1-1-2014

The Use of Tonometry as a Diagnostic Tool to Evaluate Intraocular Pressures in Normal and Abnormal California Sea Lion Eyes

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By

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A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Veterinary Medical Sciences
in the College of Veterinary Medicine

Mississippi State, Mississippi

December 2014

Use of tonometry as a diagnostic tool to evaluate intraocular pressures in clinically normal and abnormal California sea lions

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Title of Study: Use of tonometry as a diagnostic tool to evaluate intraocular pressures in clinically normal and abnormal California sea lion eyes

Pages in Study: 152

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Ocular disease is one of the most common problems encountered in sea lions at various zoos and aquariums around the world.[1] The California sea lion *(Zalophus californianus)* is one of the most common marine mammals maintained in zoos and is also the most commonly afflicted with ocular disease. Studies have shown that pinnipeds housed in captivity manifest an array of ocular lesions.[2] Eye disease can range from a pinpoint corneal opacity to loss of vision due to keratopathy, cataracts and secondary glaucoma. Glaucoma is a disease that has not been extensively studied in the sea lion.[3] Observation of clinical signs and determination of intraocular pressures (IOP) are critical for early diagnosis. IOP measurement may elucidate intraocular disease and provides information on the balance between aqueous humor production and outflow. The objective of this study is to measure IOP in California sea lions that have clinically normal eyes as well as those with varying degrees of ocular diseases, and to evaluate the incidence of secondary glaucoma in this species.

DEDICATION

I dedicate this dissertation to the people and animals in my life that have touched my heart in a way that I can't describe and because of them I wake up every day to be the best person, mom, "nieta", daughter, wife, student, teacher, colleague, friend, sister, and doctor that I can be.

The first sea lions I had ever trained were from the Memphis Zoo, TN, and I remember Dr. Bill Miller explaining to me about an ophthalmic surgery he had to do to remove the eye of one of the sea lions because the disease had progressed to a severe state and the animal could no longer see. This was the moment that I fell in love with marine mammal ophthalmology and knew I wanted to investigate something new in this area.

After graduating with a DVM from Mississippi State University, I was accepted into a Masters' program at Institute for Marine Mammal Studies, Gulfport, MS, measuring the first intraocular pressures in sea lions. Three months into the program, Hurricane Katrina hit the coast of Mississippi, completely destroying the marine facility where my study had started. On the day of August 29, 2005, my life changed forever. My husband flew to Mississippi to be with me during the storm and I will never forget going outside with him after the "eye" of the storm had passed, walking to Marine Life and finding out that a 40- foot storm surge had devastated the entire marine facility and swept the dolphins and sea lions in the Gulf of Mexico, not to mention my instrumentation for

this study as well. I would like my first dedication of this dissertation to be to the lives of the people and animals that were lost that day. I would like to recognize Drs. Connie Chevis and Moby Solangi from the Institute of Marine Mammal Studies for their help in creating the original research design and continued support to help me continue moving forward with this study. I would also like to thank all of the staff and trainers from this facility and personally recognize Tim Hoffland, Marci Romagnoli, and Shannon Sharyer for the many animals that they saved in the days to come after the hurricane. They put the animals first, even before their own lives, which to me showed true dedication and compassion.

After the hurricane, my study halted, but in the back of my mind I knew the importance of finishing this work and I was determined to making this happen. I am forever grateful to all of the marine and zoo facilities that allowed me to grow my sample size of sea lions in order to collect data that can be published. I would like to honor one sea lion in particular from Dolphins Plus that is no longer with us, but she allowed us to learn so much about sea lion ophthalmology, especially glaucoma. I am so appreciative of all of the hard work in both training and medical care that she was given. (Thank you Kyle Lane, Cory Bandy, Billy Budd, Sarah Sayre, Bob Stevens, Micah Brodsky, Natalie Noll, and Joy Middleton).

Quiero decirle las gracias a mis abuelos y mis padres. Soy la persona que soy porque ustedes siempre han estado allí para mi. Nunca me han dejado caer y siempre se que tengo un apoyo tan grande que nunca me siento sola. Siempre me han dejado seguir mis sueños y este libró lo dedico a ustedes porque yo se el sacrificio que ustedes han hecho para que yo pudiera vivir en los Estados Unidos, ir a la escuelas mejores, y triunfar

cómo ustedes lo han hecho. Mama- siempre dices que ves en mi como tu era a mi edad, pero yo esperó ser mita de la mujer y mama que eres. Gracias por ser mi amiga y mama que algunas veces no es fácil pero juntas no hay nadie que nos para. Papa- siempre será su niña pequeña. Cuando me miras con un orgullo me siento tan feliz. Espero que siempre te hago orgulloso y gracias por ayudarme llegar a esta etapa en mi vida.

Thank you sister (Chica May May). I am so lucky to have you in my life. Even though we are miles apart you have always been there to support me and know just what to say when I am feeling down. You, Todd, and my nephews Emilio, Nico, and Lorenzo have been such a help with Joseph, thank you for treating him as if he was your own son. Words can't describe how grateful I am to have you in our lives and so excited on our new working relationship. My other sisters and brothers in law, Dina, Heed, Tommy, Lori, and Ria have been such a big part of our lives and been a tremendous help with Joseph. Mom and Dad Fava I don't know how we would survive here in Athens without the both of you. Your help has been tremendous. I know your prayers are powerful and I know I am in good hands from those watching me here as well as those from above. I truly value all of my friends and family members, there are many of you, and you know who you are. You make me laugh, keep me grounded, and cook wholesome meals that I could never do for myself. All of you in your own unique way have helped me get this study completed. We are very excited to be closer to the Florida family and friends as we hopefully take our next steps to move to the Keys.

Animal Necessity team (Jen P., Ben W., Jeff M., and Sumantra S.), I can't thank you enough for holding the fort as I took some time off to complete this dissertation. Thank you for believing in me and bringing our supplements to the world. Jen- awhile

back you helped me with looking up some references and although it was a small project that you did, you are always willing and happy to help and everyday impress me more. Ino Gomez- I want to thank you for inviting me to be a guest speaker on your radio show, especially allowing me to discuss the findings of my study. Now not only will the scientific community hear about this, but you have given me an avenue to speak to your community about animal health. To my colleagues and partners Carmen C., Terri M., Debby S., and Tom D.- I have learned so much from all of you both medically and on the business end. Thank you for growing our family business and together bringing vision nutraceuticals to the animal world. We have helped so many animals and hope to never stop!

Carmen- I want to also dedicate this dissertation to you. We met right at the start of this study and everything I have learned about marine mammal ophthalmology, I know it has stemmed from you in one way or another. You are brilliant and I aspire to be like you every day. We joke and say you're my daughter, but you are more like a sister and a best friend.

To my critter friend, Sace, you are the most amazing dog. You know when I am sad and happy before I know it. I can't get enough of you when you cuddle up in my neck line and give me kisses. I have learned a few valuable lessons from you- 1. The most important thing in life is taking time out of your busy day to get your belly rubbed, 2. if you bark loud enough someone will get up and pay attention to you, and 3. pretend that you missed "the wee wee pad" because you are getting old and can't make it in time, just look cute and someone will forgive you.

To my husband- words can't describe how much you have helped me in this process. There is no way I could have ever taken this research project on without your strength and support always by my side. Thank you for all of the early morning drives to each facility that you helped take me too. You always pull me up when I am down, making me feel like I can accomplish anything. "Things have a way of working out" when I am with you that being around you is contagious and after all of these years, coming up on 20 together, I feel like it is yesterday that I met you.

To quote one of our favorite songs....The Promise (when in Rome)

If you need a friend

Don't look to a stranger

You know in the end, I'll always be there

But when you're in doubt

And when you're in danger

Take a look all around, and I'll be there

.....And if I had to walk the world, I'd make you fall for me

I promise you, I promise you I will

The most special bond we hold is our son, Joseph. I dedicate this dissertation to both of these special men in my life who have been so patient with me throughout this time. Joseph- I promise not to ever miss a family fun day, movies, and take you to your favorite jumpy jumpy place whenever you want. Yesterday, my baby drew in a coloring book and said "mommy look I have a PhD too". Then he asked when the PhD is over can the circus begin? ...and I answered, "yes let the circus begin."

Words to live by…..a very smart man told me this…..

do what you love for people who love what you do

aspire to inspire before you expire

Don't settle!

ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge all of my committee members (Skip Jack, Carmen Colitz, Don Samuelson, Caroline Betbeze, and Lora Ballweber) for their endless support throughout this long journey. Thank you for taking the time out of your busy days to help with all of your edits and for even having personal sessions with me discussing questions and hypothesis that allowed me to come up with my own explanations when it comes to the topic of marine mammal ophthalmology. Skip, as a major professor, I would not have asked for anybody else by my side, especially at times when we did not even have a working study, instrumentation, or animals to evaluate. You signify what it means to have mentor, which is someone that never gives up on his students and believes in them when no one else does, I will never forget this and your strength is what helped me continue to finish this research.

