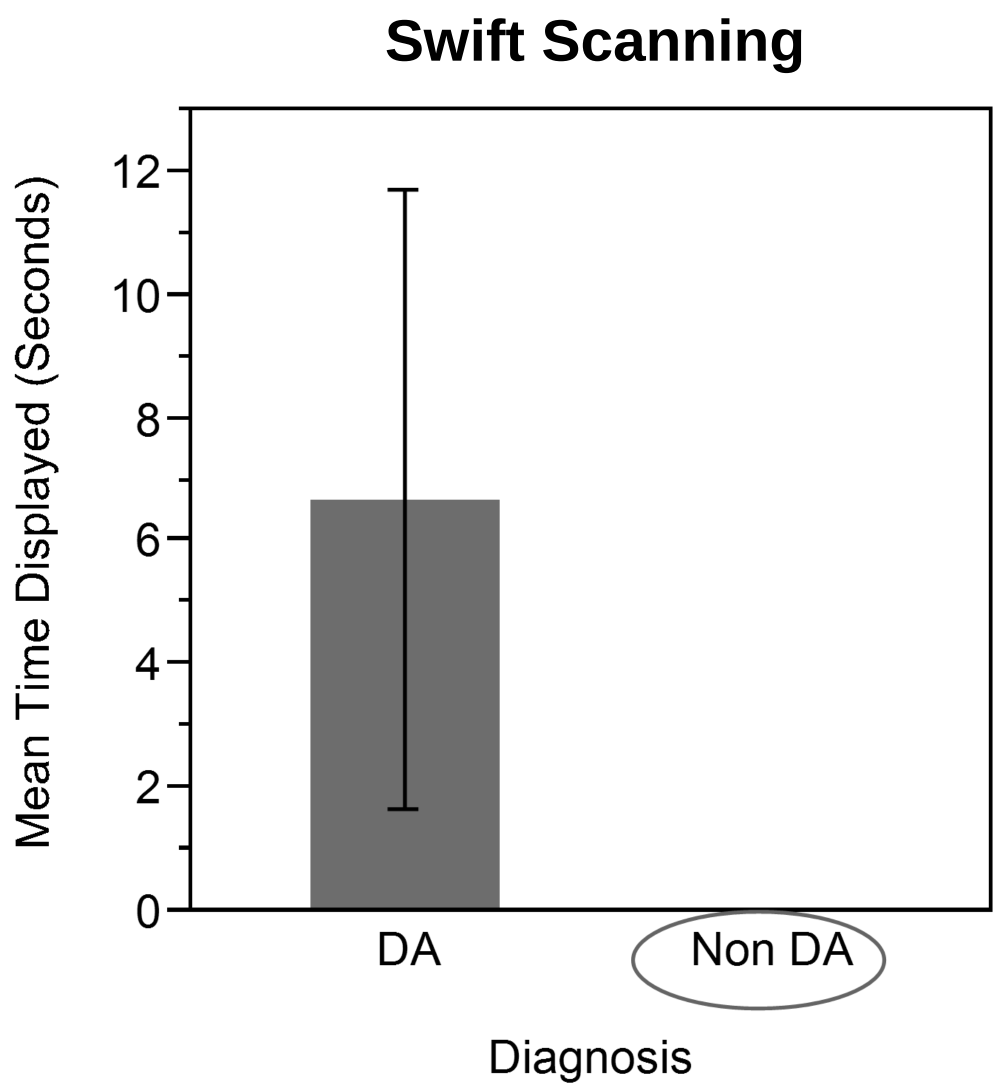


Figure 10. Mean timeframe that animals displayed dragging the hind flippers. Error bars represent one standard error from the mean. Circles represent a lack of display by a sample. n=169.

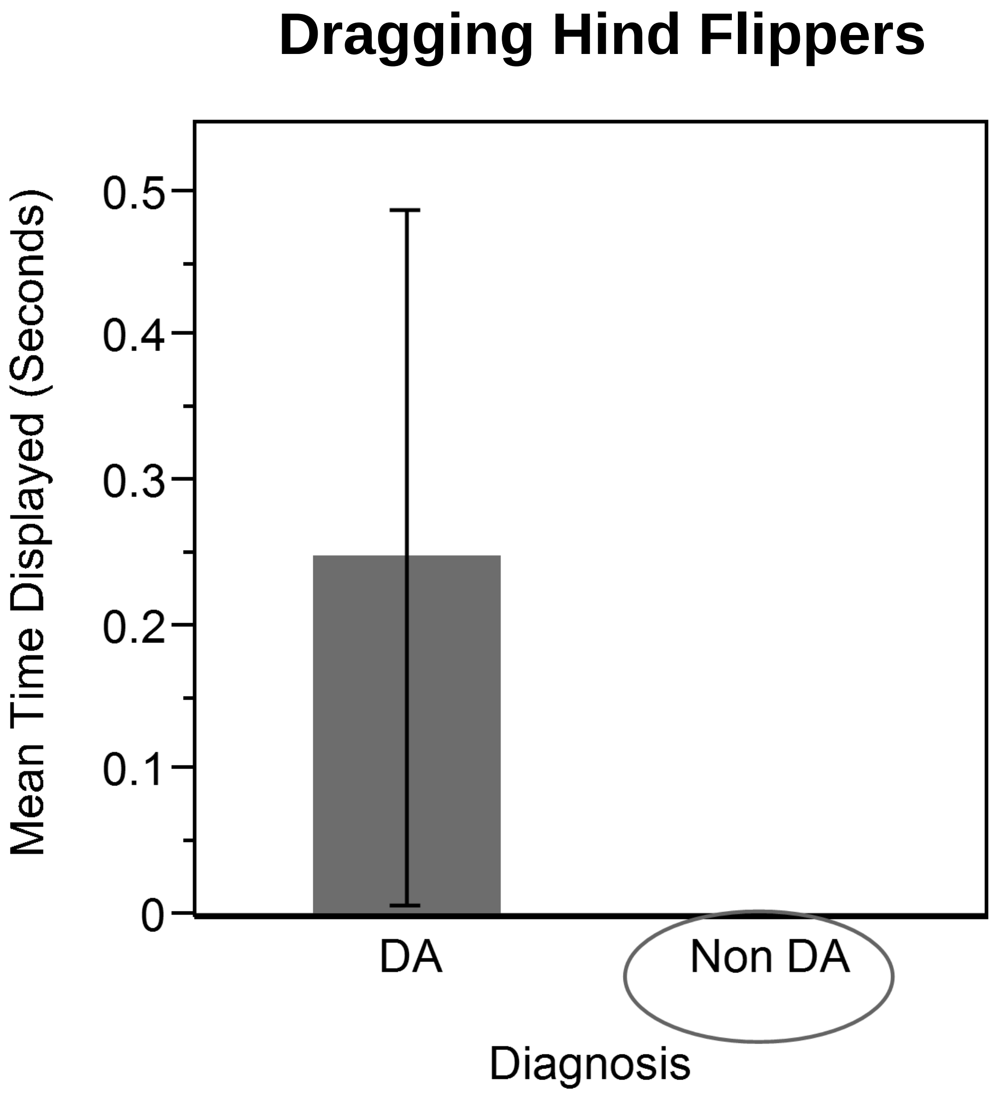


Figure 11: Mean timeframe that animals displayed muscle fasciculations. Error bars represent one standard error from the mean. N=169.

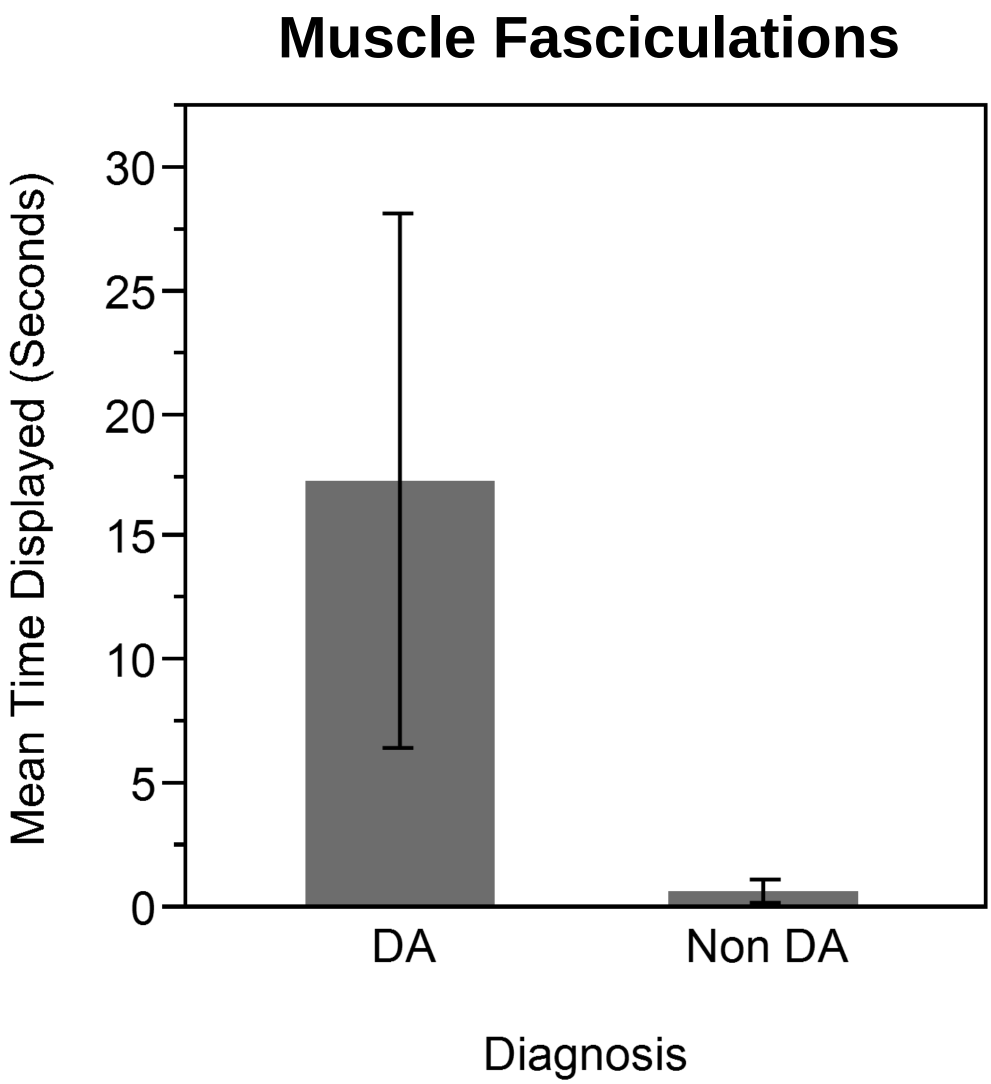


Table 11: Descriptive statistics from the Wilcoxon Signed Ranks test, testing whether animals with domoic acid toxicosis display unique abnormal behaviors.

Behavior	Number	Mean Time (Seconds)	Z Score	SD	p value
Head Weaving	24	8.86	6.5	45.18	<0.0001*
Grimacing	3	0.92	0.45	5.47	0.6518
Scratching	12	5.07	-0.04	14.73	0.9689
Muscle Fasciculations	13	5.33	3.77	40.17	0.0002*
Twitching	9	5.74	-0.78	34.14	0.4363

* Represents significance with α set to 0.05

Stranding crew and volunteers at the Marine Mammal Center documented abnormal behaviors displayed by animals before and during rescue. Because these behaviors occurred in the field, I was interested in their frequency. Stranding crew routinely documented head weaving, dragging the hind flippers, and muscle fasciculations on the stranding sheet. Stranding crew did not document swift scanning.

I used the Fishers Exact Test to determine whether animals displayed any behaviors on the beach more frequently before and during rescue than while at the center alone. The Fisher's Exact Test concurred with the Wilcoxon for head weaving (U 0.3822, DF 1, p<.0001) and for muscle fasciculations (U 0.2204, DF 1, p<.0001). Inclusion of data from the beach rose the accuracy rate of diagnostic criteria from 68% to 88%.

During the years 2011 and 2012, 10 of 41 animals within the domoic acid toxicosis sample displayed muscle fasciculations primarily on the beach before and during rescue, but not at the Marine Mammal Center. At that time, results from the Wilcoxon were not significant; however, results from the Fishers Exact Test were significant because of the tests ability to include the data from the stranding crew. Similarly, the number of animals that dragged their hind flippers at the Marine Mammal Center was small. Inclusion of data from the stranding crew doubled the number of animals displaying that behavior. The discrepancies between muscle fasciculations and dragging the hind flippers displayed on the beach versus at the center, and the 20%

increase in accuracy, demonstrates the necessity of including observations made by stranding crew into future diagnostic protocols.

Behavioral Subtypes

Behavioral subtypes existed for head weaving and muscle fasciculations. Head weaving consisted of eight subtypes whereas muscle fasciculations consisted of six subtypes.

Table 12: Descriptive statistics from the Wilcoxon Signed Rank test testing whether head weaving and muscle fasciculation subtypes were displayed significantly more often by animals from the domoic acid toxicosis sample.

Behavior	Subtype	Number	Mean Time (Seconds)	Z	SD	p Value
Head Weaving	Classic	15	2.36	4.77	18.89	<0.0001*
	Slight	2	0.14	0.62	1.26	0.5379
	Back	3	0.59	1.42	4.76	0.1547
Muscle Fasciculations	Full Body	7	0.5	1.62	4.05	0.1050
	Half Body	3	0.12	1.42	1.1	0.1569
	Head	12	4.62	2.37	39.63	0.0177*

* Represents significance with α set to 0.05.

Classic head weaving was highly significant for animals with a diagnosis of domoic acid toxicosis (Wilcoxon signed rank, S 4954, Z 4.77, $p<0.0001$). Muscle fasciculations of the head was also significant for animals with a diagnosis of domoic acid toxicosis (Wilcoxon signed rank, S 4557, Z 2.37, $p<0.05$). Therefore, animals with domoic acid toxicosis displayed these behavioral subtypes significantly more often than animals from the comparison sample. Although cannot keep head still, craning, and prolonged head weaving subtypes were exclusive to the domoic acid toxicosis sample, results were not testable due to a small subsample size. The same was true for muscle fasciculation subtypes including the eyes and both front flippers. Other behavioral subtypes were not significant.

Figure 12: Mean timeframe that animals displayed the head weaving subtype: Classic. Error bars represent one standard error from the mean. n=169.

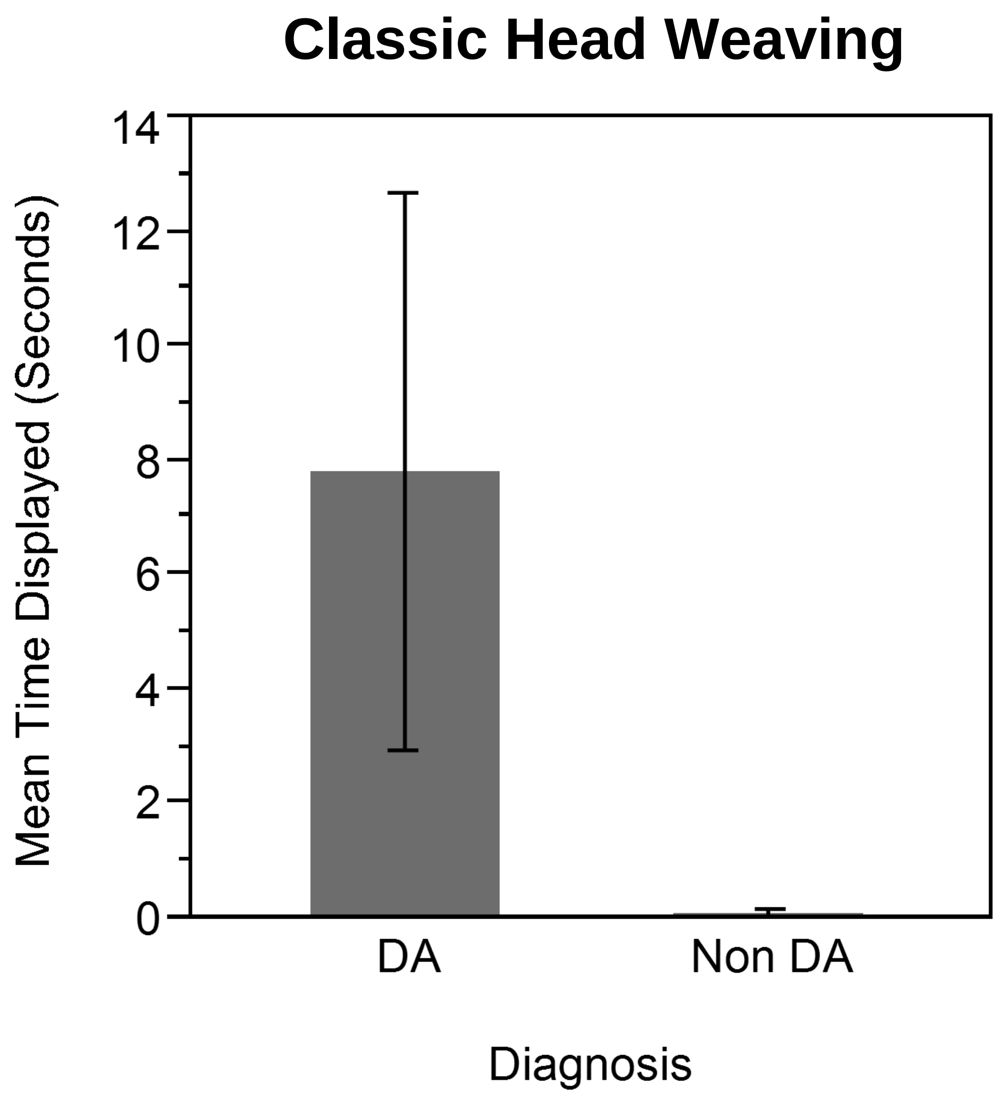
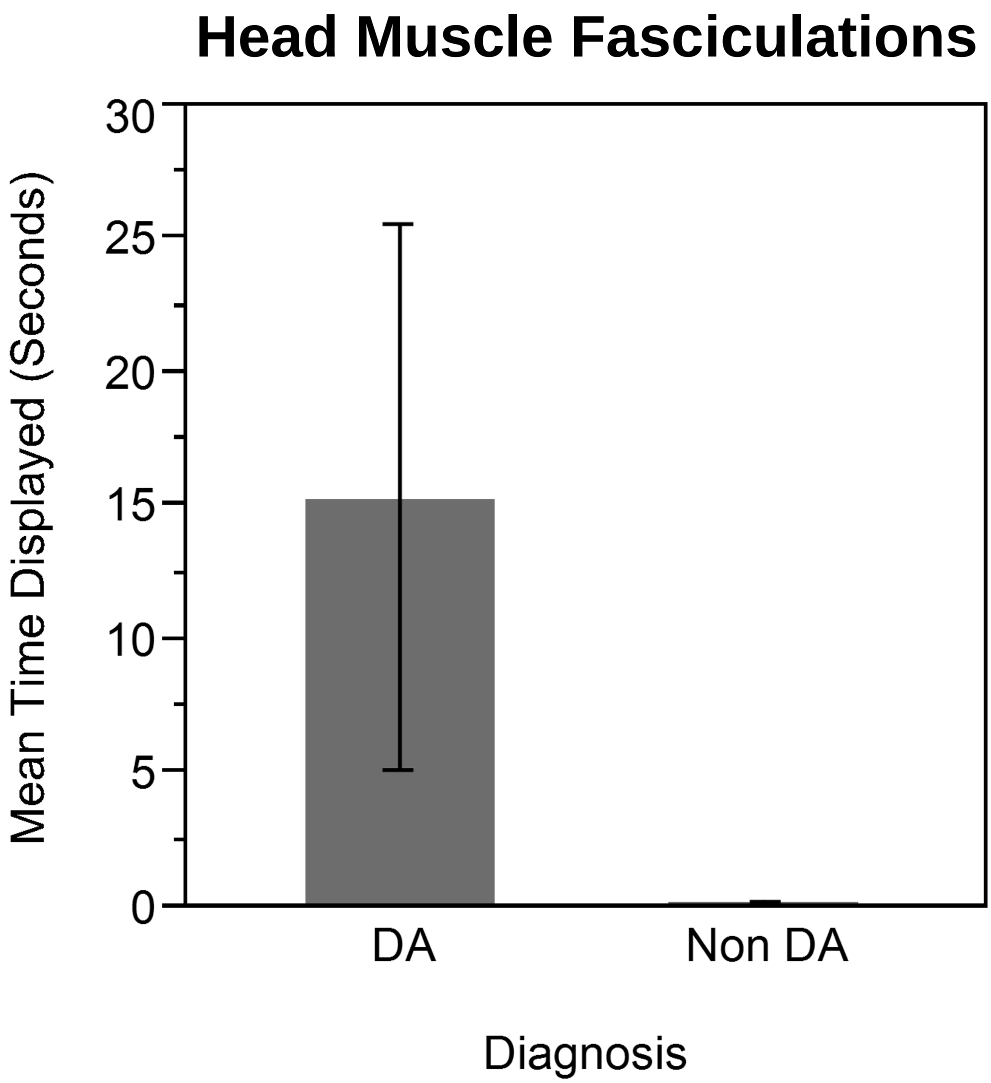


Figure 13: Mean timeframe that animals displayed the muscle fasciculation subtype: Head. Error bars represent one standard error from the mean. n=169.