After hurricane Katrina, I relocated to Miami, Florida from Biloxi, MS and I really need to acknowledge a few people and companies that without their help this study would have never started again. A special thanks to Mississippi State University, College of Veterinary Medicine and Dan Scott and Associates for donating new instrumentation, Jorgenson Laboratories for donating instrument probes, and Animal Necessity, LLC for funding travel to the various facilities in order to continue to collect intraocular pressures. Dr. Maya Rodriguez (Miami Seaquarium, FL) with the help of head trainers Heather Keenan and Sarah Graff had the vision that we could start collecting pressures on some

of the sea lions that could not see very well as medically it would be important to collect pressures and training would be easier. While we started to collect pressures on these animals, the trainers continued to help me get more animals involved knowing the importance of this project. The next facility that I will be forever grateful to that allowed me with open arms into their facility was Dolphins Plus, Key Largo, Fl (Rick Borguss, Art and Nancy Cooper, Holli Byerly). Dr. Mike Renner was instrumental in introducing me to the rest of "the Keys" family marine facilities including Theater of the Sea, Islamorada, FL (Beverley Osborne, Pam Ollen, Melissa Jaroneski) and Dolphin Research Center (Mandy Rodriguez, Pat Clough, Rita Irwin, Linda E., Loriel). At the same time, I was accepted by the Marine Mammal Center, Sausalito, CA and the Marine Mammal Care Center, Los Angeles, CA to evaluate ocular disease in rehabilitated sea lions. The following facilities joined the study after this time and I know that I am missing a few staff member names here, but please know how thankful I am to all of the veterinarians, owners, curators, trainers, and staff from each facility that welcomed me to meet their animals and continue my study. This is has been an amazing collaborative endeavor. Although animals from some of the facilities mentioned here were not entered into this study, this may have been because not enough consistent data was collected, animals were just starting to be trained, or other marine species have had pressures collected as well which will allow for future work. I remember the first time meeting Dr. Jim McBain from SeaWorld, CA and explaining my study to him. This is a doctor that aspire to be exactly like and he looked at me with such excitement explaining his experiences with pinniped ophthalmology and told me what a good study I have and to never give up, he was the first to welcome me to SeaWorld. Jenny Rego introduced me to Dr. Tom

Reidarson, another doctor that I have great respect for who also encouraged this study from the beginning and continues to challenge and motivate me to this day. Drs. Todd Schmitt, Judy St. Leger, and David Roberts (SeaWorld, CA), Chris Dold, Brad Andrews, and Todd Coffman (SeaWorld, FL), Les Dalton and Lisa Halstead (SeaWorld, TX) have been instrumental in training these animals and helping with edits and on the research side of things allowing this study to become published. Dr. David Blyde, Christina, and Cath (SeaWorld, Gold Coast, Australia) thank you for all of the video footage and pictures you were able to provide for my presentation. I would also like to acknowledge Dr. Elizabeth Hoffman and Dr. Stephanie Venn Watson from the Navy Marine Mammal Foundation, CA for collecting intraocular pressures in a different population of sea lions to have a comparison. I would also like to acknowledge other facilities such as Gulf World Marine Park, FL (Dr. Lydia Staggs), Wildlife Conservation Society (Drs. Paul Calle and Robert Moore, Kate McClave, Patricia Toledo, Martha Hiatt), Mystic Aquarium Institute for Exploration (Allison Tuttle and Mike Osborn), Dolphin Cay, Atlantis Paradise Island, Bahamas (Terri Corbett), Beth Doescher, Six Flags Great Adventure, NJ (Jessica Peranteau, Dr. Mike Renner), Atlantis Marine World Riverhead, NY (Candy Paparo), Maritime Aquarium (Dr. Barabara Mangold), New England Aquarium (Dr. Charlie Innis), Marineland, France (Dr. Manuel Garcia-Hartmann), ZooMarine, Italy, San Diego Zoo, Audubon Nature Institute, LA, Dolphin Discovery, Mexico and Grand Cayman (Dr. Roberto Sanchez), Dr. Juan Franco, Dolphin Island, DR, Six Flags Discovery Kingdom, CA (Mike and Holley Muraco, Dr. Diana Procter).

During the time while I was finishing my PhD, I also completed an aquatic internship at Boatswain's Beach Adventure Park and Turtle Farm and St. Matthew's

University as well as finished an American College of Zoological residency though the University of Georgia College of Veterinary medicine and Georgia Aquarium. I would like to thank all of my mentors for allotting me the time for additional course work to be completed at UGA as well as time to write in order to complete this dissertation. A special thanks also goes to my teachers and tutors in statistics, I could have never analyzed my data without your help. Thank you to my husband, Joe, for his amazing cartography skills.

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CHAPTER I

INTRODUCTION

Ocular disease is one of the most common medical problems seen in captive pinnipeds.[4] Studies have shown that both wild and captive pinnipeds manifest an array of ocular lesions.[1,5] Pinnipeds traditionally form their own mammalian order, Pinnipedia, and are divided into three familes: Otariidae, the eared seals; Phocididae, earless or true seals; and Odobenidae, walrus.[6] The most common pinniped maintained in zoos and aquariums in North America is the California sea lion *(Zalophus californianus)*. California sea lions are otarids (members of the Otariidae), being the only species of *Zalophus*. They inhabit sandy and rocky beaches along the coasts of the North Pacific from Mexico to British Columbia. The name "California" sea lion was given because of their high populations on the mainland shorelines of southern and Baja California.[6]

Advances in medical technology and training have allowed veterinarians and health care staff to better examine these animals improving their well-being and overall health. On average, sea lions under human care can live to be at least 20 years of age. However, some animals survive into their late twenties or early thirties,[6] likely due to improved healthcare and husbandry. Eye problems are one of the most common medical issues affecting pinnipeds under human care and some such as congenital cataracts may be present at birth (Mejia-Fava, 2014, personal observation). This is why the preservation of vision starting early in life is an important long term goal. Specific ocular diseases

described or observed in sea lion include keratopathy, cataracts, lens luxation, uveitis, and secondary glaucoma.[1,7,8] Glaucoma is an ocular disease that develops due to impaired outflow of aqueous humor. As IOP increases above that which is compatible with the normal health of the globe, the retina and optic nerve degenerate leading to blindness.[9-11] Glaucoma may be primary or secondary, and clinically may manifest with varying degrees of pain and vision loss. Primary glaucoma is an inherited disease that is not common in pinnipeds, however secondary glaucoma can develop due to chronic uveitis and lens luxation in all species, including sea lions. Glaucoma is a disease that has not been extensively described in the California sea lion. This study will evaluate methods of measuring IOP in live, captive, sea lions through rebound tonometry, thus providing a range for normal IOP and elucidating IOP ranges for the variety of ocular diseases affecting these animals. Documenting IOP measurements as these animals mature and age will help us to identify changes in IOP during disease progression and allow earlier medical, and surgical, intervention, when necessary.

Tonometry is a non-invasive method that reveals information on the aqueous humor dynamics within the eye. A measurement of IOP is an important diagnostic tool, which reflects the balance between aqueous humor production and outflow. This study was divided into 2 phases. The objective of the first phase was to estimate the range of normal IOPs in California sea lions without ocular pathology and to determine which variables affect IOP measurements. In the second phase, the objectives were three-fold: (1) to compare IOPs in five different groups of sea lions diagnosed with varying degrees of ocular disease, (2) to compare IOP of two cases with clinical signs of glaucoma, and (3) to compare IOP from one sea lion where IOP was collected in different locations.

Colleting IOPs in “at risk” sea lions and determining increases would be helpful (at risk being the ones that have cataract or lens subluxation). Charting IOPs throughout an animal’s life can help a clinician not only understand normal physiologic baseline values for a particular animal, but also recognize early IOP changes allowing medical intervention.

CHAPTER II

LITERATURE REVIEW

California sea lions have the capability to see in aquatic and terrestrial environments. Vision on land is integral during their breeding season to find their pups left on rookeries while mothers hunt for food. Various species develop sight at different ages possibly related to a need for survivability. The sense of sight develops gradually over 6 to 8 months in humans.[12] In puppies, full vision is only developed at about 8 weeks of age and in kittens visual acuity continues to improve until approximately 16 weeks of age.[13] Sea lion pups are born with their eyes open and use sight and vocalization to recognize their mothers. The importance of vision begins at a very young age in the California sea lion.

Sea lions learn quickly to dive and catch their own food. They can dive to depths of 203-900 feet (62-274 m) because of several anatomical and physiological adaptations.[5,6] These include the ability to hold their breath on average 8-20 minutes by shunting and conserving blood flow to vital organs such as the brain and heart through a mechanism of peripheral vasoconstriction.[5,6] A high tolerance for carbon dioxide allows them to dive to great depths in which they swim at speeds of up to 25-35-knots (30 miles/hr).[5,6] Other diving adaptations include a profound bradycardia resulting in a lower consumption of oxygen. [14] Muscles will still function in an oxygen poor environment because they are rich in myoglobin.[5,6] In comparison to similarly sized terrestrial

mammals, the blood volume of a sea lion is 1.5 to 2 times greater and their red blood cells are larger with a greater mass and concentration of hemoglobin.[5,6]

Pressure changes during diving can cause tissue compression in gas-filled spaces in the body.[15] When marine mammals dive, it has been suggested that the trans-thoracic pressure gradient (ambient pressure-pressure inside the respiratory system) sharply increases during a dive, compressing air out of the alveoli, into the rigid upper airways.[15] Alveolar collapse occurs at approximately 40-80 m, reducing the amount of N_2 available to equilibrate with the tissues.[16] Small pressure differentials between gas filled spaces such as air sinuses, lungs, and middle ear cavity and the surrounding tissue can cause tissue distortion known in human divers as "the squeeze."[15] Marine mammals and penguins that dive to great depths have a special adaptation to help regulate changes in pressure in these air filled cavities. The middle ear cavity is lined with an extensive venous plexus called the venous cavernous sinus, a type of corpus cavernosum which becomes engorged at depth and thus reduces the air space and prevents the development of the squeeze.[17] A large vascular supply is also found in the air cavities of the cranium which moderate air pressure within these spaces.[15] Sea lions also do not have paranasal sinuses, lacrimal bones (identified in domestic species as the ventromedial aspect of the orbit adjacent to the nasal cavity), and nasolacrimal ducts, which may be an anatomical adaptation to help decrease problems with equalizing pressures.[18]

Animals under human care do not dive to these great depths and are exposed more to shallow areas reflecting blue light colors.[19] These light colors are very effective at reflecting most of the UV light energy back towards the animals as they dive and swim.[19] Whereas diving marine mammals do not experience significant light reflected from the

bottom back into their eyes. The sandy ocean floor is non-reflective and on land these animals are usually found on rookeries interacting with other animals or sleeping or resting with their eyes closed.[19]