Severity Scores

I used severity scores to determine the consistency that an animal displayed an abnormal behavior compared to the observation period. Severity scores ranged from 1-3 (see pages 13-14 for detailed information on severity scores). Severity scores for head weaving and muscle fasciculations ranged from 1-3 whereas the scores for dragging the hind flippers ranged from 1-2 and all animals who displayed swift scanning received a score of 3.

Severity scores for head weaving were positively correlated to the timed, behavioral data (Spearman, $p<0.0001$). Although there was a low probability that the data was derived by chance, the relationship between the continuous data and severity scores was weak with a Spearman's r-value of 0.30. Severity scores for muscle fasciculations were also correlated (Spearman, $p<0.05$). The relationship between the continuous data and severity scores was negligible, with an r-value of 0.09. These results indicate that although it was unlikely that the sample values were derived by chance, the actual positive, relationship between the data is weak or negligible. I recommend a larger sample size for further analysis.

Table 13: Descriptive statistics from the Spearman test. Dragging the hind flippers was not included due to a small sample size.

Behavior	Severity Scores Given	Spearman r	Spearman ρ	p value
Head Weaving	1,2,3	0.30978	0.833	<0.0001*
Muscle Fasciculations	1,2,3	0.0996	0.4142	0.0363*

**Represents significance with α set to 0.05.*

Figure 14: Head weaving severity scores versus time displayed.

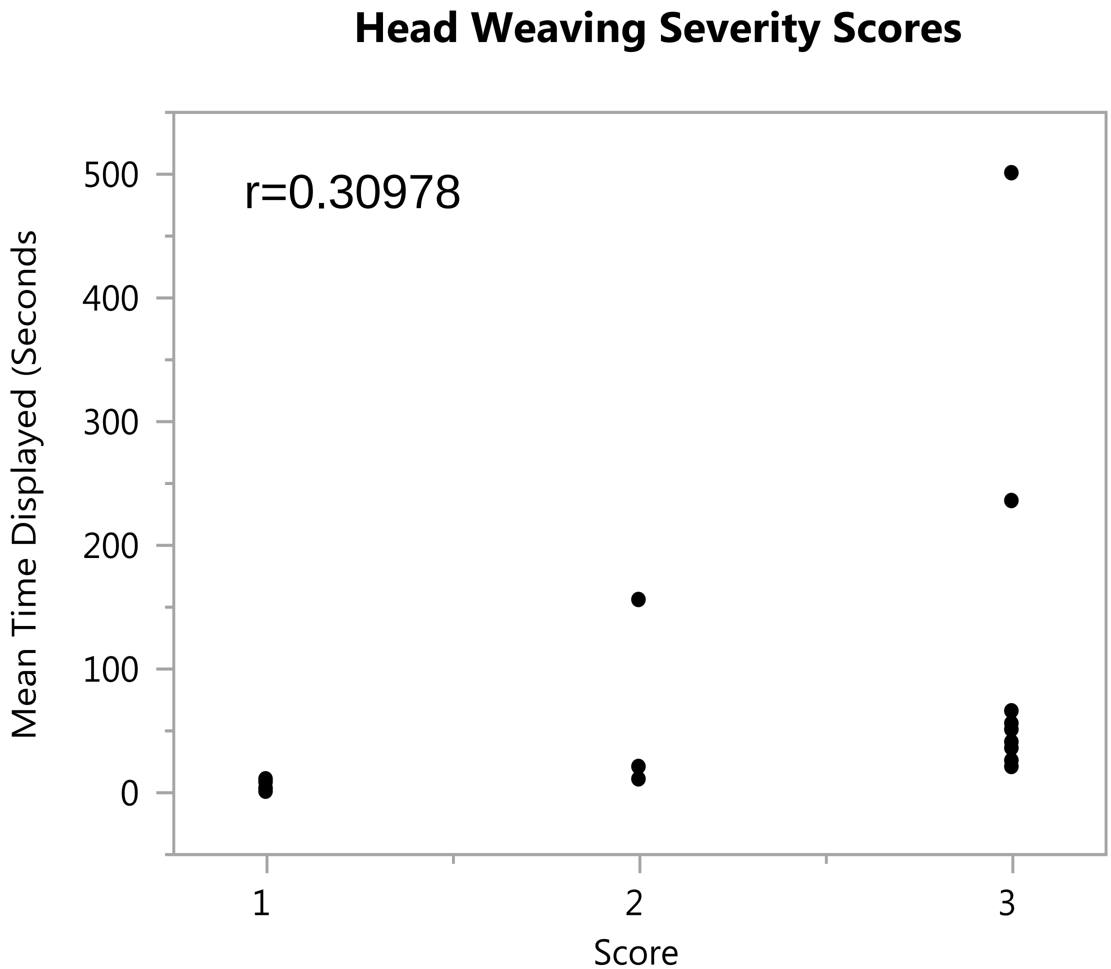
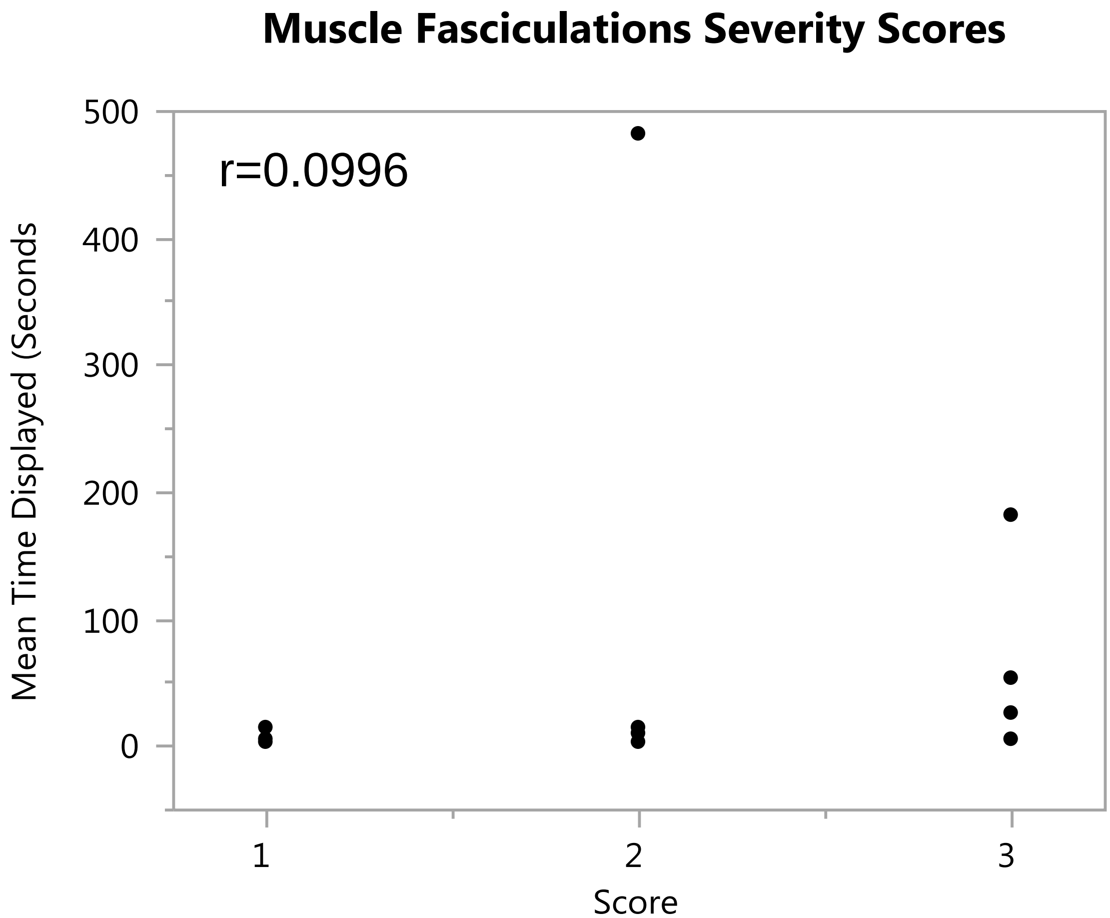


Figure 15: Muscle fasciculation severity scores versus time displayed. n=13.



Animals with domoic acid toxicosis had scores of 1 (Fishers exact test, U 0.1323, $p<0.001$) and 3 (Fishers exact test, U 0.2728, $p<0.0001$) for head weaving significantly more often than animals with other diagnoses. These animals experienced mild or severe head weaving bouts with only rare occurrences of moderate bouts. Animals with domoic acid toxicosis displaying muscle fasciculations received severity scores of 2 (Fishers exact test, U 0.1249, $p<0.05$), significantly more often than animals from the comparison sample. This indicates that animals from the domoic acid toxicosis sample displayed muscle fasciculations semi consistently throughout the observation. All animals that displayed swift scanning received a severity score of 3 (Fishers exact test, U 0.2636, $p<0.01$).

Table 14: Descriptive statistics for the severity scores associated with head weaving and muscle fasciculations.

Behavior	Score	Number	U	p Value
Head Weaving	1	9	0.1323	0.001*
	2	3	0.022	0.3625
	3	12	0.2728	<0.0001*
Muscle Fasciculations	1	5	0.0257	0.1631
	2	3	0.1249	0.0273*
	3	4	0.0705	0.0637

* Represents significance with α set to 0.05

Figure 16: Comparison of animals receiving severity scores for head weaving.

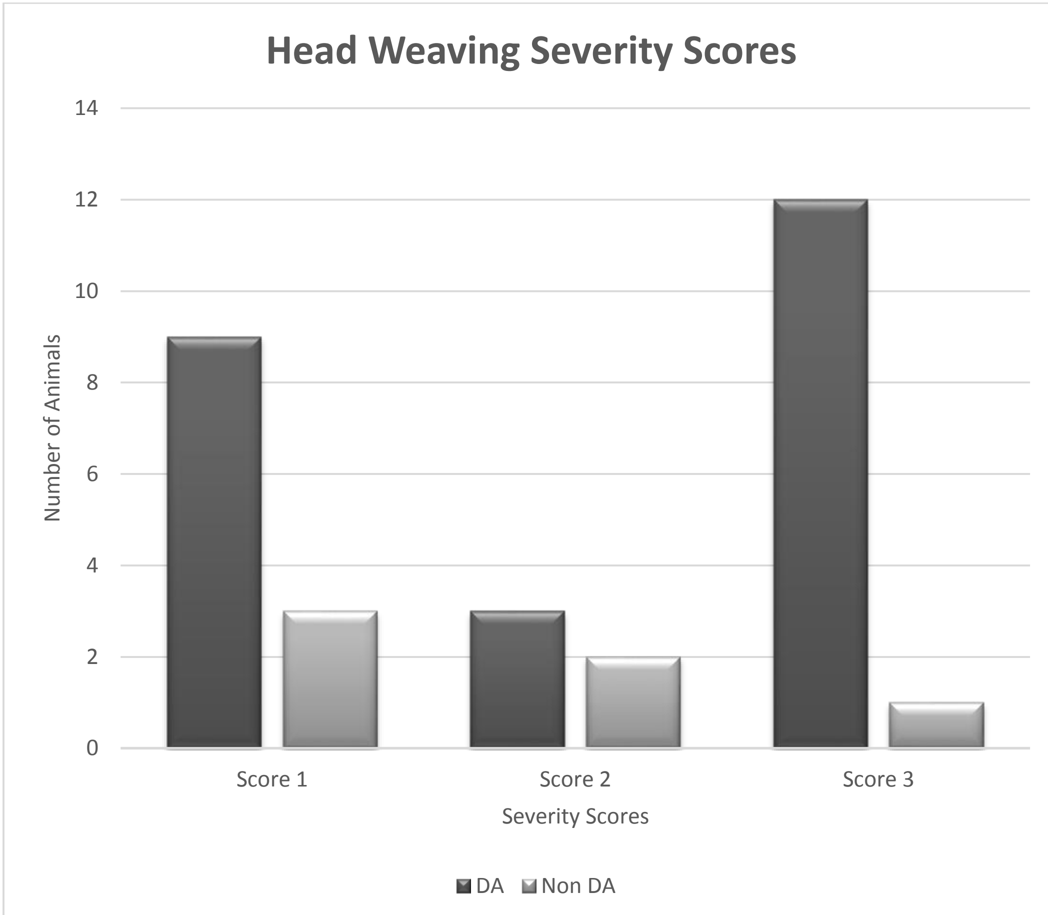
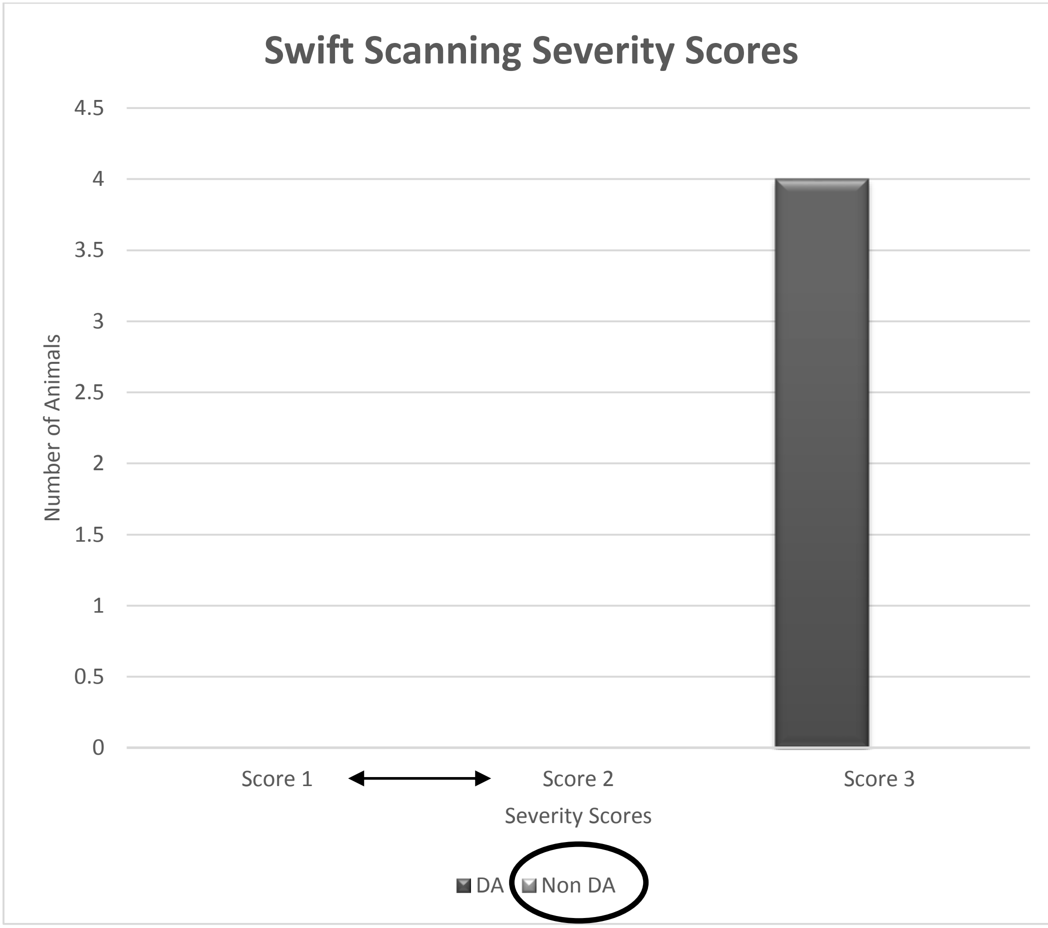


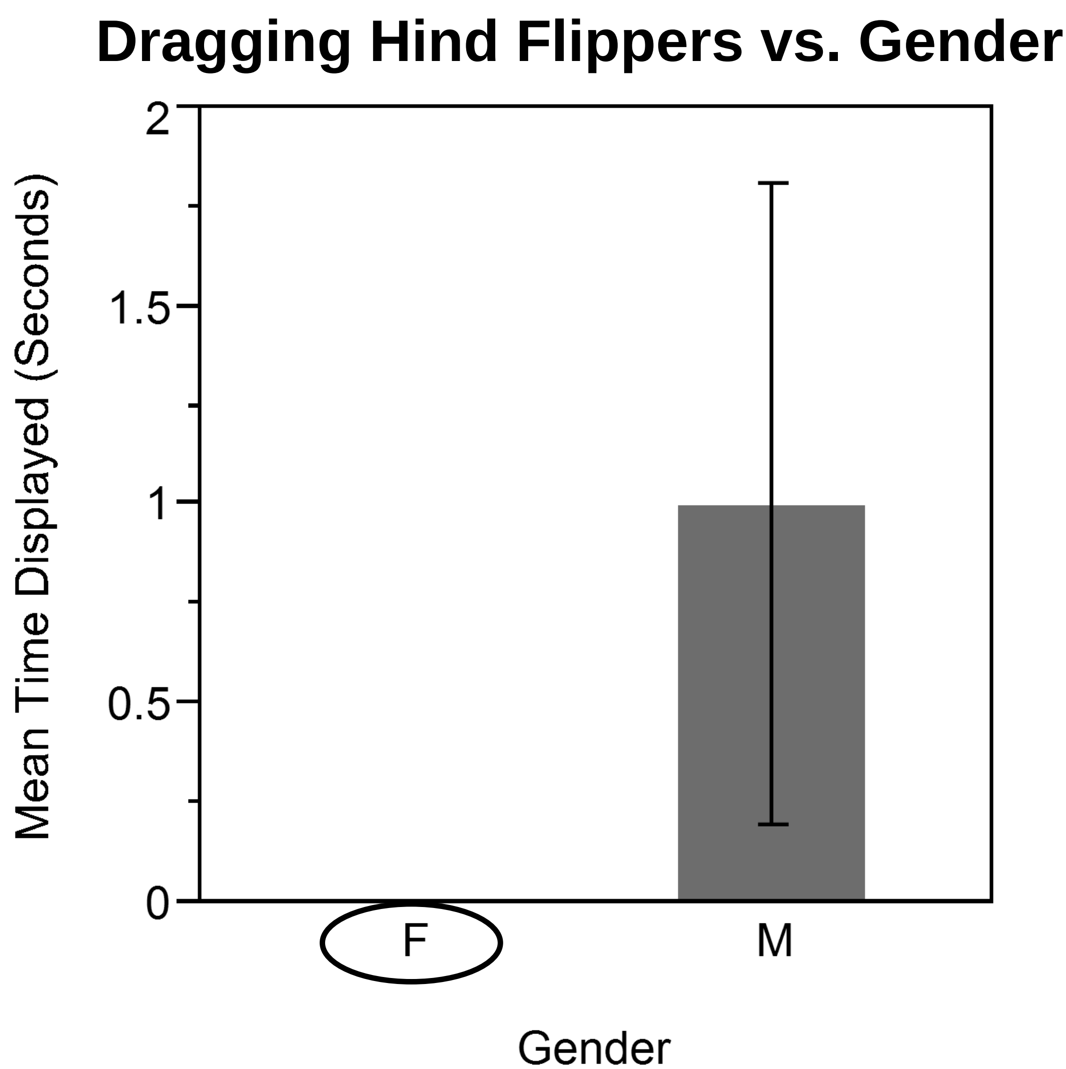
Figure 17: Severity scores for swift scanning. Severity scores of one and two are absent. Additionally, scores are absent from the comparison sample due to the behavior's exclusiveness to the domoic acid toxicosis sample.



Age and Gender

I tested whether differences existed between age and gender and the types and lengths of time that an abnormal behavior lasted. Dragging the hind flippers was exclusive to males at the Marine Mammal Center. No other results were significant.

Figure 18: Mean time that males and females dragged their hind flippers. Note that the behavior is exclusive to males at the Marine Mammal Center. However, stranding crew reported a single female displaying the behavior as well. The small number of animals displaying the behavior most likely influences results. Further research is needed to increase the dataset. The error bar represents one standard error from the mean. n=50



Discussion

Based on the results from this study, abnormal behavioral criteria is an effective tool in the diagnosis of domoic acid toxicosis in *Zalophus californianus*. Four abnormal behaviors: head weaving, muscle fasciculations, dragging the hind flippers, and swift scanning, positively correlate to the diagnosis.

Current laboratory diagnostics detected domoic acid in 28% of animals from the domoic acid toxicosis sample. In a previous study, Cook *et al.* (2011) developed diagnostic methodology that involved animal orientation in response to acoustic stimuli. In that study, Cook *et al.* (2011) identified domoic acid toxicosis in 50% of animals and rejected the diagnosis in 93% of animals from the comparison sample. Behavioral diagnostic criteria from my study had the highest level of accuracy. Observations made at both the Marine Mammal Center and by volunteers in the field, accounted for an accuracy rate of 88% whereas observations made only at the Marine Mammal Center fell to 68%. Rejection rates were slightly lower than methods developed by Cook *et al.* (2011), with 84% accuracy for both the Marine Mammal Center and field observations and 86% for observations occurring only at the Marine Mammal Center. Currently, behavioral diagnostic criteria is the strongest method of diagnosis for domoic acid toxicosis in *Z. californianus*.

The 84-86% rejection rate calls for further tightening of the diagnostic requirements. My data show that animals within the comparison sample displayed 0-1 abnormal behaviors from the diagnostic criteria. Within the domoic acid toxicosis sample, animals displayed between 0-3 abnormal behaviors. Specifically, 90% of animals displayed at least a single abnormal behavior whereas 51% displayed two or more. Therefore, animals displaying two abnormal behaviors within the diagnostic criteria (see next section) can receive the diagnosis of domoic acid toxicosis with confidence.

Diagnostic Criteria

Within the domoic acid toxicosis sample, 78% of animals displayed head weaving (M 27.66, SD 79.95, 95%CI 66.78, 99.63). Within the comparison sample 8% of animals displayed head weaving (M 0.96, SD 6.24, 95%CI 5.53, 7.15). The diagnosis of domoic acid toxicosis can be considered if head weaving lasts ≥12.4 seconds (two standard deviations from the mean of the comparison sample) during a 15 minute period. Within the comparison sample, 2% of animals reached or exceeded the cutoff threshold. This cutoff threshold lowers the risk of false diagnosis. Within the domoic acid toxicosis sample, however, 45% of animals that displayed head weaving did not reach the cutoff threshold. This leaves room for diagnostic error. In these circumstances, other diagnostic variables from the diagnostic criteria should be evaluated. When factors such as the display of two or more behaviors from the diagnostic criteria are displayed or behavioral subtypes such as prolonged, craning, classic, or cannot keep head still are displayed, the diagnosis of domoic acid toxicosis can be considered, as these subtypes are exclusive or correlated to the diagnosis. When these factors were taken into account, only 2% of the animals in the domoic acid toxicosis sample, that displayed head weaving, did not reach the cutoff threshold.

Exactly 48% of animals within the domoic acid toxicosis sample displayed muscle fasciculations (M 17.60, SD 75.13, 95%CI 62.32, 94.63). From the comparison sample, 7% of animals displayed muscle fasciculations (M 0.62, SD 4.68, 95%CI 4.15, 5.36). The diagnosis of domoic acid toxicosis can be considered if an animal displays muscle fasciculations ≥9.36 seconds (two standard deviations from the mean of the comparison sample) during a 15-minute period. Within the comparison sample, 2% of animals reached or exceeded this cutoff threshold. From the domoic acid toxicosis sample, only 1% of animals displaying muscle fasciculations did not meet the cutoff threshold.

A strong indicator of domoic acid toxicosis is dragging the hind flippers. Within the domoic acid toxicosis sample, 8% of animals displayed the behavior compared to 0% from the comparison sample. Therefore, an animal that drags the hind flippers should receive a diagnosis of domoic acid toxicosis with confidence. However, I advise caution in extreme circumstances that could affect movement of the lower limbs – not seen in this study - (e.g. spinal injuries causing lower torso paralysis).

Swift scanning is another strong indicator of domoic acid toxicosis. Within the domoic acid toxicosis sample, 10% of animals displayed swift scanning, versus 0% from the comparison. Due to the exclusiveness of the behavior, any animal displaying swift scanning – assuming proper environmental criteria (see chapter three) – can receive a diagnosis of domoic acid toxicosis with confidence.

The Use of Behavioral Subtypes

From the domoic acid toxicosis sample, 22% of animals displayed classic head weaving (M 7.78, SD 34.36, 95%CI 28.70, 42.81), compared to 2% from the comparison sample (M 0.08, SD 0.59, 95%CI 0.52, 0.67). This warrants the use of classic head weaving as a reliable indicator of domoic acid toxicosis. To be considered for the diagnosis an animal should display classic head weaving ≥1.26 seconds (two standard deviations from the mean of the comparison sample). The head weaving subtypes: cannot keep head still, craning, and prolonged, were exclusive to the domoic acid toxicosis sample. For these subtypes, I recommend diagnostic consideration. I caution against using controlled head weaving as diagnostic criteria. The controlled subtype was exclusive to the comparison sample. All other subtypes are acceptable measures.

Within the domoic acid toxicosis sample, 14% of animals displayed the head muscle fasciculation subtype (M 109.14, SD 175.14, 95%CI 112.86, 385.67) compared to 4% of animals in the comparison sample (M 3.2, SD 2.28, 95%CI 1.36, 6.55). An animal can be considered for the diagnosis of domoic acid toxicosis with the display of the head muscle fasciculation subtype for ≥7.76 seconds (two standard deviations from the mean of the comparison sample). Although not significant in this study, muscle fasciculations of the eye and both front flippers occurred exclusively within the domoic acid toxicosis sample. I recommend consideration of the diagnosis if animals display these subtypes.

The Use of Severity Scores

My results show that 50% and 37% of animals from the domoic acid toxicosis sample were given head weaving severity scores of 3 and 1 respectively. Therefore, a severity score of 3 is a good indicator of domoic acid toxicosis and can be used if the 12.4 second time criteria is not met. A score of 3 can also be used as further evidence of domoic acid toxicosis. I urge conservativeness in the use of score 1 as the sole diagnostic indicator. In the domoic acid toxicosis sample, 37% of head weaving animals received a score of 1. From the comparison sample, 43% of animals received the same score. (This disparity is not a flaw in the statistics but rather an artifact of the differing sub sample sizes.) Animals that receive a severity score of 1 should also meet other criteria within the diagnostic protocol.

Within the domoic acid toxicosis sample, 25% of animals displaying muscle fasciculations were given a severity score of 2. Animals within the comparison sample received scores of 2, 12.5% of the time. These values leave room for diagnostic error; the observer should exercise caution and ensure that the animal meets other diagnostic criteria before assigning a diagnosis of domoic acid toxicosis.

Swift scanning was absent in the comparison sample. Additionally, 100% of all animals displaying swift scanning received a severity score of 3. I recommend a diagnosis of domoic acid toxicosis if an animal displays swift scanning. I caution against rejecting animals that receive severity scores of 1-2. In these cases, the animal should still receive a diagnosis of domoic acid toxicosis, as the behavior was exclusive to the domoic acid toxicosis sample.

Gender and Age Differences

Though very few differences between gender and age exist, dragging the hind flippers was exclusive to males at the Marine Mammal Center. Despite significant results, only 13% of males displayed the behavior, making it the least observed of all the diagnostic criteria. Although a different species, Pulido (2008) showed that male rats are more susceptible to the neurological effects of domoic acid than females. Based on my results, I recommend MRI or neural tissue samples from male *Z. californianus* that drag their hind flippers.

Inclusion of Method by Rescue Crew

The 20% disparity between observation accuracy rates at both the Marine Mammal Center and the beach and for those including only observations at the Marine Mammal Center is large enough to warrant the inclusion of reporting by stranding crews. This is subject to stranding crew receiving proper training in diagnostic criteria.

Stranding crew are often volunteers, interns, and sometimes employees. Therefore, training will need to accommodate a variety of learning curves. Most rescue and rehabilitation centers already have established training courses in place on the topics of sea lion care, basic biology, and basic behavior (Personal Observation, 2011). The inclusion of simple training concerning the identification of diagnostic criteria before and during rescue is essential in the use of field diagnostics (see appendix V for sample training outline).

Stranding crew should document the following before and during rescue operations:

- Documentation of behaviors displayed that fit diagnostic criteria (required)
- Documentation of behavioral subtypes (required)
- Documentation of time behaviors were displayed (optional)
- For documentation of dragging the hind flippers and swift scanning: Conformation that environmental assumptions were met (required)

Comparison Facility: Marine Mammal Care Center

The Marine Mammal Care Center is a marine mammal hospital and rehabilitation facility located in San Pedro California. The center began treating marine mammals in 1992. The National Marine Fisheries Service permits the Marine Mammal Care Center to rehabilitate, place, euthanize, and release both seals and sea lions. Common species include:

- California sea lion, *Zalophus californianus*,
- Northern elephant seal, *Mirounga angustirostris*
- Harbor seal, *Phoca vitulina*
- Northern fur seal *Callorhinus ursinus* (Marine Mammal Care Center, 2009).

The Marine Mammal Care Center is in partnership with the Oiled Wildlife Care Network. Permitted by the Office of Spill Prevention and Response, the Marine Mammal Care Center responds to all species of marine mammals exposed to petroleum products along the coasts of Los Angeles and Ventura Counties (Marine Mammal Care Center, 2009).

The center's rescue range includes Ventura and Los Angeles Counties (Marine Mammal Care Center, 2009). Rescue crews consist of volunteers from various wild animal rescue organizations. These volunteers capture animals on the beach for transport to the center. The Marine Mammal Care Center is a smaller facility than the Marine Mammal Center, although the capacity is equal at 200 animals (Palmer, Pers Comm, 2013). The Marine Mammal Care Center does not rehabilitate oiled cetaceans; instead, they transport the animals to specialized facilities for further treatment (The Marine Mammal Care Center, 2009).

The majority of staff consists of trained volunteers. These volunteers work closely with Dr. Palmer D.V.M. with daily husbandry procedures. Unlike the Marine Mammal Center, the Marine Mammal Care Center does not have a full veterinary staff. Dr. Laura Palmer is the only veterinarian onsite who oversees all animals and volunteers (Personal observation, 2013).

The Marine Mammal Care Center has six in ground freshwater pools in pens 1-6. The pools in pens 1-4 hold a capacity of 3,000 gallons of water each. Pen 5 has a single pool with a total capacity of 5,000 gallons of water. The largest pool is located in pen 6, with a capacity of 13,000 gallons of water. All other enclosures are dry. Hard plastic, wading pools take the place of in ground pools in large dry pens. These wading pools have a constant supply of running water from a pipe above the pool. Volunteers hose down pens without wading pools to keep the animals cool. During periods of high admit loads, corridors are closed off and supplied with wading pools and access to shade. These serve as additional pen space.

Tarps cover a portion of all enclosures to provide shade. Dog carriers or custom-built wooden platforms provide shelter. Wading pools or small plastic basins provide freshwater (Personal Observation, 2013).

The public can view animals from behind a fence that is about 6 feet away from animal enclosures. The public is unable to enter the animal care area and is restricted to the right side of the facility at all times (Personal observation, 2013).

Dr. Palmer is responsible for all medical care whereas volunteers conduct all husbandry care including the administration of medications and fluids. Pen cleaning takes place daily. Once all husbandry procedures are complete and animals have received all food, medications, and exams, further physical contact is limited.

Methods

I conducted continuous focal animal scans (methodology approved by IACUC control #147-398-13-0605) on *Z. californianus* in three dry pens. Criteria for observation was identical to those at the Marine Mammal Center. Dr. Palmer provided a verbal list of available pens with animals, without listing specific animals. The single blinded survey techniques were identical to those at the Marine Mammal Center as were all diagnostic procedures.

During the time of the observation, a bloom of *Pseudonitzschia* was present off the coast of Southern California (Palmer, Pers. Comm. 2013); however, I became aware of the bloom after the fact. Observations took place between 14:00-16:00. Animal care volunteers were present during the entire observation period. During periods of volunteer and animal interaction, I halted observations until the interaction terminated. During these times, I upheld the 15-minute maximum observation length, to prevent methodological bias. I ended the observation if the 15-minute timeframe expired while the interaction continued. Volunteer personnel were also present in the same walkways as myself. I did not terminate observations due to volunteer presence in the walkway, as this would have prevented all observation, (the Marine Mammal Care Center has a higher level of volunteer activity than the Marine Mammal Center).

I conducted my observations outside the pen, behind a canvas blind. The blind was identical to that used at the Marine Mammal Center (see page 14) except for shorter individual pipe length (requiring more couplings to attain height used at the Marine Mammal Center) due to airline travel restrictions. This made the blind a little less sturdy. All observation termination protocols were identical to those used at the Marine Mammal Center.

Data recorded during the observation included the start and stop time of the observation, the animal's last three field number digits that were clipped into the fur along the back, along with behavioral states. Roto tags were not present on all animals.

Abnormal behavioral criteria, observational time increments, and severity score methodology were identical to those used at the Marine Mammal Center. Once I had observed five animals, I halted the observation day due to travel and sunset time. Patient files were unavailable to me until this time, to prevent bias. Data recorded were identical to recordings done at the Marine Mammal Center with the exception of stranding locations and the reports from stranding crew. For stranding locations, I logged specific beaches within Los Angeles County. I was unable to use all data reported by the stranding crews, as the stranding sheet did not include consistent abnormal behavioral information seen on the beach compared to the Marine Mammal Center.

Statistical Analysis

I categorized and logged data using methodology identical to that used at the Marine Mammal Center. I ran the Wilcoxon Signed Rank test against the mean values of each abnormal behavior seen at the Marine Mammal Center by the comparison group.

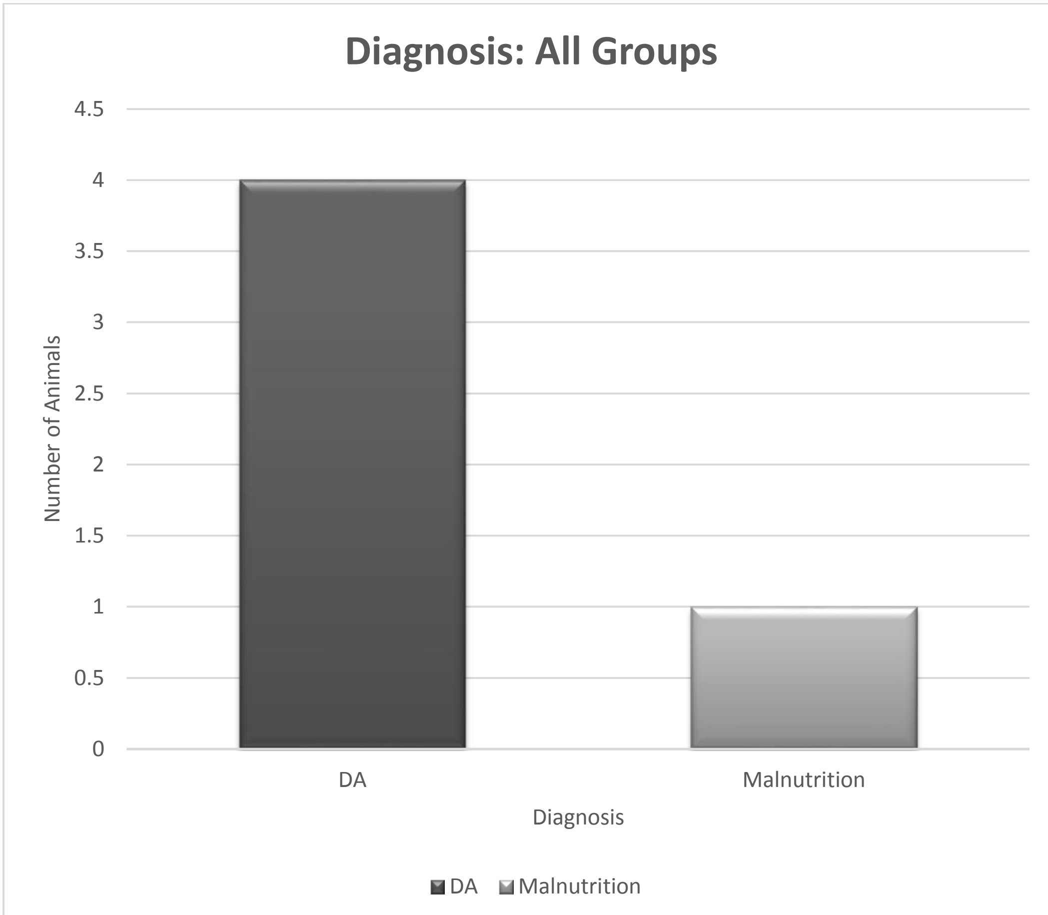
Results

I was in weekly contact with the Marine Mammal Care Center regarding the admit load of animals with domoic acid toxicosis. My criteria for traveling to the center was an admit number of >2 animals with a diagnosis of domoic acid toxicosis. In previous years, the center had seen admit loads in the 10s of animals (Palmer, Pers. Comm. 2013). Between February-August 2013, a single stranding event occurred that resulted in four animals with domoic acid toxicosis being onsite at one time. Consequently, I only had a single opportunity to observe the animals. The paucity of animals with domoic acid toxicosis at the Marine Mammal Care Center was unexpected but unavoidable. In an attempt to broaden the dataset, I maintained weekly contact with the Pacific Marine Mammal Center in Laguna Beach and the North Coast Marine Mammal Center in Crescent City, using identical admit load criteria; however admit loads remained low throughout the period.

I conducted five focal animal continuous scans on *Z. californianus* at the Marine Mammal Care Center on 5/16/2013 for a total of one and half hours. Data collection began once Dr. Palmer gave consent at around 14:25, which was before the final feed of the day. Animal care volunteers were sporadically present during the observation. Personnel did not leave the area until just before sunset when visibility and time became limiting. Therefore, I could not wait for quiet conditions to conduct my study. I conducted observations between 14:33-15:58. Of the five animals observed, four had a diagnosis of domoic acid toxicosis.

Diagnoses included domoic acid toxicosis and malnutrition. Because of the smaller sample size, the range of diagnoses seen at the Marine Mammal Center was not present in this dataset. All animals within the domoic acid toxicosis sample had a single diagnosis of domoic acid toxicosis. The animal in the comparison sample had a diagnosis of malnutrition. Within this dataset, diagnoses did not span the two groups. This is most likely the result of the small sample size. Although not intended by the observer, the study included proportionally more domoic acid toxicosis animals to comparison animals than were represented by the population at the Marine Mammal Care Center. This was the result of pre designated observation pen locations and availability of animals during observations. Therefore, a ratio of animals with a diagnosis of domoic acid toxicosis to animals with another diagnosis would be heavily biased and not representative of the population at the Marine Mammal Care Center.