Despite many of the advances in husbandry practices, life support systems, nutrition, and medicine, ocular disease is common in sea lions under human care.[19] Sea lions have been reported to have various ocular diseases including: otaradid keratopathy, cataract development with and without luxation, cataract-induced uveitis, phthisis bulbi, neoplasia, amyloid deposition in the corneal stroma and/or ciliary body, and secondary glaucoma.[1,7,8,20] Sea lions living in a non-shaded enclosure are 10 times more likely to develop cataracts or lens luxation.[1] Age, history of any eye problems, and history of fighting are other risk factors.[1] Keratitis or keratopathy was identified in 142 eyes of 113 sea lions (62.8%) captive otariids.[7] This 6-year study showed that keratitis can manifest in young animals as 21% under the age of 10 years were affected.[7] Flare-ups occurred when sunlight was increased or more intense and in northern latitudes in winter sunny days from reflection of the snow.[7] Animals housed indoors have less severe corneal disease and flare-ups for their age.[19]

Glaucoma is almost impossible to definitively diagnose through gross examination. Depending on the classification and severity of glaucoma, clinical signs may or may not be seen. In human medicine, patients who become blind from glaucoma may not seek treatment until they are significantly visually impaired (night blindness, loss of peripheral vision), as the disease often progresses insidiously where symptoms do not readily exhibit or are slow to present. To date, there have been no studies evaluating glaucoma in the California sea lion.[1] The ocular vascular anatomy, iridocorneal angle and

ciliary body physiology, and aqueous humor dynamics are still areas in which further information is needed.[20] Ocular studies in wild sea lions are impractical as it is dangerous to get close to these animals and special permits are needed to anesthetize them in order to collect data. Rehabilitation facilities show that an average (8%) of animals come in from the wild with cataracts, etc.[21] Animals that might develop cataracts or glaucoma with vision loss most likely will become part of the food chain and be very difficult to diagnose or follow-up**.**

Ocular anatomical and physiological adaptations

The California sea lion- anatomy and optics

Vision is a very important for pinnipeds as they predominately use this sense along with hearing and tactile vibrissae for behavioral functions such as catching their prey, socially communicating with other sea lions, predator avoidance, and traveling the deep ocean while diving.[22] The California sea lion has been observed to stay close to land and forage both during the day and night.[23,24] Hobsen[25] concluded that many pinnipeds, including California sea lions, are nocturnal feeders and use a hunting behavior in which they detect their prey by the silhouette of the fish against the brighter surface light, while keeping themselves out of sight by using the dark background of the deep waters. They catch fish and squid in the **neritic** (all bolded words found in Appendix A) waters, where blue green wavelengths penetrate the water column to depths over 20m (Appendix B).[26]

Sea lions are amphibious animals that have the capability to see in aquatic and terrestrial environments. Their eyes have also adapted to function in a variety of ambient light environments, from bright light intensity during the day to lower levels of light at different depths of submersion as well as night.[24] Riedman[27] suggested that pinnipeds,

with the exception of walruses, have eyes directed forward and probably have good binocular vision. Sea lions tend to have large eyes in comparison to the size of their skulls allowing better vision at depths where light is dim.[5] In contrast, a walrus has much smaller eyes in comparison to other pinnipeds, but they forage on the murky bottom and do not need to see well to find their prey.[5] On land, the nictitating membrane provides eye protection against sand and debris.[5] Sea lions also possess very active lacrimal glands that constantly produce mucin rich tears, although they do not have meibomian glands.[28]

The sea lion corneal epithelium is stratified and more keratinized towards the periphery. The Descemet's membrane is thin with a corneal stroma that is thick peripherally and thin centrally. Compared to the dog, the cornea curvature is less. The cornea is the primary refractive structure in humans and most terrestrial animals. Aquatic mammals display **emmetropia** (i.e., refraction of light to focus on the retina) while underwater.[29] Vision above water in these animals would result in aerial myopia because the corneal refractive power increases due to the larger difference in refractive index.[29] However, these aquatic species have unique mechanisms to attain emmetropia in air which include a flattened area, or plateau, in the inferonasal paraxial cornea and a stenopeic or vertically positioned slit-like pupil.[29] The uniquely delimited area (6-10mm in diameter) of almost flat surface (zone 1) is located in the central part of the cornea (Figure 1).[30,31]

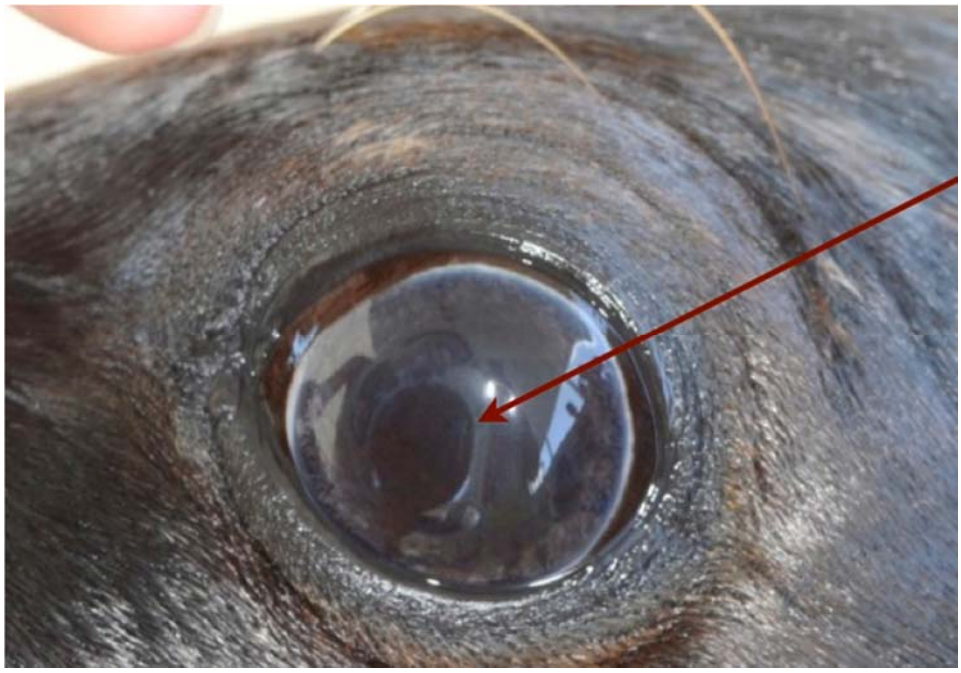

Figure 1 The red arrow depicts Z1= Zone 1 (medial paraxial flattened plateau) of the sea lion cornea.

This inferonasal paraxial flattened plateau acts as an emmetropic "window" in which refraction remains almost equal both in air and water. Since the refractive index of aqueous humor and water are very similar, the cornea does not contribute to refraction when the animals are under water.[24,32,33]

Pinnipeds, other marine animals, including fish, have a spherical or almost spherical lens which translocates in accommodation to allow focusing under water.[20,34]

Marine mammals possess strong rounded lenses that more resemble a fish lens than a terrestrial animal lens. The lens is attached to the ciliary body via both delicate zonular ligaments and by direct attachment to the ciliary processes (Figure 2).[20]

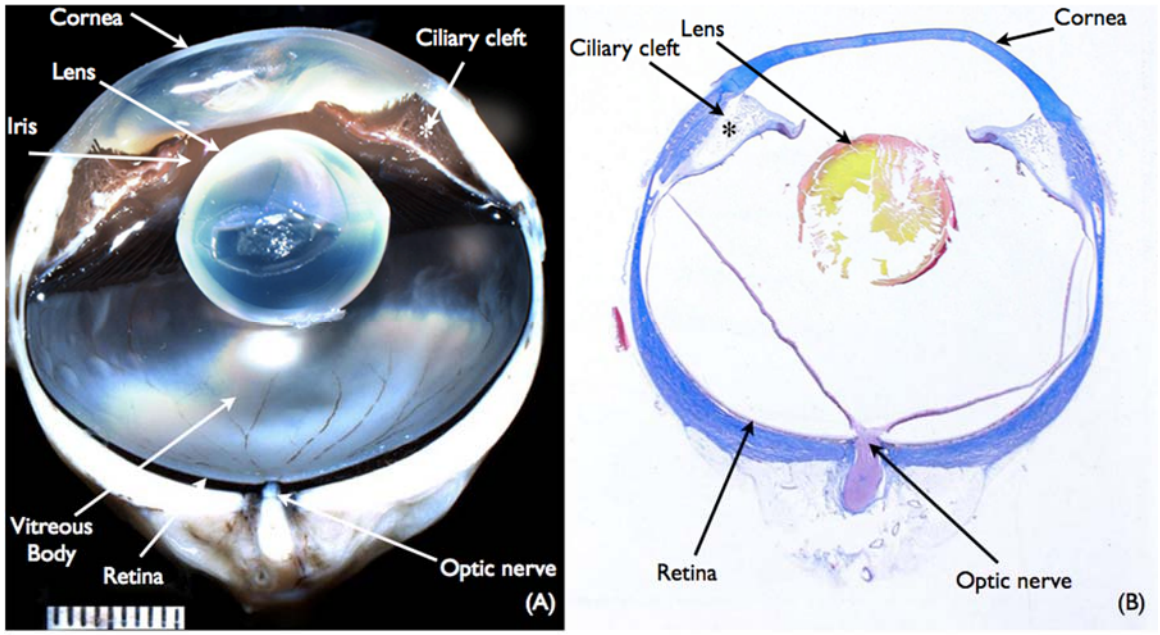

Figure 2 (A) California Sea lion Gross image. (B) Subgross image of same globe stained with Masson s trichrome.

Sea lions have a round lens and one of the widest ciliary cleft (*) compared to other marine mammals.[20] Courtesy of Dr. Richard R. Dubielzig.