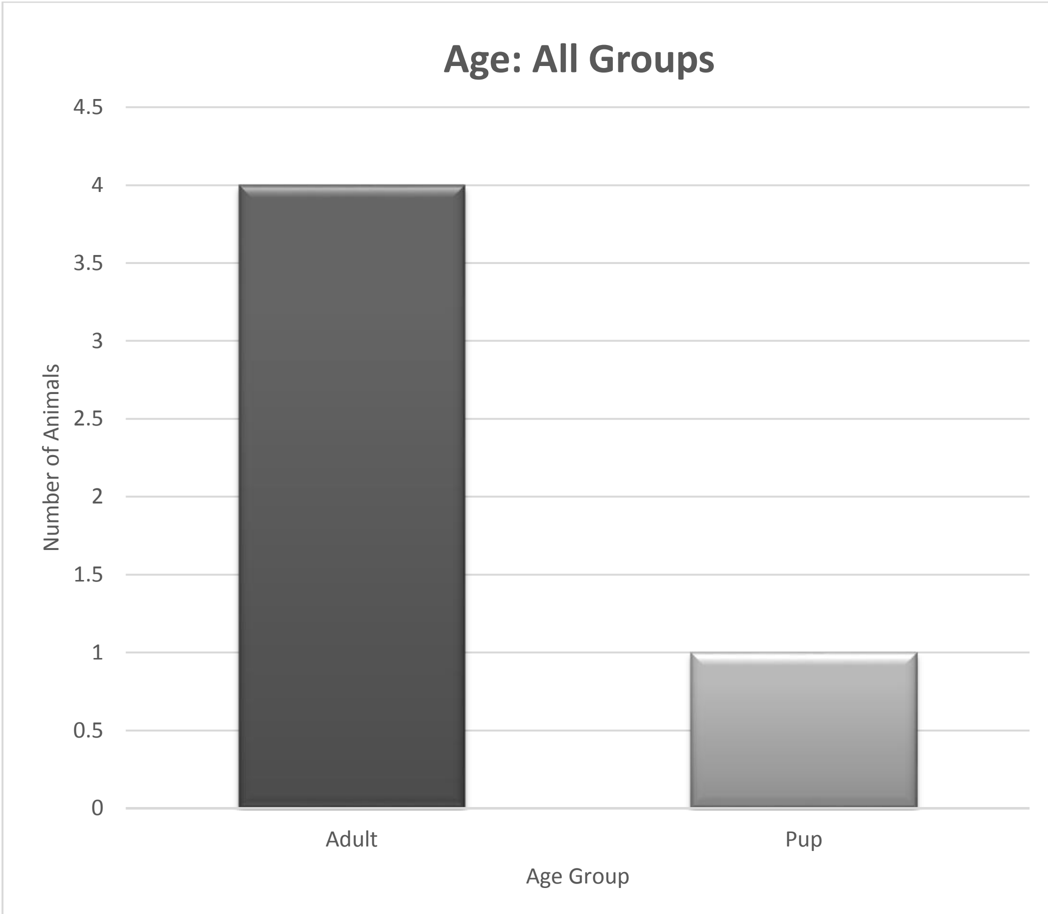
Figure 19: Frequency of diagnoses seen in animals spanning both the domoic acid toxicosis and comparison groups. All animals had only a single diagnosis.



DA represents domoic acid toxicosis.

The sample consisted of four adults and one pup. Age was determined via length, tooth eruption, and the presence of a sagittal crest in males. All adults were in the domoic acid toxicosis sample whereas the pup was in the comparison sample. I did not observe juveniles, yearlings, and sub adults due to either a lack of animals within the age group onsite or the small sample size.

Figure 20: Age groups of all animals observed at the Marine Mammal Care Center.



The stranding location of all animals remained within Los Angeles County. The Marine Mammal Care Center's rescue range - at that time - was limited to the county lines. Therefore, I did not consider locations within the statistics of this study.

The average length of animals within the domoic acid toxicosis sample was 157.5cm with the min at 155cm and the max at 160cm. The animal within the comparison group measured 86cm in length. The average weight of the animals within the domoic acid toxicosis sample was 85.33kg with the min at 71.5kg and the max at 108.5kg. The single animal within the comparison sample weighed 10kg.

Table 15: Marine Mammal Care Center sample:

Animal Dataset from the Marine Mammal Care Center					
ID	Age	Gender	Length (cm)	Weight (kg)	Diagnosis
13-541	Adult	Female	155	71.5	DA
13-540	Adult	Female	160	108.5	DA
13-539	Adult	Female	155	75.3	DA
13-544	Adult	Female	160	86	DA
13-533	Pup	Female	86	10	Malnutrition

I used the same abnormal behavioral criteria as was used at the Marine Mammal Center. During focal animal continuous scans, documentation of three abnormal behaviors occurred. Abnormal behaviors were as follows, with an asterisk-representing occurrence across groups:

- Head Weaving
- Muscle Fasciculations
- Scratching*

Rescue crew volunteers documented seizing in three of the five animals. The single animal from the comparison sample (ID 13-533) seized on the beach before or during rescue. Animals 13-540 and 13-539 seized during admit. A distinction between grand maul seizures and muscle fasciculations was lacking; therefore, I could not assume that all seizing events were grand maul. I did not observe grand maul seizing during focal animal continuous scans.

Because of the small sample size, I tested my results against the mean values obtained from the comparison sample at the Marine Mammal Center. Similar to my results from the Marine Mammal Center, animals with domoic acid toxicosis, at the Marine Mammal Care Center, displayed head weaving significantly more often than the comparison sample at the Marine Mammal Center (Wilcoxon, S 2, Z 8.8981, $p<0.0001$). Results for muscle fasciculations were also significant (Wilcoxon, S 2, Z 1.9660, $p=0.0493$). I did not observe animals dragging their hind flippers. This could be due to a small enclosure with multiple animals. I also could not determine whether animals were displaying swift scanning due to constant activity around the pen by staff, volunteers, and animals. This calls for an evaluation of surroundings before diagnostic criteria are determined.

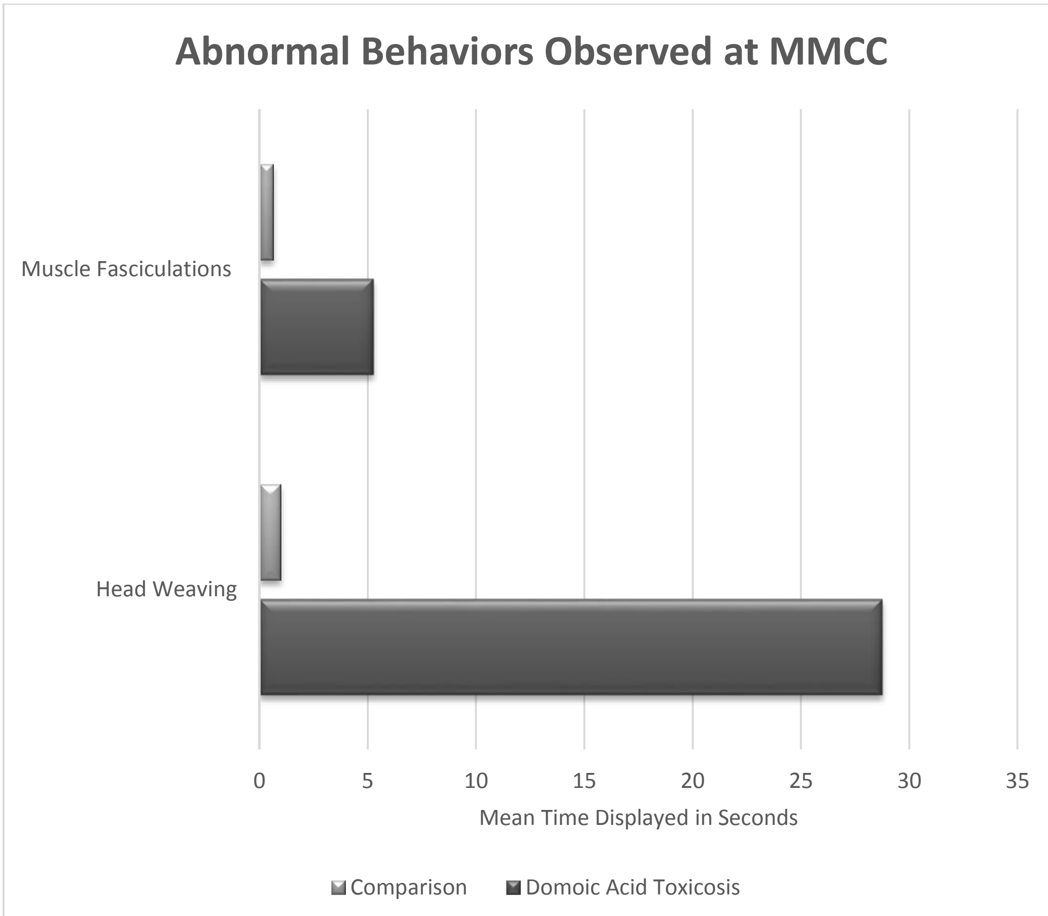
Table 16: Descriptive statistics from the Wilcoxon test for head weaving and muscle fasciculations. Significant results indicate that the behavior is a relevant diagnostic indicator in differing environments.

Behavior	Number	Mean Time (Seconds)	SD	Singed Rank	Z	p Value
Head Weaving	2↓	28.75	56.17	2	8.8981	<0.0001*
Muscle Fasciculations		5.25	7.08	2	1.966	0.0493*

** Represents significance with α set at 0.05.*

↓Represents identical subsample sizes.

Figure 21: Mean amount of time that animals from the domoic acid toxicosis sample, at the Marine Mammal Care Center, displayed head weaving and muscle fasciculations compared to the comparison sample at the Marine Mammal Center.



I observed two head weaving subtypes, classic and circle and two muscle fasciculation subtypes, head and full body. The small sample size restricted the use of statistics. Further research and a larger sample size may determine whether subtypes carry throughout differing environments.

I assigned severity scores for all abnormal behaviors observed. All animals that displayed head weaving received a severity score of 3. Only scores 2 and 3 were assigned to animals observed with muscle fasciculations. Because of the small sample size, I was unable to test these scores statistically.

Discussion

My data indicate that environmental conditions did influence diagnostic criteria. Although some of the criteria were observed (head weaving and muscle fasciculations), others were not discernable due to limited pen space and heightened visual and audio stimuli. Implications of these results demand careful examination of environmental conditions prior to the use of diagnostic criteria.

Environmental conditions did not affect head weaving and muscle fasciculations. My results concurred with those from the Marine Mammal Center. Differences between the two hospitals included: the use of freshwater versus seawater, high versus low volunteer activity, and crowded versus uncrowded enclosures.

I did not observe animals dragging their hind flippers. Although it is possible that animals with domoic acid toxicosis did not display this behavior at the Marine Mammal Care Center, I could not come to that conclusion, as the enclosures were either smaller or filled to a higher capacity than those at the Marine Mammal Center. Animals did not have proportionate space to walk. If an animal did drag the hind flippers, the movement was too small or too quick for me to discern. An animal must have ample space (I recommend 3 animal lengths or more) to move. If space is lacking, dragging the hind flippers is unsuitable as diagnostic criteria.

Discerning swift scanning from normal scanning was impossible. The assiduous environment at the Marine Mammal Care Center consists of busy volunteers and sometimes-boisterous animals (visual and auditory stimuli were constant during observations, whether from a volunteer caring for an animal or from an animal vocalizing). Swift scanning behavior is appropriate for use as diagnostic criteria only when conditions are quiet and animals are still. If any type of stimuli that alerts other animals or has the potential to is present, normal, reactive scanning is confusable with swift scanning.

Behavioral subtypes and severity scores were not significant. Two possibilities may explain this inconsistency. The first is the sample size, 169 animals at the Marine Mammal Center versus 5 animals at the Marine Mammal Care Center. The small sample size and the range of subtypes and severity scores restricted statistical testing, limiting analysis. The second possibility is that environmental conditions influence behavioral subtypes and severity scores. Because of the inconclusiveness of the results, I purpose a continuation of the study to expand the sample size. This is achievable by using the diagnostic criteria method and logging the results into a spreadsheet or database for later analysis (see appendix V).

Field Haul out Location: Pier 39

Pier 39 is located in San Francisco California. A popular tourist destination, Pier 39 consists of shops, restaurants, and attractions such as whale watching, sea lion watching, and carnival style rides, along with a large marina. In January of 1990, *Z. californianus* began hauling out onto K dock (Pier 39, 2013).

There are 41 small floating docks at the pier (see appendix IV). Between August and May, up to 1,701 *Z. californianus* haul out on the floating docks at any given time. The supply of baitfish in the area is normally plentiful. A sea wall prevents most predators from entering the marina (Pier 39, 2013). A two deck viewing area allows visitors to observe sea lions 50 feet away from the floating docks (Personal Observation, 2013). Docents from the Aquarium of the Bay, a local attraction at the pier, are available to answer questions from tourists and provide guests with informative lectures about the sea lions (Pier 39, 2013). Vehicles drive over the viewing deck in times of emergency or heightened security. Food venders are present during opening hours. Vessels including private and public tour vessels pass through the marina, as does the United States Coast Guard (Personal Observation, 2013). The marina manager hoses down the floating docks during routine dock cleanup work (Pier 39, 2013).

Z. californianus is the most common species at Pier 39 however; a single harbor seal (*Phoca vitulina*) has hauled out on a floating dock away from *Z. californianus* since 05/2013. Additionally, western gulls (*Larus occidentalis*) and double crested cormorants (*Phalacrocorax auritus*) land on the floating docks.

<u>Methods</u>

I conducted focal animal continuous scans from the bottom platform directly in front of the sea lion viewing area. Tourists and docents were present, as were food venders, pier personnel, security, and vessel traffic. I stood within the crowd, without the blind, to avoid being conspicuous. Because the animals at this location were accustomed to the presence of humans, the blind would have drawn unnecessary attention to me.

To gain a clear view of the animals, I used Tasco 7 x 35 mm Zip Focus binoculars with a clear visible range of 140-1,000 meters. If the animal hauled out onto the front dock (<50 ft. from the observation deck) binoculars were not used.

Animals with either identifiable markings (blotches, tags, brandings, coloration differences, etc.) or single animals were included in the study. I photographed each animal with a Kodak Easy Share c330 digital camera prior to observation with the exception of one occasion when the camera malfunctioned. I then conducted focal animal continuous scans on each identifiable animal for up to 15 minutes. Abnormal behavioral criteria were identical to that used at the Marine Mammal Center, as were observation and documentation techniques. I terminated the observation if an animal showed signs of aggression towards visitors or paced the dock for at least 20 seconds. On one occasion, a visitor jumped into the harbor with the animals. I terminated the observation until 5 minutes after personnel had removed the perpetrator because of animal excitement.

Data recorded during the observation included the start and stop time of the observation, any identifying marks or identifying dock locations, along with behavioral states. I documented all legible tags and branding numbers for later follow-up with the tagging organization. I obtained algal bloom information from the California Department of Public Health via their website. A delay in water testing results and the publication of harmful algal bloom locations along with concentration ensured a single blinded survey technique.

Statistical Analysis

I recorded data including identifying marks, behavioral state, abnormal behavior displayed, total time of displayed abnormal behavior, severity scores, and identifying information such as roto tag and brand numbers in a field journal. I then transferred that information to a spreadsheet. I used the harmful algal concentration classification system that the California Department of Public Health uses, which separates concentrations into the following categories: absent, rare (<1%), present (1-9%), common (10-49%), and abundant (>50%) (California Department of Public Health, 2013).

<u>Results</u>

Between 3/21/2013-8/12/2013, I conducted focal animal continuous scans for a total of five hours on 19 animals. Observations took place between 15:15-18:09, on four separate observation days. Animal health could not be determined for the entire sample. Some animals displayed tags and or brands, indicating inclusion in various field studies; however, I could not reliably discern brand numbers; discoloration of tags was also prevalent. Therefore, I was unable to identify those animals.

Table 17: Field haul out location sample. Note that "Given" refers to an animal receiving a temporary ID number for this study only.

		Animal Dataset from Pier 39			
ID	ID Type	Identifying Characteristics	Gender	Age	Date
1	Given	Alone on dock	Male	Adult	3/21/2013
2	Given	Gray Face	Unknown	At least a juvenile	3/21/2013
3	Given	Mottled Face	Unknown	At least a juvenile	3/21/2013
4	Given	Alone on dock	Male	Juvenile	3/21/2013
5	Given	Missing patches of fur	Unknown	At least a juvenile	3/21/2013
6	Given	Blond	Unknown	Juvenile	3/21/2013
7	Given	Alone on dock	Unknown	Juvenile	4/11/2013
8	Given	Face in water	Female	Adult	4/11/2013
9	Given	Alone on dock	Male	Adult	4/11/2013
10	Given	Blond	Female	Adult	4/11/2013
11	Given	Drooling off dock	Male	Adult	4/11/2013
12	Given	Under Sign	Male	Juvenile	6/6/2013
U288 / 28?	Orange Roto / Brand	Brand and roto tag	Male	Adult	6/6/2013
TMMC 1	TMMC Orange Roto	Orange Roto	Male	Juvenile	6/6/2013
1611 / J391?	Orange Roto / Brand	Brand and roto tag	Male	Adult	6/6/2013
13	Given	Alone on dock	Juvenile	Male	8/12/2013
14	Given	Alone on dock	Unknown	At least a juvenile	8/12/2013
15	Given	Light brown	Male	Juvenile	8/12/2013
16	Given	Blond	Unknown	At least a juvenile	8/12/2013

I determined animal age by the estimation of length and the presence or absence of a sagittal crest in males. The sample lacked pups and yearlings. Within this sample, I placed all animals of unknown gender in the “at least juvenile” age category. This age category implied that the animal was not a pup or yearling but could be a juvenile, sub adult, or adult. Gender was determined from the presence of a sagittal crest, the placement of a roto tag from the Marine Mammal Center (LFF for males and RFF for females) or from the observation of external genitalia.

Between the observation days of 3/21/2013 and 6/6/2013 the level of domoic acid in the water was at the present level (1-9%) as determined by the California Department of Public Health. Exact levels on the 8/12/2013 observation day are not available. Even so, bloom levels of *Pseudonitzschia* were not present during the month of August (Langlois, Pers. Comm. 2013). Animals did not display abnormal behavior on 8/12/2013.

The only abnormal behavior within the diagnostic criteria observed was muscle fasciculations. Animal 2 displayed muscle fasciculations of the head for 7 seconds. These muscle fasciculations may have been in response to the presence of multiple houseflies (*Musca domestica*) around the animal. I did not observe head weaving, swift scanning (too much activity), or dragging the hind flippers within the sample. Consequently, I could not test abnormal behaviors against levels of domoic acid present in the water.

Although not a part of the diagnostic criteria, animals at Pier 39 displayed significantly more scratching than did comparison animals at the Marine Mammal Center (One Way Wilcoxon, DF 18, Test Statistic 11.2686, $p<0.0001$) with a mean of 43.26 seconds. Although not prevalent to this study, I observed that many of the animals were missing patches of fur in circular patterns. I recommend further investigation into the health of the skin and fur, as the animals may be suffering from lice or other skin and fur disorders.

Discussion

My results are inconclusive. Although I did not observe animals displaying diagnostic criteria - with the exception of animal 2 - I also did not conduct observations during blooms of domoic acid (because of the single blinded survey method, I was unaware of bloom events until approximately two weeks post observation). Therefore, I was unable to fully test whether diagnostic criteria are applicable to field diagnostics.

I was able to determine that, during periods of non-bloom level concentrations of domoic acid, animals did not display diagnostic criteria, and therefore, I was able to rule out domoic acid toxicosis in these animals. However, during the week of 3/17/2013, animal 2 did display muscle fasciculations. It is possible that animal 2 had domoic acid toxicosis and had traveled from a bloom (during that week, the Marine Mammal Center admitted at least one animal from Monterrey with domoic acid toxicosis). Because this was an isolated event, I can conclude that animal 2 was an outlier.

Z. californianus with domoic acid toxicosis may haul out in locations void of blooms. Research concerning the travel of affected animals from bloom locations is lacking. If travel away from bloom sites is common, then diagnostic criteria is impractical in the determination of potential bloom locations. However, Dr. Palmer has seen many cases of *Z. californianus* strandings occurring before the detection of nearby blooms (Palmer, Pers. Comm. 2013), suggesting sentinel feasibility.

Like results from the Marine Mammal Care Center, I was unable to identify swift scanning. Because *Z. californianus* normally haul out in groups, deciding whether an animal is displaying swift scanning behavior instead of scanning is challenging. Proper environmental conditions must exist before swift scanning is confirmable.

Results from this study suggest that it may be possible to determine the absence of domoic acid toxicosis in a hauled out population of *Z. californianus*. I recommend further research with a larger dataset and an increased geographical range to raise the chance of observation days occurring at sites of blooms.

Urine, Fecal, and Blood Domoic Acid Levels

The main study site, the Marine Mammal Center, has an extensive database of every animal admit that includes all laboratory test results. All animals that receive an admit examination have blood drawn by veterinary staff. Veterinary staff may obtain urine through a catheter or volunteers may obtain urine from the pen floor during routine pen cleaning procedures. Additionally, volunteers may obtain feces from the animal via the pen floor. Veterinarians test these samples for the presence of domoic acid. A positive result will indicate that the animal has a diagnosis of domoic acid toxicosis. A negative result does not exclude the diagnosis because of the 2-48 hour circulatory, digestive, and urinary excretion window.

Methods

I used the File Maker database system at the Marine Mammal Center, Veterinary Science Department, to obtain domoic acid levels in feces, urine, serum, and blood, within my dataset. I documented the type of sample taken and the levels of domoic acid (measured in ng/ml) in a spreadsheet.

Results

Of the 50 animals within the domoic acid toxicosis sample at the Marine Mammal Center, tests for domoic acid in feces, urine, and serum tested positive in 14 animals or 28% of the sample. Test results using blood were not positive for any animals within the sample. Consequently, standard diagnostic procedures accounted for 28% of all diagnoses of domoic acid toxicosis at the Marine Mammal Center. MRI imagining, necropsy, or the process of elimination functioned as the diagnostic methods of choice in at least 72% of the sample.

Feces was the most reliable specimen for domoic acid testing, with 12 positive results ranging between 4-21,804ng/g. Urine and serum specimens tested positive on a single occasion with values of 3.7 and 1.2 ng/g respectively.

Table 18: The Marine Mammal Center subsample that tested positive for domoic acid:

Animal Dataset

Tag #	DA Level ng/g	Type of Sample
27152	30.2	Feces
27054	3.7	Urine
27196	60.8	Feces
27162	4.1	Feces
27070	4	Feces
27065	1.2	Serum
25976	1874	Feces
25952	21804	Feces
25982	6.2	Feces
27135	22.5	Feces
27268	487.7	Feces
27508	1980	Feces
27522	1620	Feces
27360	249	Feces

Although 12 animals tested positive for domoic acid in fecal samples, the data was insufficient for statistical analysis. Only two animals within the sample displayed swift scanning. Three animals displayed muscle fasciculations. Although seven animals displayed head weaving, the data was highly skewed. Therefore, a correlation was not evident. Behavioral subtype data was also limited and not suitable for statistical analysis. Finally, severity score data was too limited for statistical testing.

Table 19: Descriptive statistics depicting a lack of relationship between the levels of domoic acid detected in the feces and the length of time that a behavior was displayed.

Tag #	DA ng/g	Head Weaving (Seconds)	Muscle Fasciculations (Seconds)	Swift Scanning (Seconds)
27070	4	1	0	0
27162	4.1	8	6	0
25982	6.2	21	0	0
27135	22.5	53	0	25
27152	30.2	0	0	240
27196	60.8	0	2	0
27360	249	36	0	0
27268	487.7	501	0	0
27522	1620	0	0	0
25976	1874	0	0	0
27508	1980	0	181	0
25952	21804	10	0	0

Table 20: Descriptive statistics for the relationship between fecal levels of domoic acid and behavioral subtypes.

Tag #	DA ng/g	Head Weaving Subtype	Muscle Fasciculations Subtype
27070	4	Classic	
27162	4.1	Craning	Whole Torso
25982	6.2	Classic	Whole Torso
27135	22.5	Back	
27196	60.8		Both Front Flippers
27360	249	Cannot Keep Head Still	
27268	487.7	Prolonged	
27508	1980		Whole Torso
25952	21804	Slight	

Table 21: Descriptive statistics for head weaving and muscle fasciculation severity scores and levels of fecal domoic acid.

Tag #	DA ng/g	Head Weaving Severity Score	Muscle Fasciculations Severity Score
27070	4	1	
27162	4.1	1	3
25982	6.2	3	
27135	22.5	3	
27196	60.8		1
27360	249	3	
27268	487.7	3	
27508	1980		3
25952	21804	2	

Discussion

The results from this study are inconclusive. Because of the small percentage of animals that tested positive for domoic acid toxicosis through feces and the range of abnormal behaviors within the diagnostic criteria, I was not able to test my results. Upon visual analysis, it appears that a correlation between the levels of domoic acid detected in the feces and the type, severity, and subtype of behaviors displayed is lacking. This may be the consequence of a knowledge gap in the degeneration rate of domoic acid in the digestive track or the small sample size.

To date, studies have not addressed the issue of domoic acid levels over time in feces for *Z. californianus*. The toxin remains detectible for at least 2-48 hours post ingestion (Monte, Pers. Comm. 2012). However, whether toxin reduction occurs over time and the rate of that reduction remains unknown. Furthermore, because pinnipeds have a rate of digestion totaling under 5 hours (Helm, 1984), it appears that in some instances, domoic acid may remain in the system after the initial digesta has been egested. I recommend a study investigating the issue of domoic acid degeneration within feces over time. Because feces was the most reliable indicator of domoic acid in this study, it may also prove useful in future analysis. Only with this information will the possibility of future triage studies using feces be possible.

Conclusion

Since the identification of domoic acid toxicosis in 1998, veterinarians have lacked tools that help them reliably make this diagnosis. Often, the only option is the process of elimination, leaving room for scrutiny and uncertainty. (Although MRI technology is available and highly reliable, the cost and risks associated with it make it impractical for routine diagnostic use.) Even though the veterinary sciences rarely employ behavior, my research has shown that abnormal behavioral criteria is an effective tool in the diagnosis of domoic acid toxicosis in *Z. californianus*.

The studies at the Marine Mammal Center, the Marine Mammal Care Center, and Pier 39 were robust because of the large sample size. Methodology was consistent throughout the various environments. The use of a single observer (myself) reduced the possibility of observer bias. Furthermore, veterinarians assigned all diagnoses using standard, accepted, protocols.

Potential limitations included possible errors in documentation via stranding crew. The Marine Mammal Center trains all-stranding crew; however, it is possible that errors occurred within the filing system. To compensate, I compared both written and database notes against each other for inconsistences. When an error occurred, I used the data from the database because the employees flag and correct mistakes before data entry.

Guidelines

For consideration as an accurate form of diagnostics, practitioners must adhere to the following guidelines: Observations should take place by the stranding crew – if possible - (before or during rescue) and veterinary personnel/crew members (at the rehabilitation center). Observations at the center should last 15 minutes per animal (this period does not include observations conducted by stranding crew). Observers should stand in a secluded area or behind a blind. The animal should remain in view for the duration of the observation. Interaction between the observer and the animal should not occur until after the observation is complete.

The observer should document the following: Basic animal information such as ID, age, gender, and medications prescribed. Documentation must contain diagnostic criteria including abnormal behavior, subtype, and severity scores (see Appendix V for diagnostic forms). Timing of all abnormal behaviors is highly recommended and observers should use a stopwatch or other reliable device and record in units of seconds.

Observers should ensure that environmental assumptions are met. If enclosures are too small or too crowded to allow ample movement (3 animal lengths of free space in a single direction) then dragging the hind flippers is not a suitable measure of domoic acid toxicosis. The same is true for animals displaying hind limb or back torso paralysis. In these circumstances, animals must meet other diagnostic requirements. Observers should never move an animal from a crowded enclosure and encourage it to walk to determine if the animal drags the hind flippers. If a larger area is available, animal care crew may place the animal in the enclosure and allow it to settle for at least 30 minutes prior to observation.

To consider swift scanning as a valid measure of diagnostics, the animal enclosure and adjacent areas must be free of excessive audio and visual stimuli. To determine the level of stimuli, observers should scan the area prior to conducting observations. The area should be clear of stimuli that could reasonably alert neighboring animals (e.g. boisterous animals, loud noises, or presence of personnel). If other animals are not present, then the observer should use his or her own judgment and experience to determine whether stimuli is high enough to warrant increased alertness. Additionally, either the observer or an assistant should scan the surroundings if the focal animal begins displaying swift scanning behavior. The behavior is only valid if audio and visual stimuli are below levels that alert other animals.

<u>Diagnostic Method</u>

Z. californianus should receive the diagnosis of domoic acid toxicosis with the display of at least one of the following:

- Head weaving lasting ≥12.4 seconds. Head weaving with a severity score of 3 regardless of the time threshold. Classic head weaving lasting ≥1.26

seconds. Additionally, I recommend consideration for animals displaying the subtypes cannot keep head still, craning, and prolonged.

- Muscle fasciculations lasting ≥9.36 seconds or head muscle fasciculations lasting ≥7.76 seconds. Additionally, I recommend consideration for animals displaying the subtypes, eyes and both front flippers.
- Dragging the hind flippers for any length of time with any severity score.
- Swift scanning for any length of time with any severity score.

Z. californianus can receive the diagnosis of domoic acid toxicosis with confidence if two abnormal behaviors are present. This does not apply to dragging the hind flippers and swift scanning. If animals display any of these two behaviors in the absence of all other diagnostic criteria, then the diagnosis of domoic acid toxicosis is highly appropriate assuming environmental assumptions have been met. Animals may be candidates for the diagnosis of domoic acid toxicosis if they display head weaving or muscle fasciculations but do not reach the timed cutoff value. In these cases, practitioners must substantiate diagnosis with other criteria listed above or from other diagnostic methodology.

In Field Use of the Diagnostic Criteria

Observers may be able to use diagnostic criteria to rule out domoic acid toxicosis within a group of *Z. californianus.* The observer should take care to conceal his or herself behind vegetation, rocks, or a blind in areas unfrequented by people. Concealment is not appropriate in areas of high human traffic, such as docks, as the act of hiding oneself may draw unnecessary attention.

Required documentation is identical to that listed above with the exception of animal information. Observers should list location and leave gender and age blank unless known. Environmental criteria for dragging the hind flippers and swift scanning is identical except that enclosure space is not applicable. In this instance, the amount of room the animal has in relation to other animals (enough room to get up and maneuver) is sufficient. If the animal must walk on top of other animals or rocks to move, then dragging the hind flippers is not a reliable measure until the animal reaches a clear area, as statistical data from this study did not include climbing.

Use of Method for other Diagnoses and other Species

The methods described in this study are applicable for use in similar studies aiming to use abnormal behavior as a diagnostic tool. Most species are candidates as long as they are readily observable in captive or natural settings. These methods are most suitable to neurological disorders including infection, intoxication, and trauma.

The pros of behavioral diagnostics include low cost and ease. The con is observer error. Any future diagnostic protocols should include comprehensive testing and strict guidelines. Furthermore, all protocols should complement current diagnostic techniques and not serve as a replacement. Behavioral criteria is suitable in cases of low diagnostic reliability and urgent treatment needs (diagnosis can take 15 minutes compared to hours, days, or weeks). In extreme circumstances, behavioral criteria is also suitable when monetary funding does not allow for diagnostic testing.

Future Directions

In the case of *Z. californianus*, behavioral criteria is an effective tool for the diagnosis of domoic acid toxicosis. Future studies should focus on:

- Field diagnostics.
- Reduction rates of domoic acid in feces over time.
- Applicability of diagnostic criteria in other species with domoic acid toxicosis. (Similarities and differences between species may shed further insight into the disorder).
- The use of the methodological framework for identifying behavioral criteria for different diagnoses in *Z. californianus* and other species.
- Neuroanatomical and neurophysiological causes of gender disparities identified in this study.

If used appropriately, behavioral diagnostics for domoic acid toxicosis may help solve the problem that has been plaguing veterinarians since 1998: inconclusive test results. Behavioral diagnosis is quick, inexpensive, and reliable. If used in conjunction with standard procedures, the success rate of diagnosing domoic acid toxicosis in *Z. californianus* should improve dramatically. This will not only aid veterinarians in determining proper treatment in a timelier manner but also bolster future research efforts. This study showed that head weaving, muscle fasciculations, dragging the hind flippers, and swift scanning are all indications of domoic acid toxicosis in *Z. californianus*.

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Appendix I

Table 22: Sample from the Marine Mammal Center.

Animal Dataset from the Marine Mammal Center					
Tag #	Age	Gender	Length (cm)	Weight(kg)	Diagnoses
LFF 27167	Juvenile	Male	149	52.5	DA, Malnutrition
LFF 27168	Juvenile	Male	152	58.5	Malnutrition
LFF 25970	Juvenile	Male	140	42.5	Malnutrition
LFF 25900	Pup	Male	91	14	Malnutrition, Pneumonia, Abscess
RFF 27152	Sub Adult	Female	132	38	Malnutrition, DA
LFF 25924	Pup	Male	103	25	Abscess, Malnutrition
RFF 27195	Pup	Female	93	24.5	Abscess
RFF 27145	Adult	Female	150	65	Lepto
LFF 27143	Juvenile	Male	168	65	Lepto
RFF 25833	Pup	Female	111	18.5	Abscesses, Malnutrition
RFF 25940	Pup	Female	100	24	Malnutrition, Abscess
RFF 27190	Pup	Female	101	17	Malnutrition, Pneumonia
LFF 27187	Juvenile	Male	144	39.5	Head Trauma
RFF 27174	Sub Adult	Female	151	50	Entanglement
RFF 25919	Yearling	Female	108	22.5	Malnutrition, Lepto, Abscess
RFF 27165	Yearling	Female	99	17.5	Malnutrition, Pneumonia
LFF 27141	Juvenile	Male	131	52	Unknown
LFF 25554	Yearling	Male	115	25.5	Lepto, Pneumonia
RFF 27085	Juvenile	Female	120	26	Lepto
LFF 24485	Juvenile	Male	126	27	Lepto, Abscess
LFF 27066	Juvenile	Male	131	31	Lepto, Abscess
LFF 27045	Yearling	Male	128	31.5	Lepto
RFF 25907	Yearling	Female	109	19	Abscess, Pneumonia, Malnutrition
RFF 27162	Adult	Female	166	77.5	DA
RFF 27054	Adult	Female	167	59	DA , Seizures
LFF 27196	Juvenile	Male	167	58.5	DA
RFF 27013	Adult	Female	153	54	DA, Abscess
LFF 27100	Juvenile	Male	156	50.5	Lepto
LFF 27071	Yearling	Male	119	30.5	Pneumonia, Lepto
RFF 27159	Juvenile	Female	155	58.5	Lepto
LFF 27507	Yearling	Male	127	32.5	Entanglement
RFF 27091	Yearling	Female	124	29	Malnutrition, Lepto
LFF 27056	Juvenile	Male	137	39.5	Lepto

LFF 27043	Juvenile	Male	121	36	Lepto, San Miguel Sea Lion Virus
LFF 27019	Yearling	Male	118	27	Pneumonia
RFF 27070	Adult	Female	168	65.5	DA
RFF 27072	Adult	Female	168	87.5	DA, Corneal Edema
RFF 27025	Adult	Female	166	74	DA
LFF 27024	Yearling	Male	114	27.5	Malnutrition, Lepto
LFF 25803	Yearling	Male	99	18.5	Malnutrition
LFF 27039	Yearling	Male	112	29.5	Malnutrition, Lepto
LFF 27089	Juvenile	Male	135	29	Lepto
LFF 25980	Juvenile	Male	148	63	DA
LFF 25959	Juvenile	Male	156	50	Lepto
LFF 25914	Juvenile	Male	163	79	DA , Seizures, Trauma, Abscess
RFF 25988	Adult	Female	145	76.5	DA, Trauma
LFF 25996	Sub Adult	Male	188	125	DA
RFF 25998	Adult	Female	145	74	DA, Trauma
RFF 25969	Adult	Female	164	82.5	DA, Abscess
RFF 27093	Adult	Female	165	77.5	DA
RFF 27011	Adult	Female	171	81	DA
RFF 25923	Adult	Female	187	85	DA
LFF 27021	Juvenile	Male	139	37	Lepto
RFF 27065	Adult	Female	168	84	DA
RFF 25976	Adult	Female	159	69.5	DA
RFF 25976	Adult	Female	159	69.5	DA
RFF 25938	Sub Adult	Female	155	54.5	DA
RFF 27084	Adult	Female	167	92	DA
LFF 25997	Juvenile	Male	130	31	Lepto
LFF 25960	Sub Adult	Male	169	85.5	Lepto
LFF 25946	Juvenile	Male	130	29.5	Lepto, Septicemia, Abscess
LFF 25982	Adult	Male	190	128	DA
RFF 25952	Juvenile	Female	144	54	DA
LFF 25971	Juvenile	Male	148	47.5	DA
LFF 27136	Juvenile	Male	145	34.5	Malnutrition, Lepto
LFF 27118	Juvenile	Male	143	40	Lepto, Head Trauma
RFF 27135	Adult	Female	136	72	DA
RFF 25938	Juvenile	Female	157	49	DA
RFF 27132	Adult	Female	163	53.5	DA, Malnutrition
LFF 27130	Juvenile	Male	133	41	Lepto
RFF 27107	Juvenile	Female	135	40	Malnutrition, Lepto

LFF 27078	Juvenile	Male	121	28	Malnutrition
LFF 25965	Juvenile	Male	121	29.5	Malnutrition
LFF 27005	Juvenile	Male	129	30.5	Lepto, Malnutrition
LFF 27001	Juvenile	Male	134	42.5	Lepto, Malnutrition
LFF 27009	Juvenile	Male	149	47	Malnutrition, Lepto
LFF 27018	Juvenile	Male	201	64.5	Lepto
LFF 27122	Juvenile	Male	150	43	Malnutrition, Lepto
LFF 27076	Juvenile	Male	180	65	Lepto
RFF 27064	Sub Adult	Female	126	34.5	Lepto, Pneumonia
LFF 27026	Sub Adult	Male	190	92.5	Lepto
LFF 27268	Juvenile	Male	150	63.5	DA
LFF 27297	Juvenile	Male	198	77.5	Malnutrition, Lepto
RFF 27452	Yearling	Female	126	22.5	Malnutrition, Lepto
RFF 27289	Sub Adult	Female	127	27.5	Lepto
LFF 27279	Juvenile	Male	185	100.5	Lepto
LFF 27261	Juvenile	Male	150	39.5	Trauma, Pneumonia
LFF 27451	Juvenile	Male	160	64.5	Lepto, Pneumonia
LFF 27263	Juvenile	Male	149	50.5	Trauma, Abscess
LFF 27260	Juvenile	Male	145	55.5	Lepto
LFF 27453	Juvenile	Male	141	42	Lepto
LFF 27272	Juvenile	Male	180	84	Lepto
LFF 27293	Juvenile	Male	140	44.5	Lepto
LFF 27282	Juvenile	Male	122	32	Lepto
LFF 27278	Sub Adult	Male	151	41.5	Malnutrition
LFF 27284	Adult	Male	190	91	DA
RFF 27301	Adult	Female	161	53.5	DA, Oil/Tar
LFF 27335	Yearling	Male	94	15	Pneumonia, Malnutrition
LFF 27020	Juvenile	Male	172	92	Trauma
RFF 27313	Pup	Female	84	14	Lice, Malnutrition
LFF 27387	Adult	Male	202	164.5	Head Trauma
LFF 27402	Adult	Male	216	191	Trauma
LFF 27490	Sub Adult	Male	193	148.5	DA,
LFF 27365	Pup	Male	86	12.5	Pneumonia, Malnutrition
LFF 27478	Pup	Male	84	13.5	Malnutrition, Oil/Tar
RFF 27487	Pup	Female	81	11	Malnutrition
LFF 27400	Pup	Male	99	16.5	Malnutrition
LFF 27394	Pup	Male	77	10	Oil/Tar, Malnutrition
LFF 27407	Pup	Male	90	15.5	Abscess, Malnutrition
LFF 27399	Yearling	Male	104	16.5	Abscess, Malnutrition