The nearly spherical lens is **multifocal** which has been suggested to improve the focus over a range of viewing distances in monochromatic animals and therefore reduces the need for a broad accommodative range.[29] In order to focus an image onto the retina, while under water, light retains its original trajectory through the cornea and bends as it passes the highly curved lens then strikes the area centralis of retina[34] The refractive power of the shape of the lens is unfavorable in air because light is focused in front of the retina; this is compensated for by the flattened corneal plateau and the stenopeic or upside down, pear-shaped pupil which constricts to form a narrow vertical slit (Appendix C).[34]

Accommodation is achieved by adjusting either the shape of the lens or changing the distance of the lens to the retina to focus on various distances. In humans and young

dogs, the accommodative mechanism uses the ciliary body musculature to alter the curvature of the lens in biconvex lenes, but in spherical lenses will most likely not influence its shape.[33,34] Although since the lens of the sea lion is round and rigid, changing the shape of the lens may be less effective for accommodation.[20] Pinnipeds have a unique suspensory apparatus with a better developed ciliary muscle than cetaceans and various researchers have suggested the use of this muscle in accommodation.[35-37] At the equator, the inner aspect of the long ciliary processes makes direct contact with the lens capsule and also has thin and delicate zonular ligaments.[20] There is a thin circumferential smooth muscle within the ciliary body at the base of the ciliary processes. It has been suggested that this musculature is involved in accommodation and when contracted actively constricts the posterior chamber surrounding the lens equator. Miller et. al, also suggests that the lens moves anteriorly during diving via contraction of the well developed ciliary body musculature, thus physically dilating the pupil aperture.[20] The position of the uveal muscles and the suspension of the lens suggest that these structures may be involved in accommodation. Constriction of the lens using the ciliary muscles may also impact the curvature of the lens. In cetaceans, research shows that the ciliary muscle is not well developed. This muscle is lacking in most whale eyes.[33,37] This characteristic may suggest that accommodation is not achieved by change of the lens shape as is seen with terrestrials.[31,38] A theory behind the mechanism of how dolphins accommodate is by axial displacement of the lens due to changes in intraocular pressures.[31] It is thought that dolphins use their massive musculus retractor bulbi producing axial displacement of the globe within the orbit changing (i.e. increasing) the IOP.[31] Mass and Supin[31] suggest that when the eye is pulled back into the orbit, IOP

increases thus shifting the lens forward. When the eye is allowed to move forward, the pressure decreases thus shifting the lens backward.[31] Another theory suggested by Dawson[39] is that dolphins use their well developed extraocular muscles to alter the diameter of the eye and thus compensate for the changed focal length, although research has not been performed to confirm this.[39] The sea lion is exposed to continuous external water pressure changes that occur on a daily basis. As they dive, they may rely on increasing IOP, which may facilitate repositioning of the lens. It may not be a coincidence that the equator of the eye is the thinnest part of the eye in most species, possibly resulting in small changes in axial length at that region of the eye (Samuelson, 2014, personal communication). As extraocular muscles contract, the lens moves forward, potentially raising the IOP, as aqueous continues to move into the prominent iridocorneal angle. (Colitz, Samuelson, 2014, personal communication). (Figure 3).

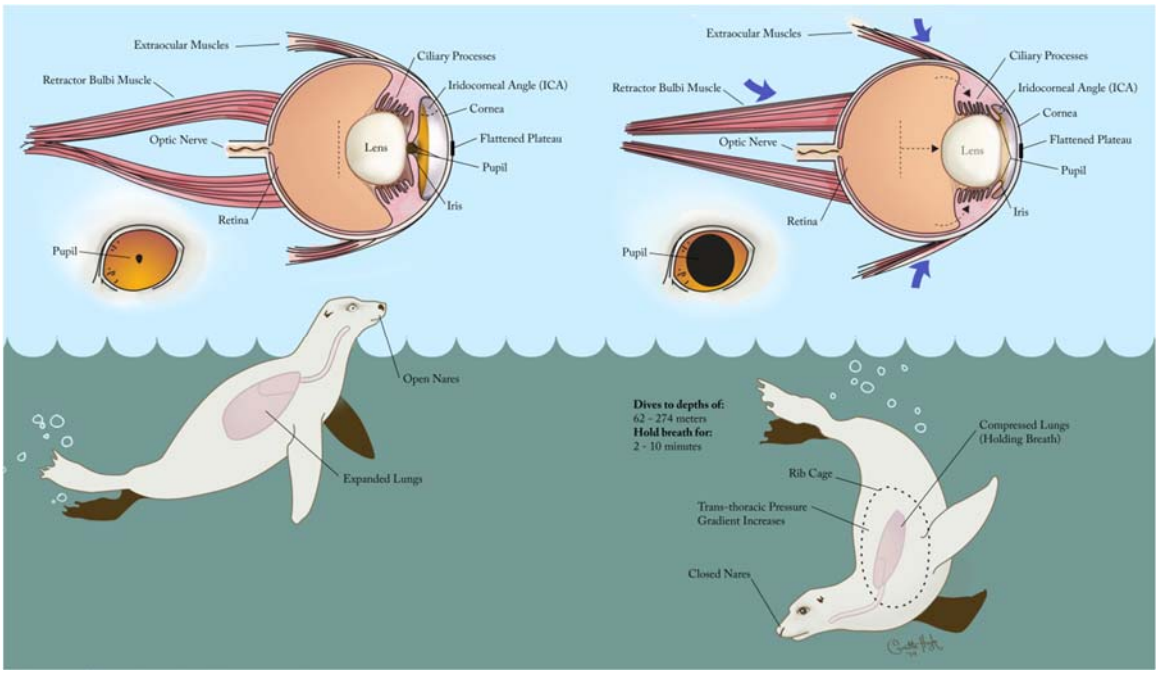

Figure 3 This image is showing a possible mechanism of how intraocular pressures may increase when sea lions dive.

Sea lions hold their breath during a dive, the trans-thoracic pressure gradient increases, causing IOP to increase, which may due to an autonomic response. One hypothesis includes that sea lions accommodate by moving the lens forward with assistance of the extraocular muscles, retractor bulbi muscle, and ciliary musculature (shown by blue arrows).

The sea lion pectinate ligament extends from the base of the iris toward the limbus with intermittent open channels between the anterior chamber and the ciliary cleft. The ciliary cleft shows a strong reticular pattern supporting stromal columns.[20] A corneoscleral and uveal trabecular meshwork was observed similar to other caniforms.[20] Ocular ultrasonography is a medical behavior that has been trained in sea lions.[40] This ultrasonography technique has been performed above and below water, showing that underwater the lens of the sea lion does move forward into the anterior chamber compared to on land.[40]

The anterior iris was delineated by pigmented cells and grossly appears brown with some animals having purple color variation.[20] The dilator muscle extends posterior to the base of the iris, extending over the widened base of each ciliary process until the edges of the plicata.[20] The dilator muscle is thin within the iris itself and nonexistent near the pupillary margin, which may be one reason why, topical mydriatics such as tropicamide, atropine, or sympathomimetics do not dilate the eyes of pinnipeds. However, the pupil can be dilated using intracameral undiluted epinephrine (1:1000).[20] This lack of pharmacological response to these topical agents may suggest poor penetration or too few cholinergic and adrenergic receptors, respectively to respond as they do in other mammals.[20] The posterior and more robust portions of the dilator muscle suggest that this muscle may have more of a function affecting accommodation rather than mydriasis.[20] A robust, circumferential sphincter muscle is well developed all along its entire length from the pupillary margin to a short distance posterior to the iris base. Miosis is achieved when this segment of the sphincter contracts, but the thickest segment of this muscle posterior to the iris base suggests a possible role in accommodation.[20]

In the underwater environment, the eye must adjust to wide variations in luminosity. In order to achieve this mechanism, the iris muscle contractions create species-specific pupil shape to regulate the amount of light entering the pupil. Mass and Supin[31] showed that pupil size changed over a wider range in animals that that are deep divers versus shallow ones (Appendix C **).** The pupil will constrict in very bright, illuminated areas and dilate as the animal moves into darker waters. Another role of the pupil is to prevent the bleaching of the photoreceptor pigment in bright light.[29] The range of pupillary area variation is rather small in shallow-diving animals; 70.5 times in the

harbor seal (*Phoca vitulina*), and 26 times in the California sea lion than normal resting size.[31,41,42] The pupil size of the deeper diving northern elephant seal (*Mirounga angustirostris*) varied within an extremely wide range (Appendix C).[31,41,42] In dark-adapted conditions, there was a giant area of 422 mm^2 (approximately 23 mm in diameter) to a pinhole opening of 0.9 mm^2 in light-adapted conditions. The range of variation is almost 470 times. [31,41,42] Most vertebrates have two types of photoreceptors, cones and rods. Cone cells contain visual pigments that function at high light intensities and provide resolution i.e., fine detail, and color vision. In colorless, dim light, rods provide the less acute night vision and motion.[24] The relative number of cones and rods depends on whether an animal is diurnal, arrhythmic, or nocturnal.[34] In nocturnal animals, rods are the predominant photoreceptor; while the number of cones is few or lacking completely.[34] Diurnal animals have a larger number of cones and lower number of rods. [34] The eyes of arrhythmic, also known as cathemeral, species are designed to operate equally in bright and dim light. Arrhythmic animals (most mammals – dogs, cats, herbivores) possess a developed area centralis with relatively high cone density, but not exclusively cones as in foveate species. The California sea lion has a high density of photoreceptors of 200,000 to 260,000 per mm^2 which maximize their visual sensitivity.[15,43] The retinas of aquatic mammals are similar to those of nocturnal terrestrial mammals as they have mostly rod photoreceptors and a limited number of cones.[31]

Light penetrates the ocean column and it is absorbed, refracted, and scattered.[44] The amount of light that passes depends on its wavelength, the concentration of chlorophyll, and the concentration of dissolved organic matter.[44] As light is scattered and

absorbed; contrast and brightness are reduced.[26] In open waters, the shorter wavelength, blue light, is transmitted better. In coastal waters, light of longer wavelengths penetrate more easily.[44] Modified visual rod pigments have been observed in deep diving species that provide increased sensitivity to the blue-green wavelength (Appendix B).[45,46] Deep sea fish tend to have a wavelength of maximum absorbance in the range of 477-487nm which uses a blue sensitive visual pigment.[24,47] The southern elephant seal, which dives to depths of 200m, has a similar deep-sea, blue-shifted visual pigment with wavelength of maximum absorbance between 485-486nm.[24,47] They also suggested that the visual pigment of the elephant seal can be considered adaptation not so much to the ambient light of the sun, but to that emitted by the bioluminescent squids on which it feeds.[24,45] *Zalophus californianus* and *Callorhinus ursinus* are two shallow diving otariids species which have visual pigments with wavelengths of maximum absorbance shifted toward the longer wavelengths near 500nm.[24]

Immunochemical studies in five species of seals and sea lions revealed that cones comprise only 1% of photoreceptors.[31,48] Pinnipeds and cetaceans are monochromatic, containing only blue-shifted green cones, which are different than most terrestrial primarily arrhythmic species which have at least two spectrally sensitive cone types (dichromats), and primates which have three cone types (trichomats) (Appendix B).[31,49] S cone opsins have not been isolated in pinnipeds or cetaceans and this has been confirmed through molecular techniques and electroretinography. One pinniped (the harp seal) has an SWS(short wave-length-sensitive) pseudogene, similar to Cetacea, and another species (the harbor seal) appeared to have an intact SWS gene. These marine species only possess M/L cone opsins and rod opsins.[20,46,50] The loss of S opsins represents a

mutational event at the basal point of pinniped lineage suggesting an adaptation situated for living in a marine environment.