LFF 27573	Yearling	Male	107	16	Malnutrition, Renal failure, Pneumonia
LFF 27575	Yearling	Male	111	20	Malnutrition, Abscess
LFF 27599	Yearling	Male	110	24	Abscess, Malnutrition, Pneumonia
RFF 27541	Yearling	Female	106	20	Trauma
RFF 27530	Yearling	Female	107	23.5	Abscess, Malnutrition, Osteomyelitis
LFF 27550	Yearling	Male	101	20	Pox Virus
LFF 27532	Yearling	Male	112	20	Osteomyelitis, Malnutrition
RFF 27543	Yearling	Female	111	18.5	Malnutrition
RFF 27549	Yearling	Female	104	17.5	Abscess, Malnutrition, Pneumonia
LFF 27538	Sub Adult	Male	170	96.5	DA
LFF 10387	Juvenile	Male	108	30.5	Lepto
RFF 27508	Adult	Female	161	61	DA
LFF 27545	Yearling	Male	110	18.5	Malnutrition, Head Trauma
RFF 27525	Adult	Female	164	78.5	DA, Head Trauma
LFF 27522	Sub Adult	Male	169	54	Trauma, DA, Head Trauma
RFF 27358	Sub Adult	Male	138	46	Trauma
LFF 27524	Yearling	Male	108	22.5	Abscess, Malnutrition
LFF 27360	Juvenile	Male	182	91.5	DA
RFF 27546	Adult	Female	167	84.5	DA
LFF 27509	Yearling	Male	104	20.5	Malnutrition, Pneumonia
LFF 27555	Juvenile	Male	157	72	Shark Bite
RFF 27518	Yearling	Female	103	19	Malnutrition, Head Trauma
RFF 27624	Sub Adult	Female	128	31	Lepto, Malnutrition
RFF 27639	Yearling	Female	121	24.5	Shark Bite
LFF 27644	Sub Adult	Male	161	87	DA
LFF 27634	Juvenile	Male	132	42	Lepto
RFF 27576	Juvenile	Male	125	26	Abscess
RFF 27533	Juvenile	Female	160.5	64	Lepto
LFF 27537	Juvenile	Male	125	35	Malnutrition
RFF 27628	Sub Adult	Female	134	27	Malnutrition
RFF 27643	Sub Adult	Female	148	54.5	Malnutrition, Azotemia

LFF 27646	Juvenile	Male	125	35	Malnutrition, Pneumonia, Trauma
LFF 27633	Juvenile	Male	163	70	Entanglement
LFF 27526	Juvenile	Male	123	37.5	Lepto, Pneumonia
LFF 27637	Sub Adult	Male	184	75	Lepto, Malnutrition
LFF 27699	Juvenile	Male	145	48	Malnutrition, Corneal Ulcer
LFF 27700	Sub Adult	Male	122	27.5	Malnutrition
LFF 27690	Juvenile	Male	183	81	Trauma
RFF 27667	Adult	Female	168	74.5	DA, Oil/Tar
LFF 27664	Yearling	Male	137	38	Malnutrition, Pneumonia
LFF 27687	Juvenile	Male	130	27	Malnutrition, Pneumonia
RFF 27652	Adult	Female	172	63	DA, Cardiomyopathy
RFF 23802	Adult	Female	167	56	DA, Cardiomyopathy, Head Trauma
RFF 23837	Adult	Female	149	74	DA, Cardiomyopathy
RFF 23958	Adult	Female	158	59	DA, Heart Murmur
None	Sub Adult	Male	142	51	Blind, Trauma, Malnutrition
RFF 23999	Adult	Female	154	66	Malnutrition, Cardiomyopathy
LFF 20779	Pup	Male	94	16.5	Malnutrition, Head Trauma
LFF-RFF 1964	Pup	Male	97	16.5	Malnutrition, Head Trauma
None	Juvenile	Male	128	33	Unknown
LFF 23557	Juvenile	Male	157	59	Pox Virus, Malnutrition, Dehydration
RFF 23545	Juvenile	Female	141	59	DA
RFF 23823	Adult	Female	170	81.5	DA
LFF 23931	Sub Adult	Male	168	74.5	Pneumonia, Trauma
RFF 23619	Sub Adult	Female	123	28.5	Malnutrition, Abscess, Osteomyelitis
RFF 23616	Yearling	Female	98	18.5	Entanglement, Malnutrition
LFF 23633	Sub Adult	Male	172	124	DA
None	Adult	Female	155	76	DA
RFF 23623	Adult	Female	177	110	DA

LFF represents the left front flipper
RFF represents the right front flipper
DA represents domoic acid toxicosis
Lepto represents leptospirosis

Appendix II

Table 23: Raw data from the Marine Mammal Center including animal ID, diagnoses, abnormal behaviors observed (time increments of seconds), severity scores assigned, and abnormal behaviors observed by stranding crew.

Animal ID	Diagnosis	Abnormal Behaviors	Severity Scores	Observations by Stranding Crew
LFF 27167	Domoic Acid Toxicosis	Grimacing 1	None	None
RFF 27152	Malnutrition, Domoic Acid Toxicosis	Twitching 180: Swift Scanning 240	Twitching 3: Swift Scanning 3	None
RFF 27054	Domoic Acid Toxicosis, Seizures	Scratching 6: Swift Scanning 56	Scratching 1: Swift Scanning 3	Head Weaving: Rolling
LFF 27196	Domoic Acid Toxicosis	Muscle Fasciculations (Both Front Flippers 1, Left Eye 1): Twitching 2	Muscle Fasciculations 1: Twitching 1	None
RFF 27162	Domoic Acid Toxicosis	Head Weaving (Craning) 8: Muscle Fasciculations (Entire Torso 3, Face 3): Twitching 3	Head Weaving 1: Muscle Fasciculations 3: Twitching 1	None

RFF 27013	Domoic Acid Toxicosis, Abscess	Head Weaving (Cannot Keep Head Still) 63: Grimacing 8	Head Weaving 3: Grimacing 1	None
RFF 27070	Domoic Acid Toxicosis	Head Weaving (Classic) 1	Head Weaving 1	Head Weaving
RFF 27072	Domoic Acid Toxicosis, Corneal Edema	Head Weaving (Classic) 9	Head Weaving 1	Head Weaving: Seizures
RFF 27025	Domoic Acid Toxicosis	Scratching 5	Scratching 1	Dragging Hind Flippers: Head Weaving: Muscle Fasciculations
LFF 25914	Domoic Acid Toxicosis, Seizures, Trauma (Flipper), Abscess	None	None	Muscle Fasciculations
LFF 25996	Domoic Acid Toxicosis	Dragging Hind Flippers 12: Scratching 129	Dragging Hind Flippers 2: Scratching 3	Head Weaving: Muscle Fasciculations

RFF 25998	Domoic Acid Toxicosis, Trauma (Flipper)	None	None	None
RFF 25969	Domoic Acid Toxicosis, Abscess	None	None	None
RFF 27093	Domoic Acid Toxicosis	Head Weaving (Classic) 23: Scratching 39: Doughnut 189	Head Weaving 3: Scratching 3: Doughnut 3	None
RFF 27011	Domoic Acid Toxicosis	Twitching 14	Twitching 1	Head Weaving: Muscle Fasciculations
RFF 25923	Domoic Acid Toxicosis	None	None	Head Weaving: Muscle Fasciculations
RFF 25988	Domoic Acid Toxicosis	Twitching 1	Twitching 1	Head Weaving: Muscle Fasciculations
LFF 25980	Domoic Acid Toxicosis	Head Weaving (Classic) 2: Circling 7: Floating 180	Head Weaving 1: Circling 1: Floating 2	Head Weaving: Muscle Fasciculation: Flapping Flippers: Seizures

RFF 27065	Domoic Acid Toxicosis	Head Weaving (Classic) 64	Head Weaving 3	Head Weaving: Muscle Fasciculations
RFF 25976	Domoic Acid Toxicosis	None	None	Head Weaving
RFF 25976	Domoic Acid Toxicosis	Head Weaving (Cannot Keep Head Still) 50: Scratching 18	Head Weaving 3: Scratching 1	None
RFF 25938	Domoic Acid Toxicosis	Head Weaving (Cannot Keep Head Still) 41: Muscle Fasciculations (Head) 26	Head Weaving 3: Muscle Fasciculations 3	None
RFF 25938	Domoic Acid Toxicosis	Head Weaving (Classic) 1: Scratching 20: Twitching 3: Head Shaking 15	Head Weaving 1: Scratching 1: Twitching 2: Head Shaking 3	None
RFF 27084	Domoic Acid Toxicosis	None	None	Head Weaving
RFF 25952	Domoic Acid Toxicosis	Head Weaving (Slight) 10: Scratching 30	Head Weaving 2: Scratching 2	None

LFF 25971	Domoic Acid Toxicosis	Head Weaving (Classic) 3	Head Weaving 1	Head Weaving
LFF 25982	Domoic Acid Toxicosis	Head weaving (Classic) 21: Twitching 67	Head Weaving 3: Twitching 3	Head Weaving
RFF 27135	Domoic Acid Toxicosis	Head Weaving (Straight Back) 53: Craning 8: Swift Scanning 25	Head Weaving 3: Craning 2: Swift Scanning 3	Head Weaving
RFF 27132	Domoic Acid Toxicosis, Malnutrition	Head Weaving (Cannot Keep Head Still) 54	Head Weaving 3	None
LFF 27268	Domoic Acid Toxicosis	Head Weaving (Prolonged) 501	Head Weaving 3	Muscle Fasciculations
LFF 27284	Domoic Acid Toxicosis	Dragging Hind Flippers 3: Head Weaving (Cannot Keep Head Still) 124	Dragging Hind Flippers 1: Head Weaving 2	Head Weaving

RFF 27301	Domoic Acid Toxicosis, Oil/Tar	Head Weaving (Classic) 235	Head Weaving 3	None
LFF 27490	Domoic Acid Toxicosis	Head Weaving (Classic) 21: Twitching 41	Head Weaving 2: Twitching 3	None
LFF 27538	Domoic Acid Toxicosis	None	None	Head Weaving: Muscle Fasciculation: Twitching
RFF 27525	Domoic Acid Toxicosis, Trauma Face	None	None	Head Weaving: Rolling
RFF 27508	Domoic Acid Toxicosis	Muscle Fasciculations (Head) 181	Muscle Fasciculations 3	Twitching
LFF 27360	Domoic Acid Toxicosis	Head Weaving (Cannot Keep Head Still) 36	Head Weaving 3	None

RFF 27546	Domoic Acid Toxicosis	Head Weaving (Classic) 1: Muscle Fasciculations (Half Torso) 2	Head Weaving 1: Muscle Fasciculations 2	None
LFF 27644	Domoic Acid Toxicosis	Scratching 2: Muscle Fasciculations (Head 48 and Entire Torso 4): Twitching 2: Swift Scanning 5	Scratching 1: Muscle Fasciculations 3: Twitching 1: Swift Scanning 3	None
RFF 27667	Domoic Acid Toxicosis, Oil/Tar	Muscle Fasciculations (Head) 481	Muscle Fasciculations 3	Head Weaving
RFF 23837	Domoic Acid Toxicosis, Cardiomyop-athy	Head Weaving (Classic) 7: Scratching 2: Muscle Fasciculations (Head) 15	Head Weaving 1: Scratching 1: Muscle Fasciculations 2	None
RFF 27652	Domoic Acid Toxicosis, Cardiomyop-athy	Scratching 68	Scratching 1	None

RFF 23802	Domoic Acid Toxicosis, Cardiomyop-athy, Trauma (Eye)	Twitching 14	Twitching 3	None
RFF 23958	Domoic Acid Toxicosis, Heart Murmur	Head Weaving (Up and Back) 24: Muscle Fasciculations (Entire Torso) 5	Head Weaving 3: Muscle Fasciculations 1	None
RFF 23545	Domoic Acid Toxicosis	Scratching 5	Scratching 1	None
RFF 23823	Domoic Acid Toxicosis	Head Weaving (Classic) 1: Muscle Fasciculations (Entire Torso) 15	Head Weaving 1: Muscle Fasciculations 2	Head Weaving
LFF 23633	Domoic Acid Toxicosis	Scratching 6: Muscle Fasciculations (Head) 10	Scratching 1: Muscle Fasciculations 2	Head Weaving

Not Known	Domoic Acid Toxicosis	Muscle Fasciculations (Half Torso) 14: Uncoordinat-ed Movements 2	Muscle Fasciculations 3: Uncoordinated Movements 1	Head Weaving: Muscle Fasciculation: Flapping Flippers
RFF 23623	Domoic Acid Toxicosis	None	None	Head Weaving
LFF 27168	Malnutrition	None	None	None
LFF 25970	Malnutrition	None	None	None
LFF 25900	Malnutrition, Pneumonia, Abscess	Scratching 8	None	None
LFF 25924	Abscess, Malnutrition	Scratching 28	None	Head Weaving
RFF 27195	Abscess	None	None	None
RFF 27145	Presumed Leptospirosis	Floating	None	None
LFF 27143	Lepto	Twitching 3	None	None
RFF 25833	Abscess, Malnutrition	Craning 4	Craning 1	None
RFF 25940	Malnutrition, Abscess	None	None	Head Weaving
RFF 27190	Malnutrition, Pneumonia	None	None	None
LFF 27187	Eye Trauma	None	None	None
RFF 27174	Entanglement	Drinking Seawater 282	Drinking Seawater 3	None

RFF 25919	Malnutrition, Leptospirosis, Abscess	None	None	None
RFF 27165	Malnutrition Pneumonia	Scratching 21	Scratching 2	None
LFF 27141	Unknown	None	None	None
LFF 25554	Leptospirosis, Pneumonia	Twitching 4	Twitching 1	None
RFF 27085	Leptospirosis	Grimacing 40: Muscle Fasciculations (Head) 2	Grimacing 3: Muscle Fasciculations 1	None
LFF 24485	Leptospirosis, Abscess	Grimacing 35	Grimacing 3	None
LFF 27066	Leptospirosis, Abscess	None	None	None
LFF 27045	Leptospirosis	Scratching 11	Scratching 1	None
RFF 25907	Abscesses, Pneumonia, Malnutrition	None	None	None
LFF 27100	Leptospirosis	Scratching 17: Muscle Fasciculations (Half Torso) 2	Scratching 3: Muscle Fasciculations 1	None
LFF 27071	Pneumonia, Leptospirosis	Floating 25	Floating 1	None
RFF 27159	Leptospirosis	None	None	None

LFF 27059	Entanglement	Muscle Fasciculations (Head) 1	Muscle Fasciculations 1	None
RFF 27091	Malnutrition, Leptospirosis	Drinking Seawater 133	Drinking Seawater 3	None
LFF 27056	Leptospirosis	Scratching 27	Scratching 2	None
LFF 27043	San Miguel Sea Lion Virus, Leptospirosis	None	None	None
LFF 27019	Pneumonia	Scratching 3	Scratching 1	None
LFF 27024	Malnutrition, Leptospirosis	None	None	None
LFF 25803	Malnutrition	Muscle Fasciculations (Whole Torso) 15: Drinking Seawater 5	Muscle Fasciculations 2: Drinking Seawater 1	None
LFF 27039	Malnutrition, Leptospirosis	Muscle Fasciculations (Whole Torso) 3	Muscle Fasciculations 1	None
LFF 27089	Leptospirosis	None	None	None
LFF 25959	Leptospirosis	Head Weaving (Straight Back) 22: Twitching 2	Head Weaving 2: Twitching 1	None

LFF 27021	Leptospirosis	Twitching 17	Twitching 2	None
LFF 25997	Leptospirosis	None	None	None
LFF 25960	Leptospirosis	Scratching 4	Scratching 1	None
LFF 25946	Leptospirosis, Abscess, Septicemia	None	None	None
LFF 27136	Malnutrition, Leptospirosis	Twitching 53	Twitching 3	None
LFF 27118	Leptospirosis, Trauma (Eye)	Grimacing 3	Grimacing 1	None
LFF 27130	Leptospirosis	None	None	None
RFF 27107	Malnutrition, Leptospirosis	Muscle Fasciculations (Head) 3: Twitching 5	Muscle Fasciculations 3: Twitching 3	None
LFF 27078	Malnutrition	None	None	None
LFF 25965	Malnutrition	None	None	None
LFF 27005	Leptospirosis, Malnutrition	None	None	None
LFF 27001	Leptospirosis, Malnutrition	Twitching 33	Twitching 2	None
LFF 27009	Malnutrition, Leptospirosis	None	None	None
LFF 27018	Leptospirosis	Scratching 31	Scratching 3	None

LFF 27122	Leptospirosis, Malnutrition	Muscle Fasciculations (Head) 3: Twitching 5	Muscle Fasciculations 1: Twitching 1	None
Not Known	Leptospirosis	None	None	None
RFF 27064	Pneumonia, Leptospirosis	None	None	None
LFF 27297	Malnutrition, Leptospirosis	Flapping Flippers 5	Flapping Flippers 1	None
RFF 27452	Malnutrition, Leptospirosis	Twitching 1: Head Shaking 9	Twitching 1: Head Shaking 3	None
RFF 27289	Leptospirosis	Head Weaving 5	Head Weaving 1	None
LFF 27279	Leptospirosis	Scratching 38: Flapping Flippers 4	Scratching 3: Flapping Flippers 1	None
LFF 27261	Trauma, Pneumonia	Grimacing 3: Scratching 45	Grimacing 2: Scratching 3	None
LFF 27451	Leptospirosis, Pneumonia	Scratching 36: Twitching 7	Scratching 3: Twitching 1	None
LFF 27263	Trauma, Abscess	Scratching 32	Scratching 3	None
LFF 27260	Leptospirosis	None	None	Mouth Chattering
LFF 27453	Leptospirosis	Scratching 15	Scratching 3	None
LFF 27272	Leptospirosis	None	None	None
LFF 27293	Leptospirosis	Rump Weaving 12	Rump Weaving 1	None

LFF 27282	Leptospirosis	Twitching 4	Twitching 2	None
LFF 27278	Malnutrition	Stretching and Waving Flippers 8: Scratching 12	Stretching and Waving Flippers 3: Scratching 2	None
LFF 27335	Pneumonia, Malnutrition	None	None	None
LFF 27020	Trauma	None	None	None
RFF 27313	Lice, Malnutrition	None	None	None
LFF 27387	Trauma	Muscle Fasciculations (Head 7, Whole Torso 4): Twitching 2	Muscle Fasciculations 3: Twitching 1	None
LFF 27402	Unknown	None	None	None
LFF 27365	Pneumonia, Malnutrition	Scratching 64	Scratching 3	None
LFF 27478	Malnutrition, Oil/Tar	None	None	None
RFF 27487	Malnutrition	None	None	None
LFF 27400	Malnutrition	Nursing 135	Nursing 3	None
LFF 27394	Oil/tar, Malnutrition	None	None	None
LFF 27407	Abscess, Malnutrition	None	None	None
LFF 27399	Abscess, Malnutrition	None	None	None

LFF 27573	Malnutrition, Renal Failure, Pneumonia	Head Weaving 1	Head Weaving 1	None
LFF 27575	Malnutrition, Abscess	Scratching 37	Scratching 2	None
LFF 27599	Abscesses, Malnutrition, Pneumonia	None	None	None
RFF 27541	Trauma	Twitching 17	Twitching 2	None
RFF 27530	Abscess, Malnutrition, Osteomyelitis	Nursing 7	Nursing 3	None
LFF 27550	Pox Virus	None	None	None
LFF 27530	Osteomyelitis Malnutrition	Scratching 8	Scratching 1	None
RFF 27543	Malnutrition	None	None	None
RFF 27549	Abscess, Malnutrition, Pneumonia	None	None	None
LFF 10387	Lepto	Twitching 25: Drinking Seawater 24	Twitching 3: Drinking Seawater 2	None
LFF 27545	Malnutrition, Trauma (Eye)	None	None	Twitching
LFF 27522	Trauma (Eye and Flipper), Domoic Acid Toxicosis	Grimacing 25	Grimacing 3	Head Weaving
RFF 27525	Trauma Flipper	Scratching 4	Scratching 1	None

LFF 27524	Abscess and Malnutrition	Drinking Seawater 1	Drinking Seawater 1	None
LFF 27509	Malnutrition and Pneumonia	Scratching 2	Scratching 1	None
LFF 27555	Shark Bite	Scratching 2: Twitching 4	Scratching 1: Twitching 3	None
RFF 27518	Malnutrition, Trauma (Face)	Head Weaving (Slight) 13: Scratching 5	Head Weaving 2: Scratching 1	None
RFF 27624	Leptospirosis, Malnutrition	None	None	None
RFF 27639	Shark Bite	Twitching 395	Twitching 3	None
LFF 27634	Leptospirosis	Twitching 53: Head Shaking 43	Twitching 2: Head Shaking 3	None
RFF 27576	Abscess	Scratching 6: Twitching 30	Scratching 1: Twitching 3	None
RFF 27533	Leptospirosis	Head Weaving (Circle) 7: Scratching 24: Twitching 10	Head Weaving 1: Scratching 1: Twitching 1	None
LFF 27537	Malnutrition	Twitching 6	Twitching 1	None
RFF 27628	Malnutrition	Open Mouth Breathing 6: Twitching 5	Open Mouth Breathing 1: Twitching 2	None
RFF 27643	Malnutrition, Azotemia	Scratching 7: Twitching 2	Scratching 1: Twitching 2	None

LFF 27646	Malnutrition, Pneumonia, Trauma	Stretching and Waving Flippers 2: Scratching 4: Twitching 1	Stretching and Waving Flippers 2: Scratching 1: Twitching 1	None
LFF 27026	Leptospirosis	Head Weaving 4	Head Weaving 2	None
LFF 27633	Entanglement	Twitching 2	Twitching 1	None
LFF 27526	Leptospirosis, Pneumonia	Twitching 2	Twitching 1	None
LFF 27637	Leptospirosis, Malnutrition	Stretching and Waving Flippers 2: Twitching 1	Stretching and Waving Flippers 1: Twitching 1	None
LFF 27699	Malnutrition, Corneal Ulcer	Head Weaving (Controlled) 63	Head Weaving 3	None
LFF 27700	Malnutrition	Scratching 11: Drinking Seawater 29	Scratching 1: Drinking Seawater 1	None
LFF 27690	Trauma Flipper	None	None	None
LFF 27664	Malnutrition, Pneumonia	Scratching 11: Drinking Seawater 72	Scratching 2: Drinking Seawater 1	None
LFF 27687	Malnutrition, Pneumonia	None	None	None

Not Known	Malnutrition, Trauma, Blind	Scratching 16: Twitching 2	Scratching 3: Twitching 1	None
RFF 23999	Malnutrition, Cardiomyop-athy	None	None	None
LFF 20779	Malnutrition, Trauma (Face)	None	None	None
LFF-RFF 1964	Malnutrition, Trauma (Face)	None	None	None
Not Known	Unknown	Grimacing 40: Head Shaking 395	Grimacing 3: Head Shaking 3	None
LFF 23557	Pox virus, Dehydration, Malnutrition	None	None	None
LFF 23931	Pneumonia, Trauma	None	None	None
RFF 23616	Malnutrition, Abscess, Osteomyelitis	None	None	None
RFF 23616	Entanglement Malnutrition	None	None	None

Table 24: Raw data from the Marine Mammal Center including gender, age, weight, length, stranding location, and date of observation.

Animal Name	Tag#	Gender	Age	Weight (kg)	Length (cm)	Location	Date
Kombucha	LFF 27167	Male	Juvenile	52.5	149	MBO	5/22/2011
JessAvila	RFF 27152	Female	Juvenile	38	132	SLO	5/29/2011
Babe	RFF 27054	Female	Adult	59	167	SLO	7/9/2011
Midway	LFF 27196	Male	Juvenile	58.5	167	SLO	7/9/2011
Muscat	RFF 27162	Female	Adult	77.5	166	SLO	7/9/2011
Imogen	RFF 27013	Female	Adult	54	153	SLO	7/17/2011
Crusty	RFF 27070	Female	Adult	65.5	168	SLO	7/31/2011
Firefighters	RFF 27072	Female	Adult	87.5	168	SLO	7/31/2011
Arafel	RFF 27025	Female	Adult	74	166	SLO	7/31/2011
Copernicus	LFF 25914	Male	Juvenile	79	163	MBO	8/7/2011
Matrim	LFF 25996	Male	Sub adult	125	188	SLO	8/7/2011
Syrah	RFF 25998	Female	Adult	74	145	SLO	8/7/2011
Hani	RFF 25969	Female	Adult	82.5	164	SLO	8/7/2011

Osana	RFF 27093	Female	Adult	77.5	165	SLO	8/7/2011
BassetHound	RFF 27011	Female	Adult	81	171	SLO	8/7/2011
Slovakia	RFF 25923	Female	Adult	85	187	SLO	8/7/2011
SixPence	RFF 25988	Female	Adult	76.5	172	SLO	8/7/2011
Jamara	LFF 25980	Male	Juvenile	63	148	SLO	8/7/2011
Arbela	RFF 27065	Female	Adult	84	168	SLO	8/14/2011
Perrin	RFF 25976	Female	Adult	69.5	159	SLO	8/14/2011
Perrin (Restrand)	RFF 25976	Female	Adult	69.5	159	SLO	8/21/2011
Aramon	RFF 25938	Female	Sub adult	54.5	155	SLO	8/14/2011
Aramon (Restrand)	RFF 25938	Female	Juvenile	49	157	SAUS	9/4/2011
Kuuipa	RFF 27084	Female	Adult	92	167	SLO	8/14/2011
Piece of Me	RFF 25952	Female	Juvenile	54	144	SLO	8/21/2011
Calypso	LFF 25971	Male	Juvenile	47.5	148	SLO	8/28/2011
Hit and Miss	LFF 25982	Male	Adult	128	190	SLO	8/21/2011
Tizer	RFF27135	Female	Adult	72	136	SLO	9/4/2011

Trevor	RFF 27132	Female	Adult	53.5	163	SLO	9/4/2011
Christopher	LFF 27268	Male	Juvenile	63.5	150	SAUS	8/16/2011
Bumble	LFF 27284	Male	Adult	91	190	MBO	12/11/2011
Hitchhiker	RFF 27301	Female	Adult	53.5	161	SLO	3/11/2012
Farewell	LFF 27490	Male	Sub adult	148.5	193	MBO	4/29/2012
Foggy Day	LFF 27538	Male	Sub adult	96.5	170	SLO	8/5/2012
Real Fire	RFF 27525	Female	Adult	68.5	164	MBO	8/12/2012
Roshi	RFF 27508	Female	Adult	61	161	SLO	8/5/2012
Ki	LFF 27360	Male	Juvenile	91.5	182	SLO	8/19/2012
Nui Wahini	RFF 27546	Female	Adult	84.5	167	MBO	8/19/2012
Clean Shores	LFF 27644	Male	Sub adult	87	161	MBO	9/16/2012
Coral Elayne	RFF 27667	Female	Adult	74.5	168	MBO	11/18/2012
Princess Daisy	RFF 23837	Female	Adult	74	149	SLO	3/10/2013
Cyndy	RFF 27652	Female	Adult	63	172	SLO	3/10/2013
Doug	RFF 23802	Female	Adult	56	167	SLO	3/10/2012

Branuik	RFF 23958	Female	Adult	59	158	MBO	3/24/2013
Frebec	RFF 23545	Female	Juvenile	59	141	SLO	8/11/2013
Rhapsody	RFF 23823	Female	Adult	81.5	170	SLO	8/11/2013
Surfer	LFF 23633	Male	Sub adult	124	172	SLO	8/25/2013
Cologne	Unknown	Female	Adult	76	155	SLO	8/25/2013
Perfume	RFF 23623	Female	Adult	110	177	SLO	8/25/2013
Wet Feet	LFF 27168	Male	Juvenile	58.5	152	SLO	5/22/2011
Wixom	LFF25970	Male	Juvenile	42.5	140	MBO	5/22/2011
Wotan	LFF 25900	Male	Pup	14	91	SLO	5/29/2011
Selva	LFF 25924	Male	Pup	25	103	MBO	5/29/2011
Calamity	RFF 27195	Female	Yearling	24.5	93	SLO	5/29/2011
Uphill	RFF 27145	Female	Adult	65	150	SAUS	5/29/2011
Flying Leap	LFF 27143	Male	Juvenile	65	168	SAUS	5/29/2011
Ivanho	RFF 25833	Female	Pup	18.5	111	SLO	6/5/2011
Egwene	RFF 25940	Female	Yearling	24	100	SLO	6/5/2011

Kayler	RFF 27190	Female	Pup	17	101	SLO	6/5/2011
Haku	LFF27 187	Male	Juvenile	39.5	144	MBO	6/5/2011
Stewball	RFF 27174	Female	Sub adult	50	151	MBO	6/5/2011
Sharla	RFF 25919	Female	Yearling	22.5	108	MBO	6/12/2011
Indo	RFF 27165	Female	Yearling	17.5	99	MBO	6/12/2011
Timor	LFF 27141	Male	Juvenile	52	131	MBO	6/12/2011
Dickens	LFF 25554	Male	Yearling	25.5	115	MBO	6/19/2011
Bowtie	RFF 27085	Female	Juvenile	26	120	MBO	6/19/2011
Elkers	LFF 24485	Male	Juvenile	27	126	MBO	7/3/2011
Snarly	LFF 27066	Male	Juvenile	31	131	MBO	7/3/2011
Squiggles	LFF 27045	Male	Yearling	31.5	128	MBO	7/3/2011
Arlene	RFF 25907	Female	Yearling	19	109	MBO	7/3/2011
Milestone	LFF 27100	Male	Juvenile	50.5	156	MBO	7/17/2011
Zooly	LFF 27071	Male	Yearling	30.5	119	MBO	7/17/2011
Fiano	RFF 27159	Female	Juvenile	58.5	155	SLO	7/17/2011

Orseycorn	LFF 27059	Male	Yearling	32.5	127	MBO	7/17/2011
Swell	RFF 27091	Female	Yearling	29	124	SAUS	7/24/2011
Zodiac Girl	LFF 27056	Male	Juvenile	39.5	137	MBO	7/24/2011
Naji	LFF 27043	Male	Juvenile	36	121	MBO	7/24/2011
Sowin	LFF 27019	Male	Yearling	27	118	MBO	7/24/2011
Mushrooms	LFF 27024	Male	Yearling	27.5	114	SAUS	7/31/2011
Yemanya	LFF 25803	Male	Yearling	18.5	99	MBO	7/31/2011
Kaweah	LFF 27039	Male	Yearling	29.5	112	MBO	7/31/2011
Palisades	LFF 27089	Male	Juvenile	29	135	MBO	7/31/2011
Columbia	LFF 25959	Male	Juvenile	50	156	SAUS	8/7/2011
BillyBay	LFF 27021	Male	Juvenile	37	139	SAUS	8/7/2011
Cortland	LFF 25997	Male	Juvenile	31	130	MBO	8/14/2011
Foggy Head	LFF 25960	Male	Sub adult	85.5	169	MBO	8/14/2011
Puddinhead	LFF 25946	Male	Juvenile	29.5	130	FBO	8/14/2011
Camden	LFF 27136	Male	Juvenile	34.5	145	SAUS	8/28/2011

Tennesse Shane	LFF 27118	Male	Juvenile	40	143	MBO	8/28/2011
Kymar	LFF 27130	Male	Juvenile	41	133	MBO	9/11/2011
Mandrake	RFF 27107	Female	Juvenile	40	135	MBO	9/11/2011
Liam	LFF 27078	Male	Juvenile	28	121	MBO	9/11/2011
Mimulus	LFF 25965	Male	Juvenile	29.5	121	MBO	9/11/2011
Pyramid	LFF 27005	Male	Juvenile	30.5	129	MBO	9/18/2011
Carma	LFF 27001	Male	Juvenile	42.5	134	MBO	9/18/2011
Bingo	LFF 27009	Male	Juvenile	47	149	SAUS	10/2/2011
Nusha	LFF 27018	Male	Juvenile	64.5	201	MBO	10/2/2011
Harkins	LFF 27122	Male	Juvenile	43	150	SAUS	10/9/2011
Duncan	Unknown	Male	Juvenile	22.5	180	SAUS	10/9/2011
Stegul	RFF 27064	Female	Sub adult	34.5	126	SAUS	10/9/2011
Moocow	LFF 27297	Male	Juvenile	77.5	198	SAUS	10/16/2011
Leaf Killer	RFF 27452	Female	Yearling	22.5	126	SAUS	10/16/2011
Brickell	RFF 27289	Female	Sub adult	27.5	127	MBO	10/16/2011

Eccentrica	LFF 27279	Male	Juvenile	100.5	185	SAUS	10/23/2011
Ditka	LFF 27261	Male	Juvenile	39.5	150	MBO	10/23/2011
Mariposa	LFF 27451	Male	Juvenile	64.5	160	SAUS	10/23/2011
Little V	LFF 27263	Male	Juvenile	50.5	149	MBO	10/27/2011
Steelie	LFF 27260	Male	Juvenile	55.5	145	SLO	10/30/2011
Whipstalk	LFF 27453	Male	Juvenile	42	141	SAUS	10/30/2011
Lazar	LFF 27272	Male	Juvenile	84	180	SAUS	11/6/2011
Comet	LFF 27293	Male	Juvenile	44.5	140	MBO	11/6/2011
Slater	LFF 27282	Male	Juvenile	32	122	MBO	11/6/2011
Gravy	LFF 27278	Male	Sub adult	41.5	151	MBO	11/13/2011
Chumpy	LFF 27335	Male	Yearling	15	94	SAUS	3/11/2012
Sugar Danni	LFF 27020	Male	Juvenile	92	172	MBO	3/18/2012
Puptart	RFF 27313	Female	Pup	14	84	MBO	3/18/2012
R Solo	LFF 27387	Male	Adult	164.5	202	MBO	4/15/2012
Handle It	LFF 27402	Male	Adult	191	216	SAUS	4/15/2012

Gia Pan	LFF 27365	Male	Pup	12.5	86	SAUS	4/29/2012 1
Carob	LFF 27478	Male	Pup	13.5	84	SAUS	5/6/2012
Jan	RFF 27487	Female	Pup	11	81	SLO	5/6/2012
Timkane	LFF 27400	Male	Pup	16.5	99	MBO	5/20/2012
Ledger	LFF 27394	Male	Pup	10	77	MBO	5/20/2012
Anchor	LFF 27407	Male	Pup	15.5	90	MBO	5/20/2012
Dynamite	LFF 27399	Male	Yearling	16.5	104	SAUS	5/20/2012
Karako	LFF 27573	Male	Yearling	16	107	SAUS	7/8/2012
Ishi	LFF 27575	Male	Yearling	20	111	SAUS	7/8/2012
Keegan	LFF 27599	Male	Yearling	24	110	SAUS	7/8/2012
Mint	RFF 27541	Female	Yearling	20	106	SLO	7/15/2012
Vault	RFF 27530	Female	Yearling	23.5	107	SLO	7/22/2012
Bandicoot	LFF 27550	Male	Yearling	20	101	SLO	7/22/2012
Bazingo	LFF 27530	Male	Yearling	20	112	SLO	7/22/2012
Cucu	RFF 27543	Female	Yearling	18.5	111	SLO	7/22/2012

Gulliver	RFF 27549	Female	Yearling	17.5	104	SLO	7/22/2012
Lee	LFF10 387	Male	Juvenile	30.5	108	MBO	8/5/2012
Wolverine	LFF 27545	Male	Yearling	18.5	110	MBO	8/5/2012
Shareef	LFF 27522	Male	Sub adult	54	169	MBO	8/12/2012
Maddy Right	RFF 27525	Male	Sub adult	46	138	MBO	8/12/2012
Kabebe	LFF 27524	Male	Yearling	22.5	108	SLO	8/19/2012
Wazam	LFF 27509	Male	Yearling	20.5	104	MBO	9/2/2012
Athena	LFF 27555	Male	Juvenile	72	157	MBO	9/2/2012
Ratatouille	RFF 27518	Female	Yearling	19	103	MBO	9/2/2012
Vanuatu	RFF 27624	Female	Sub adult	31	128	SAUS	9/9/2012
Zap	RFF 27639	Female	Yearling	24.5	121	SLO	9/9/2012
P. Floyd	LFF 27634	Male	Juvenile	42	132	SAUS	9/23/2012
Lefty Armstrong	RFF 27576	Male	Juvenile	26	125	MBO	9/23/2012
Callison	RFF 27533	Female	Juvenile	64	160.5	MBO	9/23/2012
JJ	LFF27 537	Male	Juvenile	35	125	MBO	9/30/2012

Wombat	RFF 27628	Female	Sub adult	27	134	MBO	9/30/2012
Achop	RFF 27643	Female	Sub adult	54.5	148	SAUS	10/7/2012
Duckduck	LFF 27646	Male	Juvenile	35	125	SAUS	10/7/2012
Rippy Roo	LFF 27026	Male	Sub adult	92.5	190	SAUS	10/9/2011
Blond Bomber	LFF 27633	Male	Juvenile	70	163	SAUS	10/14/2012
Cousin It	LFF 27526	Male	Juvenile	37.5	123	SAUS	10/14/2012
Gertrude Grace	LFF 27637	Male	Sub adult	75	184	MBO	11/4/2012
Keekee	LFF 27699	Male	Juvenile	48	145	MBO	11/11/2012
Krab Kringle	LFF 27700	Male	Sub adult	27.5	122	SAUS	11/11/2012
Mypal	LFF 27690	Male	Juvenile	81	183	FBO	11/18/2012
Drummer Boy	LFF 27664	Male	Yearling	38	137	SAUS	11/25/2012
Asante	LFF 27687	Male	Juvenile	27	130	MBO	11/25/2012
Aemon	Unknown	Male	Sub adult	51	142	SLO	5/26/2013
Grey Wind	RFF 23999	Female	Adult	66	154	MBO	5/26/2013
Cave Hermit	LFF 20779	Male	Pup	16.5	94	SLO	6/2/2013

My Mom	LFF-RFF 1964	Male	Pup	16.5	97	SLO	6/2/2013
Lovers Freedom	Unknown	Male	Juvenile	33	128	MBO	7/7/2013
Javelin	LFF 23557	Male	Juvenile	59	157	MBO	8/4/2013
Ayla	LFF 23931	Male	Sub adult	74.5	168	SBMMC	8/18/2013
Goov	RFF 23616	Female	Sub adult	28.5	123	SBMMC	8/18/2013
Kareja	RFF 23616	Female	Yearling	18.5	98	SBMMC	8/18/2013

Table 25: Raw data from the Marine Mammal Care Center including animal ID, diagnosis, abnormal behaviors observed, and severity scores assigned.

ID	Diagnosis	Abnormal Behaviors	Severity Score
13-541	Domoic Acid Toxicosis	Muscle Fasciculations (Head) 6	Muscle Fasciculations 3
13-540	Domoic Acid Toxicosis	Head Weaving (Circles) 113	Head Weaving 3
13-539	Domoic Acid Toxicosis	Head Weaving (Classic) 2: Muscle Fasciculations (Entire Torso) 15: Scratching 7	Head Weaving 3: Muscle Fasciculations 2: Scratching 1
13-544	Domoic Acid Toxicosis	Scratching 33	Scratching 2
13-533	Malnutrition	Scratching 16	Scratching 2

Table 26. Raw data from the Marine Mammal Care Center including animal ID, length, weight, gender, and age. All animals were observed on 5/16/2013.

ID	Length(cm)	Weight (kg)	Gender	Age
13-541	155	71.5	Female	Adult
13-540	160	108.5	Female	Adult
13-539	155	75.3	Female	Adult
13-544	160	86	Female	Adult
13-533	86	10	Female	Pup

Table 27: Raw data from Pier 39 including animal ID, abnormal behaviors displayed, severity scores, the concentration of domoic acid within the water (as determined by the California Department of Public Health) and date of observation.