Studies of topographic distribution of ganglion cells in the inner retina of cetaceans revealed two ganglion cell concentration, area centralis (the best vision areas), whereas sea lions only have one area.[31] These are areas of the best visual acuity. A characteristic feature of the cetacean and pinniped retinas is the large size of ganglion cells separated by wide intercellular spaces in a single row.[20,31] The pinniped ganglion cell measurement on average was 31 microns. [20] In comparison, a terrestrial caniform, such as the dog, was 28.5 microns.[20] In pinnipeds, the inner nuclear layer of the retina is thin and has **bipolar** and **amacrine** cells diffusely distributed throughout. [31] The ratio of photoreceptors to bipolar cells to ganglion cells are 100:10:1. [24,35,43,51] The outer nuclear layer is the thickest of all the layers, being more than 20 **perikarya** deep.[31] The sea lion fundus is holangiotic similar to domestic ruminants, pigs and carnivores. The retinal vessels are numerous and positioned in a spoke-like manner extending to the peripheral retina.[20] Similar to cats, the optic nerve is not myelinated. The lamina cribrosa within the optic nerve is very delicate and a prominent vascular plexus surrounds it. This ophthalmic rete is also found in cetaceans suggesting it is used to maintain appropriate temperature, and possibly oxygen concentration, to the delicate tissues found at the posterior of the globe.[20,35]

The tapetum lies within the choroid and external to the retinal pigmented epithelium (RPE). This layer of reflective tissue acts as a mirror to reflect light back through the retina a second time, effectively increasing the total amount of available light to the rod and cone photoreceptors in the retina.[33] In comparison to other animals, marine

mammals have the thickest and most well developed tapeta.[39,44] The increased scotopic sensitivity to blue-green and green wavelengths in the seal may be due to the enhanced development of this structure.[52] Pinnipeds and cetaceans have two different types of tapeta. The tapetum cellulosum found in pinnipeds is formed from intercellular reflective rodlets and on fundic exam appears green. Jamieson and Fisher[35] and Young et al.[53] showed that cetaceans contain a tapetum fibrosum, which is formed with extracellular collagen fibrils.[31,35,53] In cetaceans and pinnipeds the tapetum completely covers the fundus, whereas in terrestrial animals the tapetum covers the fundus partially in the dorsal aspect.[54,55]

A definition for visual acuity is the measurement of how well an animal can resolve features in its environment. It depends on contrast, and the perception of fine point to point detail, i.e., resolution.[24] Peterson and Bartholomew[23] suggested that the visual acuity of the California sea lion is quite poor in daylight and is even worse at night.[22,23] Schusterman[22] also observed in vision experiments that sea lions learn to orient to visual patterns slower in air than underwater.[22,52] The reason for this decreased detail in air is either because the pupil stays constricted and a low level of light enters the eye or because the pupil stays dilated in air in dim light resulting in corneal **astigmatism**.[22,24,56] Lavigne and Ronald[52] suggested that visual acuity gradually decreases underwater primarily because of decreased luminance and the accompanying transition from cone to rod vision. Although even in extreme low light, there is behavioral evidence to suggest that the nocturnally adapted eyes of sea lions see relatively sharply, at least underwater.[22] Schusterman[22] called the visual acuity of the sea lion similar to that of some visually

active carnivores on land, such as the cat, making them quite suitable to detect small prey from a distance.

General vascular pattern and aqueous humor hydrodynamics of the eye

Glaucoma is a disease of increased intraocular pressure that can be caused by impaired aqueous humor dynamics.[9] When evaluating a glaucomatous eye, it is important to understand the vascular anatomy, iridocorneal angle, and aqueous humor outflow mechanisms. In the canine, the external ophthalmic artery is the major arterial supply of the eye. The artery branches off the internal maxillary artery, a branch of the external carotid artery.[11] Numerous short posterior ciliary arteries arise from the external ophthalmic artery and penetrate the sclera around the optic nerve head.[11] The retina and choroid are supplied by these short ciliary arteries. Within the sclera, single medial and lateral long posterior ciliary arteries pass around the globe horizontally to supply the ciliary body (Appendix D).[11] The extraocular muscles are supplied by muscular branches of the orbital artery (anterior ciliary arteries) which enter the globe near the insertions of these muscles.[11] The complete ciliary arterial supply is formed when these anterior ciliary arteries anastomose with the long posterior ciliary arteries. The major arterial circle of the iris is formed from branches of the ciliary arterial network (Appendix D).[11]

The retinal veins and venules run from the peripheral retina toward the optic nerve head and are the main venous drainage of the retina.[11] The posterior ciliary veins drain this venous circle posteriorly to a dilation in the orbital vein, the superior (dorsal) ophthalmic vein.[11] Vortex veins drain the choroid exiting the globe near the equator and join the superior and inferior ophthalmic veins (Appendix D).[11] The anterior ciliary veins drain the ciliary body. These veins also drain to the same superior and inferior

ophthalmic veins that empty into the orbital venous plexus at the apex of the orbit. [11] This plexus of venous blood enters the cavernous sinus within the cranial vault. The cavernous sinus drains into the vertebral sinuses, external jugular vein, and internal maxillary vein.[11] Venous blood can also pass anteriorly by anastomoses between the ophthalmic veins, and the malar, angularis oculi, and facial veins. [11] The facial veins drain via the external maxillary and external jugular veins.[11]

Aqueous humor is a transparent liquid that fills the anterior and posterior chambers. The anterior chamber is located between the iris and cornea and the posterior chamber is between the iris and anterior lens capsule, forming a cavity for the lens to reside. The nonpigmented epithelium of the ciliary body produces aqueous humor via two mechanisms, which include passive diffusion and active secretion.[11] Passive diffusion consists of ultrafiltration and diffusion. Ultrafiltration is defined as the passage of fluid under the influence of the hydrostatic pressure of the arterial system in the ciliary vasculature against an osmotic gradient into the posterior chamber.[11] The ciliary capillaries provide the fluid which passes into the stroma of the ciliary processes through the ciliary epithelium and into the posterior chamber.[11] Aqueous humor then flows into the anterior chamber through the pupil then between the pectinate ligament, through the trabecular meshwork of the ciliary cleft, and out the drainage (iridocorneal) angle (Figure 2).[11] In the canine, approximately 85-90% of aqueous humor filters through the meshwork into the systemic venous circulation through a plexus of small veins in the sclera called the scleral venous plexus (aka angular aqueous plexus) .[11] Approximately 10-15% of the exit of aqueous occurs in the unconventional uveoscleral outflow, posteriorly through the ciliary body, and into the suprachoroidal spaces.[11]

Passive diffusion is controlled by the blood-aqueous barrier, provided primarily by the pigmented epithelial layer of the ciliary process. The blood-aqueous barrier separates the blood in the capillaries of the ciliary stroma and the aqueous humor in the posterior chamber.[11] Many substances seen in blood are also seen in aqueous humor. Substances can be broken down into three categories according to how freely they pass into the aqueous humor. Large molecules, including plasma proteins, pass very slowly giving the impression that there is an absolute barrier.[57] Thus the concentration of plasma proteins in aqueous humor is 0.02- 0.03%.[57] Damage to the blood-aqueous barrier increases permeability. Inflammation of the anterior uvea can increase the amount of proteins such as immunoglobulins and fibrinogen that enter the aqueous humor.[57]

Active secretion is the selective transport against a concentration gradient. The nonpigmented epithelium of the ciliary body has several energy dependent transport mechanisms.[11] This active transport occurs as a result of the osmotic pressure of the aqueous humor being higher than that of the plasma.[11] A sodium pump system has been identified which actively pumps sodium (Na^+) and chloride (Cl^-) ions into the aqueous humor and draws water passively along a concentration gradient.[11] The sodium pump is responsible for the majority of actively formed aqueous. Carbonic anhydrase is the main enzyme transport system that catalyzes the formation of carbonic acid (H_2CO_3) from CO_2 and water.[11] Carbonic acid dissociates and results in the secretion of bicarbonate ions into the aqueous humor. Sodium also accompanies bicarbonate.[11] Inhibition of carbonic anhydrase, can decrease bicarbonate to enter the posterior chamber which results in less water following and an overall decrease in aqueous production.[11]

The sea lion ciliary cleft is wide with a thick pectinate ligament that extends from the base of the iris toward the limbus with intermittent open channels between the anterior chamber and ciliary cleft. The corneoscleral trabecular meshwork is embedded in the sclera with the ciliary cleft in the inner margin and the uveal trabecular meshwork is a blending of the columns of the ciliary cleft and ciliary body stroma. The iridocorneal angle, vasculature, and aqueous humor outflow have been studied in two other marine mammals.[58] The West Indian Manatee, *Trichechus manatus* and short-finned pilot whale, *Globicephala macrorhynchus* were compared to their terrestrial counterparts, *Loxodonta Africana* and *Hippopotamus amphibus*, in this study.[58] The manatee, a herbivorous marine mammal, was found to have a narrow iridocorneal angle, which becomes thinner internally.[58] The pectinate ligament is radially aligned with round beams of collagen and pores separating them.[58] Most of the angle is filled with the uveal trabecular meshwork (UTM). Aqueous humor outflow runs through a plexus, with numerous vessels in some areas, and as sinus-like in others.[58] Aqueous outflow appears to be substantial through the angular aqueous plexus (AAP) where large and numerous vessels are present.[58] On average, the AAP consists of 6-7 vessels.[58]

The pilot whale has a much wider iridocorneal angle in comparison to the manatee.[58] Encapsulated sensory corpuscles (ESCs) were observed near several blood vessels, often posterior to major veins and also found within the UTM (Appendix E).[58] These ESCs have also been found in other cetaceans and are believed to be mechanoreceptors.[58] These receptors found in proximity to vessels may detect vessel volume and pressure due to diving.[58] ESCs adjacent to vessels have been found in both the pilot whale and the beaked whale, *Mesoplodon bidens*.[58]

Through evolution, these aquatic animals may have developed mechanisms to control IOP more rapidly and effectively via aqueous humor production and removal. Regulating control of IOP may be advantageous when diving. An increased ocular vascular supply and venous outflow may be a mechanism to deal with changes in the external pressure. A number of iridial and ciliary body blood vessels have also been found in other aquatic mammals such as the *Delphinapterus leucas, Monodon monoceros, and Balaenoptera physalus.*[58]

Aqueous humor provides nutrients (oxygen, glucose, and amino acids) for the tissues it contacts; iris, cornea, and lens.[58] Along with carrying nutrients, aqueous humor also removes metabolic waste products. Therefore, as aqueous humor moves out of the ciliary body and into the drainage angle, there is a change in the composition.[11,57] Aqueous humor also functions in maintaining IOP and therefore affects the shape and size of the eye.[57] IOP is an equilibrium between the rate of formation and drainage of aqueous humor.[59] Formation of aqueous humor appears to be relatively constant and pressure is controlled by the outflow mechanism and rate of formation is related to the circulation of the anterior uvea, the activity of the secretory epithelium, and the integrity of the blood aqueous barrier. The outflow mechanism is related to open communication of the pupil, open filtration angle, and unobstructed flow through the ciliary-scleral pathway.[57]

Classification of glaucoma

Glaucoma is currently being redefined, but in the past was classified under two categories (Appendix F).[60] Understanding the pathophysiology behind the cause of aqueous humor obstruction leading to elevated IOP is vital in categorizing glaucoma.

One classification is based on whether the iridocorneal angle is open, narrow, or closed. [11] The second classification describes the glaucoma as primary, secondary, or congenital.[11] In canines, primary glaucoma is often bilateral and likely hereditary. The initiating event of increased IOP begins in the trabecular meshwork or other parts of the iridiocorneal angle.[11,57] In secondary glaucoma, outflow obstruction is caused by a pre-existing ocular or systemic disease.[57,60] If possible, the underlying etiology will need to be treated in conjunction with the glaucoma. Secondary glaucoma can be unilateral or bilateral. Congenital glaucomas are present at or soon after birth and are rare in domestic animals.[11,57]

In humans and nonhuman animals nearly all of the glaucomas occur from an impairment of aqueous humor outflow rather than an increased rate of aqueous humor formation. These glaucomas can be classified as primary, secondary, and congenital glaucoma.[9,11] In humans and nonhuman animals, primary open angle disease is related to outflow resistance that occurs at the corneoscleral trabecular meshwork and/or juxta canalicular connective tissues.[61] Humans and nonhuman animals with primary narrow and closed-angle glaucoma have a similarity that there is a mechanical compromise to the filtration angle.[62] There are some differences in animals and humans, such that nonhumans have the presence of the pectinate ligaments and sclerociliary cleft which may not make them ideal for animal modeling.[62,63]

In general the primary glaucomas develop when there is no concurrent ocular disease contributing to the elvevation of IOP, usually occurs in both eyes, and has an abnormal biochemical component of the trabecular meshwork cell metabolism for the outflow system.[9] Primary glaucomas in nonhuman animals are associated with a genetic

predisposition usually from inbreeding.[61] This type most commonly is seen in domesticated species, such as the dog. The progression of this disease must be gradual enough to allow animals to reproduce and pass on the disease to future offspring. Reports of primary glaucoma in the wild are limited, although they do occur. Whereas, secondary glaucomas in wild animals is the most common type seen and usually only effecting one eye. Secondary glaucomas are associated with concurrent ocular disease that can physically obstruct the aqueous outflow pathway causing an elevation in IOP.[9] Thus far in sea lions we have only diagnosed secondary glaucoma. Congenital glaucomas are rare in the dog and usually occurs with elevated IOP after birth.[11] This type of glaucoma is associated with an angular anomaly which contributes to abnormalities in the aqueous humor outflow pathway. Rabbits have been reported to have a congenital form of glaucoma that is autosomal recessive and most frequently seen in the New Zealand albino rabbit.[64] It is interesting to note that aqueous ascorbate concentration levels in affected rabbits are 50% of control eyes before the onset of clinical signs.

Dogs are affected most frequently with primary open and closed angle glaucoma (approx. 40 breeds are predisposed to this type). [64] Primary glaucoma is most commonly seen in Caucasian humans and less frequently seen in cats and horses. Primary open angle glaucoma has been reported in a *Macaca mulatta*, although in wild animals it is not common.[64] An inherited or untreated primary open angle glaucoma has been documented in the Beagle (autosomal recessive) and Welsh Springer and Great Dane (autosomal dominant).[61] The cause is related to the metabolism of the trabecular meshwork. Beagles between 8-16 months are affected and since the progression of this disease is slow, disease and vision loss may not be detected until advanced.[61] An interesting finding was

that histochemical localization of the glycosaminoglycans within corneosleral beams and juxta canalicular zone in normal dogs revealed chondroitin sulfate was a major component.[61] In glaucomatous animals as the disease progressed, the amount of chondroitin decreased and started to produce a glycosaminoglycan-resistant enzyme material. Open angle glaucoma usually affects both eyes and on gonioscopy the angle appears normal during early stages of the disease.[61]

Primary closed angle glaucoma is the type most frequently seen in dogs in the United States. This glaucoma can be characterized as initially unilateral, acute elevation in IOP that often resolves or persists at elevated levels (50-80mmHg).[10] The acute elevation in IOP causes nerve damage inducing retinal ganglion cellular degeneration and apoptosis caused by glutamate excitotoxicity, neurotropin deprivation, accumulation of intraneuronal calcium, and formation of nitric oxide, proteases, and oxygen radicals.[61] Gonioscopy reveals an angle that is collapsed or covered with peripheral iris or connective tissue.[10] Pupillary block has been mentioned as one of the causes in which resistance from posterior chamber to the anterior chamber increases.[10] The iris is pushed forward as aqueous humor accumulates in the posterior chamber causing angle closure. In most breeds, this disease may be considered bilateral, as 50% of dogs will present a few months later with the other eye affected.[10] Goniodysgenesis has been associated with angle closure. American and English Cocker Spaniels and Basset Hounds have been shown to have narrowed iridocorneal angles and sclerociliary clefts.[10,62] Studies in Basset Hounds show dysplasia of the pectinate ligaments forming large, solid sheets with or without flow holes.[62] Angle structures deep to these flow holes may or may not be normal.[10] These animals may maintain normal IOP for years and then present with

elevated pressures middle to late years. These dogs are at greater risk of secondary glaucoma caused by uveitis because inflammatory debris may occlude the holes.

In secondary glaucomas, increased IOP is a result of impairment or obstruction of aqueous outflow caused by a pre-existing ocular or systemic disease.[10] The underlying etiology will need to be treated in conjunction with the glaucoma. Secondary glaucoma can be unilateral or bilateral, either open or closed angle and have some detectable impairment of aqueous humor outflow. Secondary glaucoma associated with anterior uveitis is the most common type seen in cats.[9] In the dog, lens luxation is the most frequent cause of secondary glaucoma.[9] Buphthalmos causes progressive stretching of the lens zonules which eventually break at the equatorial lens capsule. Terriers are commonly affected with lens luxations.[10] Mechanical anterior lens movement can impair the passage of aqueous humor through the pupil, causing anterior ballooning of peripheral iris and reduction of iridocorneal aqueous outflow. This movement of the iris causes anterior and posterior synechiae, also known as iris bombe.[10] The lens can luxate posteriorly causing pupillary blockage by allowing vitreous access into the pupil and anterior chamber.[10] Intumescent (swollen) lens may also cause acute pupillary block, pushing the iris anteriorly. Iridocyclitis is a common inflammatory glaucoma associated with pupillary occlusion and iris bombe leading to inflammatory cells, fibrin, and cellular debris which obstruct the iridocorneal angle.[10] Traumatic glaucomas are infrequent in the dog. Intraocular hemorrhage in the dog is more likely to increase IOP than complete acute hyphema which is usually associated with uveal inflammation and may decrease IOP.[10] Aphakic glaucoma may be caused by occlusion of the pupil from inflammatory membranes and closure of the iridocorneal angle and ciliary cleft by formation of pre-

iridal fibrin membranes and anterior synechia.[10] Secondary glaucomas can also be caused from intraocular tumors. The most common primary intraocular tumors found in the dog are melanomas and adenomas/adenocarcinomas of the ciliary body and iris. Lymphoma/lymphosarcoma may also commonly affect the anterior uvea.[10]

The most common type of glaucoma in pinnipeds is secondary glaucoma and typically occurs following presence of long standing cataracts with or without anterior luxation (Colitz, 2014, personal communication). Chronic lens induced uveitis may also cause secondary glaucoma.[9] Following lens removal, keepers need to be aware of possible trauma which can lead to hyphema causing secondary glaucoma and buphthalmos.