Animal ID	Abnormal Behavior	Severity Score	Concentration in Water	Date of Observation
1	Scratching 38	Scratching 3	Present 1-9%	3/21/2013
2	Muscle Fasciculations 7	Muscle Fasciculations 3	Present 1-9%	3/21/2013
3	None	None	Present 1-9%	3/21/2013
4	Scratching 618	Scratching 3	Present 1-9%	3/21/2013
5	None	None	Present 1-9%	3/21/2013
6	None	None	Present 1-9%	3/21/2013
7	None	None	Present 1-9%	4/11/2013
8	None	None	Present 1-9%	4/11/2013
9	Scratching 69	Scratching 2	Present 1-9%	4/11/2013
10	None	None	Present 1-9%	4/11/2013
11	None	None	Present 1-9%	4/11/2013
12	None	None	Present 1-9%	6/6/2013
U288 / 28?	Scratching 55	Scratching 3	Present 1-9%	6/6/2013
TMMC 1	Scratching 42	Scratching 1	Present 1-9%	6/6/2013
1611 / J391?	None	None	Present 1-9%	6/6/2013
13	None	None	Unknown	8/12/2013
14	None	None	Unknown	8/12/2013
15	None	None	Unknown	8/12/2013
16	None	None	Unknown	8/12/2013

Appendix III

Table 28: Example of data setup for the Wilcoxon Signed Rank Tests. Diagnosis serves as the X factor and is nominal. Head-weaving time serves as the Y response and is continuous.

Diagnosis	Head Weaving (Seconds)
DA	8
DA	63
DA	1
DA	9
DA	23
Non DA	5
Non DA	1
Non DA	7
Non DA	7
Non DA	4

Table 29: Example of data setup for the Fishers Exact Tests. Diagnosis serves as the X factor and is nominal. Head weaving displayed has been converted to either a yes (animal displayed head weaving) or no (animal did not display head weaving) format and is nominal.

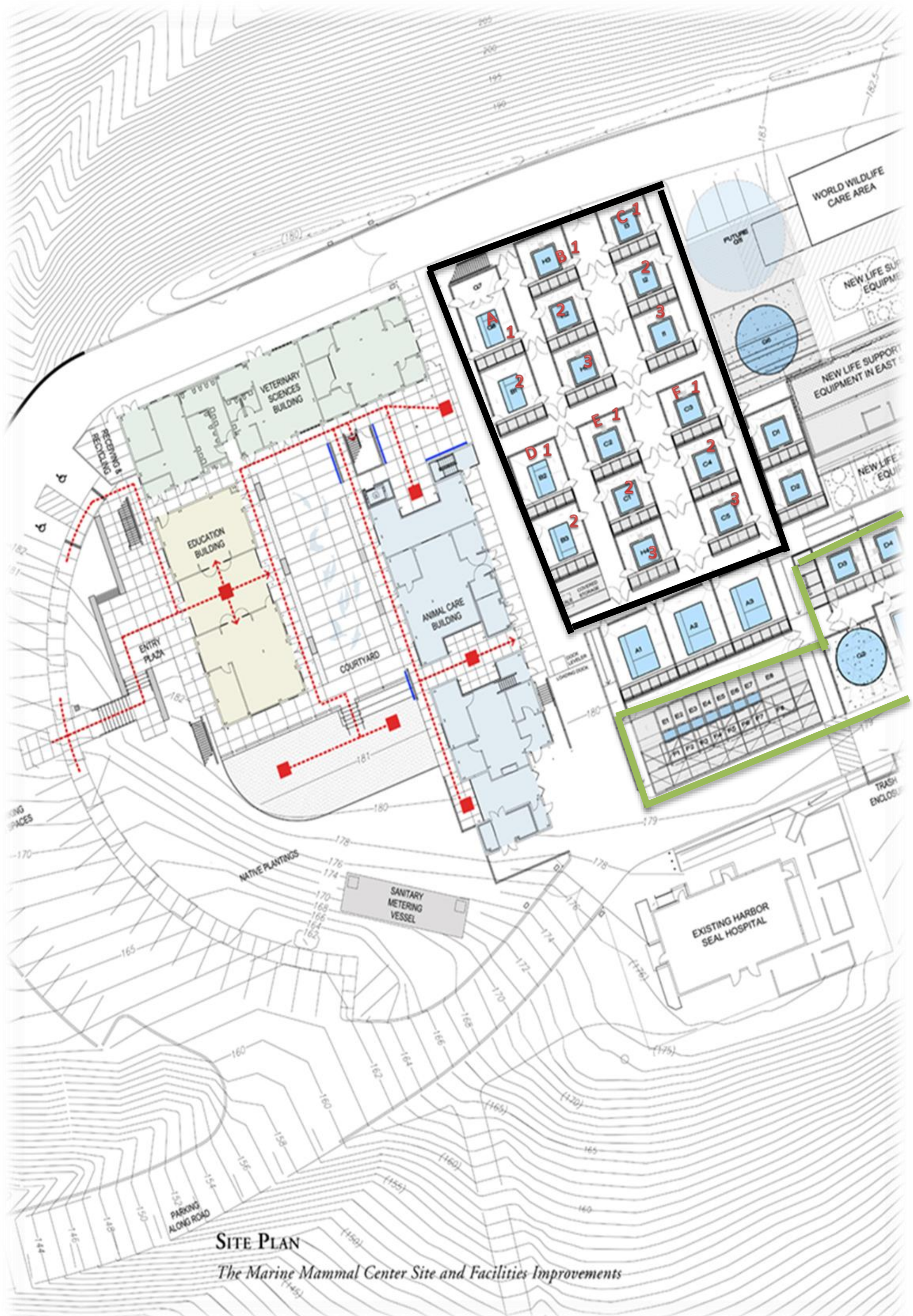
Diagnosis	Head Weaving Displayed
DA	Yes
DA	No
DA	Yes
DA	Yes
DA	Yes
Non DA	Yes
Non DA	No
Non DA	No
Non DA	No
Non DA	No

Table 30. Example of data setup for the ANOVA tests. Severity Score serves as the X factor and is nominal. Head Weaving times serve as the Y response and is continuous.

Head Weaving (Seconds)	Severity Score
8	1
63	3
1	1
9	1
23	3
2	1
64	3
50	3
41	3
1	1

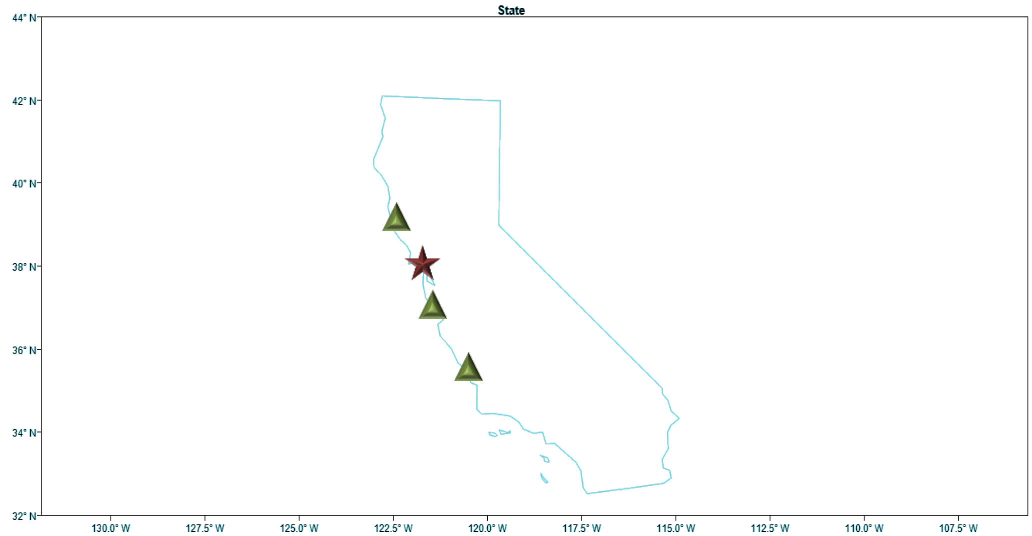
Appendix IV

Figure 22: Map (next page) of observation area at the Marine Mammal Center.



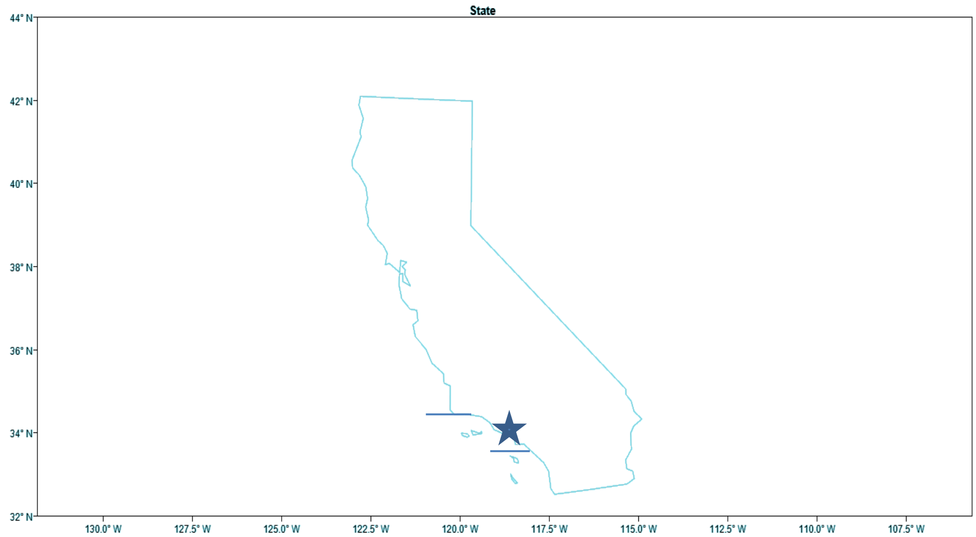
Legend: Study Area ▬ Correct Pen ID X Future enclosures not yet built ▬

Figure 23: Marine Mammal Center Rescue Range



Legend: Maine Hospital Location   Satellite Locations

Figure 24: Marine Mammal Care Center Rescue Range



Legend: Facility Location ★ Rescue Range Boarder ———

Figure 25: Location of Pier 39

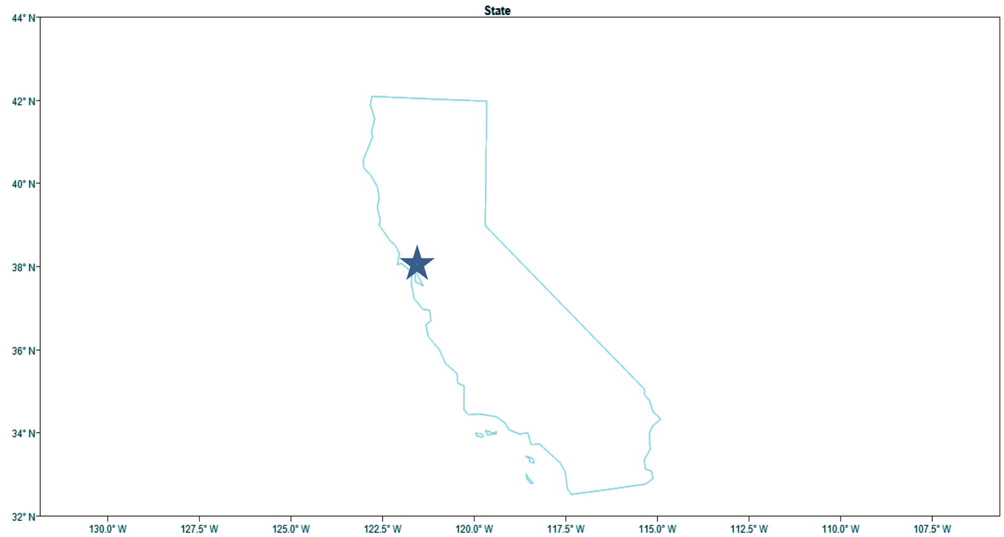


Figure 26: Lane of vessel traffic at Pier 39

Figure 27: Sea wall that inhibits predator entrance at Pier 39

Appendix V

Documenting Behavioral Diagnostic Information on the Beach for Domoic Acid Toxicosis in the California sea lion: Training Sample

- **What is domoic acid?**
    - **Domoic acid is a neurotoxin produced by diatoms of the genius *Pseudonitzschia*. After ingestion of domoic acid, brain damage can occur, including the shrinking of the hippocampus.**

- **How does domoic acid impact sea lions?**
    - **Along the west coast of the United States, sea lions come into contact with domoic acid through the consumption of contaminated fish, such as anchovies. If levels of domoic acid become toxic, the animal is diagnosable with the condition known as "domoic acid toxicosis". These animals often behave differently (convulsions, tremors, difficulty moving) because of the neurological effects of the toxin. Domoic acid is often hard to detect using blood, urine, and fecal samples which is why behavioral diagnostic criteria is important.**

- **What abnormal behaviors do sea lions typically display when they have domoic acid toxicosis?**
    - **California sea lions with domoic acid toxicosis may display head weaving, muscle fasciculations (tremors), dragging the hind flippers, or swift scanning behavior.**

- **How can I identify these behaviors before and during rescue?**
    - **The person in charge of notes should document any abnormal behavior displayed that matches diagnostic criteria. This documentation can include the period that the animal displayed the behavior, depending on the situation. Information relating to diagnostics is provided on the next three pages.**

- **Why is documentation on the beach important?**
    - **Research shows that, during some years, 20% of admits display diagnostic criteria on the beach only. Documentation on the beach will help veterinarians make a quicker diagnosis for these animals.**

Diagnostic Information

Ethogram: Abnormal Behaviors

Head Weaving	Animal sways head from side to side; front to back, or in a circular motion, often touching the torso with the back or side of the head. Neck may be loose or ridged. Sways may be prolonged or quick. Movements may be bobbing, jerking, or smooth. Head weaving can occur while animal is in any posture while on land.
Muscle Fasciculations	Visible muscular ripples or large tremors occur along the entire torso or half of the torso. The head and neck may also be involved, which can but not always, involves the facial regions. In the instance of the head and neck, the movement must be smaller than head weaving and not involve side to side swaying.
Swift Scanning	Animal scans the surroundings 360° at intervals lasting <90 seconds. Surroundings must be void of abnormal visual and auditory stimuli as similar scanning is a normal behavior observed with animals in increased levels of stimuli.
Dragging Hind Flippers	Animal uses only the front flippers for locomotion. Instead of tucking the back flippers under the body and using them to walk, the animal drags itself along with the front flippers, allowing both back flippers to drag against the ground. Often seen as a performance behavior by trained animals but never observed in healthy, wild populations.

Ethogram: Behavioral Subtypes: Head Weaving:

Head Weaving Subtypes	Description
Craning	Animal lurches head forward and down instead of from back to front or side to side.
Cannot Keep Head Still	Head wobbles in any direction.
Classic	Stiff or wobbly, side to side or front to back weaving.
Slight	Head weaves but does not touch side or back of body.
Back	Head moves up and back, does not sway from side to side.
Prolonged	Stiff movements in any direction where head makes contact with body and remains for a few seconds
Circle	Head weaves in full circles instead of from side to side or back to front.
Controlled	Animal is able to halt head weaving upon the addition of stimuli.

Ethogram: Behavioral Subtypes: Muscle Fasciculations:

Muscle Fasciculations Subtypes	Description
Full Body	All muscles of the torso ripple or jerk.
Half Body	Muscles of only the upper or lower torso ripple or jerk. Cannot include both halves of the torso.
Head	Muscles around the head and facial area ripple or jerk. May include the vibrissa and mouth.
One Front Flipper	Muscles within the front flipper pit jerk, causing the flipper to move upwards and/or outwards.
Both Front Flippers	Muscles within both front flipper pits tense, causing the flippers to move outwards.
Eye	The muscles around the eye socket jerk, causing animal to squint spastically.

End Training Sample

Sample Datasheet for Swift Scanning Recording

Animal ID	
Gender	
Age	
Length (cm)	
Weight (kg)	
Diagnosis	
Medications	
Stranding Location	
Date of Stranding	
Swift Scanning Displayed (Seconds)	
Date	
Time	

Sample Data Sheet for Subtype and Severity Score Recording

Animal ID	
Gender	
Age	
Length	
Weight	
Diagnosis	
Medications	
Stranding Locations	
Date of Stranding	
Head Weaving	
Head Weaving Subtype	
Head Weaving Severity Score	
Muscle Fasciculations	
Muscle Fasciculations Subtype	
Muscle Fasciculations Severity Score	
Dragging Hind Flippers	
Dragging Hind Flippers Severity Score	
Swift Scanning	
Swift Scanning Score	
Date	
Time	

Domoic Acid Toxicosis Behavioral Diagnostic Criteria Form

Animal Information

Animal IDClick here to enter text. Date
Facility Name: Click here to enter text. Stranding Location:
Enclosure ID Click here to enter text. Observer Name Click here to enter text.
Age: ☐ Pup ☐Yearling ☐Juvenile ☐Sub Adult☐ Adult Gender M ☐ F ☐

Criteria

Head Weaving ☐

Craning	☐	**Time**Click here to enter text.
Classic	☐	**Time**Click here to enter text.
Slight	☐	**Time**Click here to enter text.
Back	☐	**Time**Click here to enter text.
Prolonged	☐	**Time**Click here to enter text.
Circle	☐	**Time**Click here to enter text.
Controlled	☐	**Time**Click here to enter text.

Muscle Fasciculations ☐

Full Torso	☐	**Time**Click here to enter text.
Half Torso	☐	**Time** Click here to enter text.
Head	☐	**Time**Click here to enter text.
Eyes	☐	**Time**Click here to enter text.
Front Flipper One	☐	**Time**Click here to enter text.
Both	☐	**Time**Click here to enter text.

Dragging the Hind Flippers☐

Swift Scanning ☐

TimeClick here to enter text.

Time Click here to enter text.

Comments
Click here to enter text.

Number of abnormal behaviors displayed: Zero☐ One ☐ Two ☐Three ☐ Four ☐

Based on information above and any other relevant information, do you recommend a diagnosis of domoic acid toxicosis? Yes ☐ No ☐

Instructions

Fill out all known animal information. Under "Criteria" check all abnormal behaviors (located on the left) displayed. Check all displayed subtypes (if applicable) (located in the middle). Record the time each behavior and subtype was displayed in increments of seconds. Record the number of abnormal behaviors displayed (this does not include subtypes). If at least two abnormal behaviors were displayed, a diagnosis of domoic acid toxicosis is recommended. If only a single abnormal behavior was displayed and that behavior included dragging the hind flippers or swift scanning, then a diagnosis of domoic acid toxicosis is recommended only if environmental conditions have been met (see below). If only head weaving was displayed but lasted over 12.4 seconds, a diagnosis of domoic acid toxicosis is highly recommended. If only muscle fasciculations were displayed and lasted over 9.36 seconds a diagnosis of domoic acid toxicosis is highly recommended.

Environmental Conditions

Dragging the hind flippers and swift scanning are only appropriate measures for the diagnosis of domoic acid toxicosis if certain environmental assumptions have been met.

Dragging the Hind Flippers:

Enclosure must be large enough and have enough free space for animal to move freely (3 animal lengths in a single direction).

Swift Scanning

Conditions inside and around enclosure must be quiet and free from activity. This includes auditory and visual stimuli such as loud vocalizing and boisterous animals and or people.

Ethogram: Abnormal Behaviors

Head Weaving	Animal sways head from side to side; front to back, or in a circular motion, often touching the torso with the back or side of the head. Neck may be loose or ridged. Sways may be prolonged or quick. Movements may be bobbing, jerking, or smooth. Head weaving can occur while animal is in any posture while on land.
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Swift Scanning	Animal scans the surroundings 360° at intervals lasting <90 seconds. Each scan lasts <5 seconds. Surroundings must be void of abnormal visual and auditory stimuli as similar scanning is a normal behavior observed with animals in increased stimuli.
Dragging Hind Flippers	Animal uses only the front flippers for locomotion. Instead of tucking the back flippers under the body and using them to walk, the animal drags itself along with the front flippers, allowing both back flippers to drag against the ground. Often seen as a performance behavior by trained animals but never observed in healthy, wild populations.

Ethogram: Behavioral Subtypes

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