Tonometry and pathogenesis of glaucoma

Tonometry is the diagnostic tool used to measure IOP. A tonometer is a non-invasive instrument that records IOP by gently touching the cornea and measuring bulbar tonus, which is then used to estimate the force exerted by the intraocular fluid on the relatively rigid walls of the globe from within (=IOP). Currently, two different instruments are used in veterinary practice, Tono-Vet® (Lumic International, Baltimore, Md./ Webster Veterinary Supply, Inc.) (Figure 4) and Tono-pen Vet® (Dan Scott and Associates) (Figure 5).

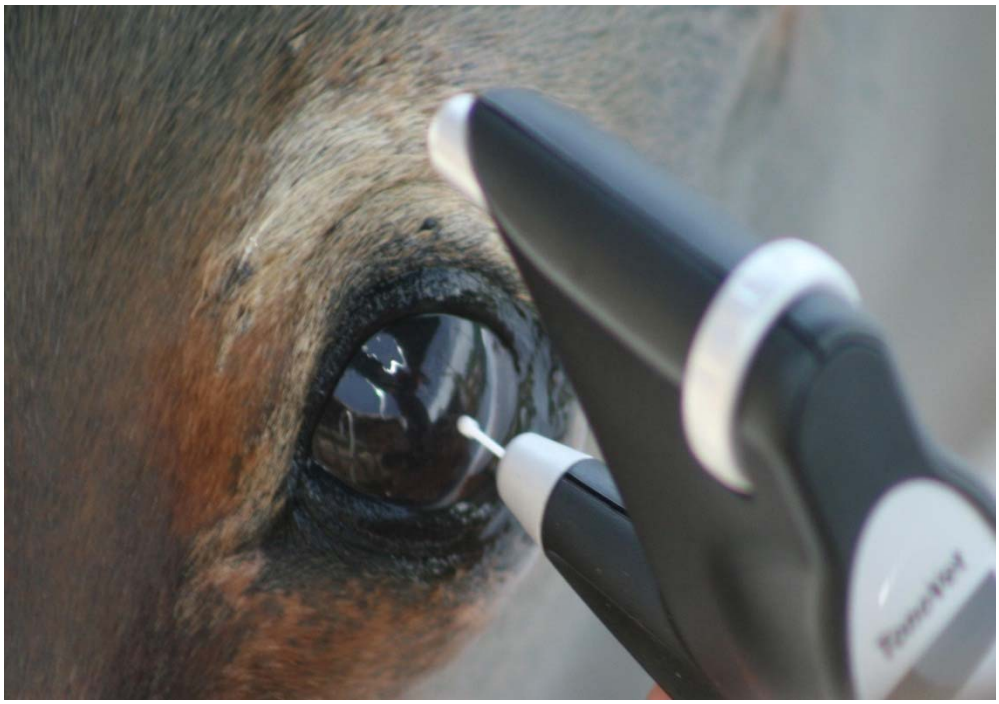

Figure 4 TonoVet® rebound tonometer being used to collect an intraocular pressure on a sea lion. Courtesy of Dolphins Plus, Key Largo, Florida.

Figure 5 Tono-pen Vet® applanation tonometer is being used to collect an intraocular pressure on a sea lion. Courtesy of Theater of the Sea, Islamorada, Florida.

Data collected regarding the etiology, pathogenesis, and classification of the glaucomas has been extrapolated from human and canine medicine. In the future, there will hopefully be more studies aimed at the exact vascular pattern of the eye, aqueous flow dynamics, and pathophysiology of glaucoma in the California sea lion. Glaucoma is diagnosed when there is an increase in intraocular pressure (IOP) beyond that which is compatible with healthy ocular tissues.[11] Regardless of the cause of elevated IOP, the detrimental effects on ocular tissues are similar.

The Tono-pen Vet® is an applanation tonometer that functions by measuring the force required to flatten, or applanate, a specific area on the surface of the cornea (Figure

5).[59] Applanation tonometers are based on principles using the Imbert-Fick law stating that the external force (W) against the sphere equals a pressure within the sphere (Pt) times the area (A) flattened by the external force.[65] When this force is converted into an estimate of IOP, there are a few assumptions that must be made about various physical factors including corneal thickness and curvature, corneal and scleral rigidity, tear film viscosity, and the effects of any topical medications that might be present.[59] A topical anesthetic should be applied to the corneal surface prior to using the instrument. Once the instrument is turned on, The Tono-pen Vet® is automatically set to a default calibration and will need to be changed depending on the species being evaluated. The Tono-pen Vet® has only one setting and not calibrated for various species. The "normal" IOP range of the Tono-pen Vet® is between 15-25 mm Hg in the dog, cat, and horse.[10] Glaucoma should be or is suspected if IOP is greater than 25 mm Hg. An IOP greater than 30 mm Hg is diagnostic for glaucoma.[66] In a study conducted by Hirst et. al. (1983), 7 pinnipeds were anesthetized with halothane and applanation tonography was conducted on 7 eyes revealing pressures between 8 and18 mmHg.[67] The applanation tonometer used was not specified and was not adjusted for scleral rigidity and corneal curvature.[67]

In 2004, a revised rebound tonometer was introduced specifically calibrated for horses, dogs, and mice. The TonoVet® does not use applanation of the corneal surface, but rather uses a new rebound method to estimate IOP by quantifying the deceleration of the probe and its impact on the corneal surface (Figure 4).[59,65] This diagnostic tool does not require topical anesthetic. An electrically magnetized tonometer probe comes into contact with and rebounds from the corneal surface to estimate an IOP using rebound kinematics.

Knollinger et. al., evaluated the TonoVet® for measuring IOP, using rebound tonometry, in dogs and horses and compared it to the Tono-Pen Vet®, using applanation tonometry.[59] The results of the study showed that mean +/- SD IOP's collected with the rebound tonometer were 10.8 +/- 3.1 mm Hg (range, 5 to 17 mm Hg) and 22.1 +/-5.9 mm Hg (range, 10 to 34 range) for the dogs and horses, respectively.[59] Mean IOPs obtained with the applanation tonometer were 12.9 +/- 2.7 mm Hg (range, 8 to 18 mm Hg) and 21.0 +/- 5.9 mm HG (range, 9 to 33), respectively.[59] The study concluded that the rebound tonometer provides an accurate estimate in clinically normal eyes in dogs and horses.[59]

Both instruments apply a stress to the corneal surface and therefore are subject to the effects of its biomechanical properties, such as corneal thickness and elasticity.[65] When you add corneal disease to the equation, such as edema, keratitis, vascularization, pigmentation, lipidosis, and scarring, IOP can increase when pressures are collected directly on the area of pathology.[65] Corneal biomechanics such as the type (edema vs scarring) and extent of abnormality need to be considered when collecting IOPs. Therefore, IOPs should be collected on normal corneal surfaces as aberrant pressures on the diseased cornea can falsely lead to a diagnosis of glaucoma.[65] The Tono-pen Vet® has a large footplate compared to the TonoVet® and therefore it has been suggested that the small tipped probe of the TonoVet® allows for better target precision enabling the examiner to strike small areas on the corneal surface. For glaucomatous patients, the TonoVet® has been recommended in dogs and cats due to the accuracy of manometrically controlled high pressure studies, in comparison to the Tono-pen Vet® performance which demonstrated a tendency to underestimate true IOP.[65] It has been suggested that the

TonoVet® is more sensitive for hypertensitive conditions allowing for early detection of glaucoma.[65]

A normal IOP is the pressure measured that will not cause optic nerve damage. A combination of tonometry and observation of ocular clinical signs is an acceptable way of diagnosing glaucoma. There are acute, subacute, and chronic clinical signs seen with glaucoma (Appendix G). All of the ocular tissues are affected in some way by an increased IOP including the retina, optic nerve, uvea, lens, cornea, and sclera.[64]

The uvea

The iris, ciliary body and choroid make up the uvea or vascular tunic of the eye.[10] The uvea is rich in blood supply which provides nutrition to internal structures. The dilator and sphincter muscles of the iris become atrophied and paralyzed with repeated elevations of IOP, resulting in **mydriasis**.[11] Mydriasis also occurs due to loss of retinal ganglion cells and optic nerve axons. In some cases of primary glaucoma, focal clusters of mononuclear inflammatory cells may be found at the base of the iris.[11] It is unknown whether inflammation has a role in precipitating primary glaucoma.[11] Occasionally in some cases of glaucoma, there may be an accumulation of melanin-containing cells found in the trabecular meshwork, iris base, ciliary body, and sclera.[68] There is evidence that this pigmentation may significantly decrease aqueous humor outflow, but the origin and cause is unknown.[68] Chronic elevations of IOP cause progressive atrophy and fibrosis of the iridal stroma and ciliary body and processes.[11] Sustained elevations of IOP decrease blood supply and, therefore, reduce aqueous humor production.[11]

Cornea and sclera

The cornea is the clear, avascular outermost layer of the anterior aspect of the eye. Corneal opacities and edema are commonly seen in captive sea lions at various aquariums.[20] An early manifestation of elevated IOP is disruption of the corneal endothelium resulting in diffuse **corneal edema**. Another cause of corneal edema is a disturbance in the balance between hydrating and dehydrating forces in the corneal stroma.[66] Primary glaucoma may cause diffuse edema; whereas, secondary glaucomas caused by anterior uveitis or lens luxations may show a focal or diffuse corneal edema.[66] Histologically, the normal lamellar pattern is disrupted due to an increase in the intercellular spaces.[69] Eyes with chronically elevated IOP with corneal edema may vascularize, develop pigmentation and/or fibrosis.[11] In advanced glaucoma, the sclera stretches and the eye enlarges (buphthalmia) with concurrent enlargement of the cornea (megalocornea).[11] Even if IOP returns to normal, this scleral stretching is irreversible. In advanced glaucomatous eyes that have scleral stretching buphthalmos, the aqueous production is decreased so these eyes stop stretching and the condition becomes tolerable despite pathological changes.[11] Commonly, corneal strectching can cause breaks in **Descemet's membrane (Haab's striae**).[68] Upon ophthalmic examination, they are seen as curvilinear double lines located in the posterior cornea.[68] The Haab's striae allow aqueous humor to be imbibed directly into the corneal stroma. The cornea may become ulcerated or perforated due to trauma to a blind eye or one with **lagophthalmos** (failure in the ability to close the eyelids completely due to enlarged globe).[68] With increased corneal exposure and reduced sensitivity, poor tear film and epithelial desiccation may result.[64] It is important for marine veterinarians to differentiate between corneal edema

and opacity seen with glaucoma versus edema seen with nonglaucomatous conditions such as uveitis, traumatic (penetrating) injuries, keratitis, corneal ulcerations and erosions. Pinnipeds with glaucoma will have a more mydriatic pupil compared to contralateral eye.

The lens

The main function of the lens is to refract incoming light to a focal point on the retina. The avascular, transparent crystalline lens contains four layers consisting of the outer lens capsule, an epithelial monolayer on the anterior aspect, the lens cortical fibers or cortex, and the central nucleus.[10] In chronic glaucoma, the lens will exhibit changes in both morphology and positioning. Sea lion cataract formation leads to subluxation and anterior or posterior lens luxation.[1] The lens does not have to be cataractous to luxate and this may be due to chronic exposure to UV and keratopathy (Colitz, 2014, personal communication). Secondary glaucoma in sea lions is typically due to either an anteriorly luxated lens or due to chronicity of inflammation wherein a pre-iridial fibrovascular membrane has developed covering the ICA[70] (Colitz, 2014, personal communication). In sea lions, buphthlamia is rarely the cause of the lens luxation (Colitz, 2014, personal communication). In canines, elevated IOP causes the sclera and adjacent anterior uvea to stretch rupturing the lens zonules resulting in lens instability and subluxation.[11] The formation of cataracts can occur with advanced glaucoma due to lens instability, mild or subclinical uveitis, or impaired nutrition to the lens via abnormal aqueous humor dynamics.[11]

The retina and optic nerve

Lesions in the optic nerve and the retina can be detrimental to vision and eventually result in blindness. In glaucoma, one area that is still controversial is the cause of optic nerve degeneration and optic disc cupping (the enlargement of the central physiologic cup of the optic disc).[64,66] In early glaucoma, the focus of the neural damage is impairment of rapid axoplasmic transport within the optic nerve axons and accumulation of cellular organelles in the scleral lamina cribosa. The lamina cribosa is a series of sieve-like openings in the posterior sclera through which the optic nerve axons pass out of the globe to the orbit.[64,66] In chronic changes, the optic nerve will become "cupped" or "excavated".[11,64] Cupping indicates that the optic nerve head has been irreversibly damaged. Also in dogs, as the level of IOP rises above 40 mmHg, blood flow is reduced.[11,64] Even brief periods of ischemia will cause damage to the retinal ganglion cells. As the nerve fiber layer degenerates, there is loss of retinal ganglion cells and thinning of the inner layers of the retina to the point where they disappear and are replaced by a glial scar.[11,64] Profound glaucoma results in tapetal hyper-reflexivity, retinal atrophy, peripapillary hyperpigmentaion, and attenuation or complete loss of retinal vessels, and extensive cupping of the optic nerve. Atrophy of the retinal pigmented epithelium in the nontapetal fundus and optic atrophy (grayish-white appearance) may also be visualized with an ophthalmoscope.[11,64]

Detrimental environmental factors

Theories to explain these ocular problems

Many factors contribute to ocular problems in sea lions. Environmental physiologic parameters such as water quality, filtration, and exposure to ultraviolent light

change when animals are brought under human care. Ocular lesions can start as minute corneal opacity and be exacerbated over time by secondary bacterial invasion. Different bacteria, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and coliforms including *Escherichia coli*, have been cultured from eyes showing traumatic lesions, conjunctivitis, and keratitis.[20] Other causes may be that there is a hereditary component associated with ocular disease (Colitz, 2014, personal communication). There are various theories as to why these animals are so prone to ocular problems. Most marine veterinarians would agree that the cause of eye disease is multifactorial.[5]

Sea lions also have a reduced ability for corneal healing. Corneal vascularization is necessary for wound healing.[71] One study evaluated the latter and results showed that sea lions do possess the functional components of the neovascularization pathway, but perhaps another factor in the cornea that moderates this process is absent or insufficient.[71]

Water quality testing is critical for maintaining a clean aquatic environment (i.e. appropriate salinity, pH, chlorine, temperature, and bacterial counts) that is essential for ocular health. Throughout the world, salinity will differ depending on the body of water being measured, the location, and the time of year.[5] Normal oceanic salinity ranges from 25 to 35 ppt. In aquaria, salinity should be measured daily.[5] The best choice is natural salt water for these animals, but some facilities only have freshwater. An open system is ideal because water is constantly replaced with new fresh seawater. In a closed or semi-closed system, the water is more prone to accumulation of toxins from biological reactions.[5] Eye lesions are seen more frequently in animals kept in freshwater, although there have been reports of some species of free-ranging phocids that live entirely in freshwater.[5] Transient cases of corneal edema can be caused by simply moving an animal to freshwater from

saltwater, or vice versa. If animals are kept in freshwater, daily eye soaks in saltwater may help prevent ocular disease (Colitz, 2014, personal communication). Griebel[26] studied a group of sea lions to evaluate if there was a difference in ocular effects in which chlorination was not used and instead water was changed once a week. Salt water was made synthetically by adding sodium chloride to avoid ocular disease. During the experiments no ocular lesions were observed in the animals studied.[26]

Bacterial contaminants should be closely monitored with a bacterial count of less than 1000 coliform bacteria per 100 ml of water.[5] To guarantee acceptable and minimal concentrations of fecal coliforms, tests should be run at least once per week. Coliform counts are required by the USDA.[5]

A measure of the hydrogen ion concentration in water is pH and should be measured daily.[5] This value indicates the acidity or alkalinity of the water and should range between 7.2 and 7.8 because chlorine disinfectant properties are ideal at this level.[10] Chlorine is used for removal of algae and killing of microorganisms. Free chlorine in the range of 0.3-0.5 ppm at a pH of 7.6 can control most bacteria levels.[5] Free chlorine refers to hypochlorous acid and hypochlorite ion or bleach. Chloramines, also known as combined chlorine, form when ammonia or organic nitrogen are also present. The sum of free chlorine plus combined chlorine equals total chlorine. Some facilities use fresh municipal water where the total chlorine frequently exceeds 2.5 ppm. One hundred percent of the animals in these pools develop corneal disease.[19] High levels and by-products of chlorine (chloramines, bromine, and other oxidizing agents) can cause ocular lesions.[5] A study was done evaluating two sea lions with corneal lesions and results showed that the concentrations of total, free and combined chlorine had a significant

influence on the size of the corneal lesions, whereas combined chlorine had the greatest influence.[72] This study recommended decreasing the organic material in the water and closely monitoring excessive oxidant use to sterilize the water. Levels of these disinfectants can spike unexpectedly and expose animals to noxious chemical byproducts.[19,21,72] Bromine and its noxious byproducts such as halogenated methanes (chloroform, bromoform, bromodichloromethane or dibromochloromethane) are often overlooked and rarely measured.[19] Liver and kidney toxicity can occur from these undesirable byproducts.[19] Oxidation occurs by the cytochrome p450 enzyme, which is present in ocular tissues at about 5% of the concentration of liver cells.[19] This enzyme is involved in free radical formation from breakdown of drug and toxins in ocular tissues.[19] These enzyme systems will begin the breakdown of drugs or toxins present in ocular tissues thereby producing free radicals, peroxide, or other intermediates in the process. Another popular disinfectant that is used is a powerful oxidant called ozone. Ocular discomfort and damage can occur if animals are exposed to residual ozone. Commercial ozone test kits are inexpensive and a valuable tool to test for the presence of ozone.

The temperature of the water is another aspect in water quality that should be monitored. Most aquariums see a trend of sea lions showing more ocular lesions in the summer versus in the winter, this is most likely due to longer days and increase exposure to UV. (Colitz, 2014, personal communication). Therefore, testing water temperature can be an indirect measurement of how much heat from the sun is penetrating the water column. Hyperthermia is always a concern when ambient temperatures start to rise, especially above 79^0F and can be fatal.[2] Low water temperatures are usually not a

problem because sea lions have a very thick layer of fat, called blubber, which keeps them insulated, stores energy, and contributes to their streamlined shape.[2]

Ultraviolet exposure is a risk for sea lions to develop ocular disease and their habitat should be modified to protect their eyes when possible. The recommendation for aquarium pool floors are multicolor granular aggregates embedded in clear epoxy resin which allows nonreflective use of multicolor choices, a slip-resistant surface, and chemical and water penetration resistance.[2] Although, not all aquariums are built this way, some floors are painted in very light colors so that animals are visualized better by the public and the water appears clearer. These lighter colors create an increase light reflection possibly causing ultraviolent hypersensitivity leading to ocular lesions.[2,19] Aquariums should be kept covered to prevent extra exposure to direct sunlight. When feeding sea lions, trainers should always feed the animals in a shaded area where they are not looking directly up to the sun to catch food.

CHAPTER III

STATEMENT OF RESEARCH AND HYPOTHESIS

Statement of research problems and objectives

There is a high level of ocular disease in captive California sea lions and the need for early detection is imperative. During this study, the objective was to track a group of sea lions over a period of several years to determine the range of IOPs in normal eyes and in eyes with different ocular lesions. Some of these sea lions were diagnosed with varying degrees of cataract formation prior to or during the study. Once present, progressive cataracts are detrimental to sight and also have the potential to luxate, causing pain, corneal fibrosis and glaucoma. Glaucoma is a chronic disease and may lead to blindness if not diagnosed immediately at the onset of clinical signs. Early detection and medical management are critical to maintaining vision. Today, due to the advancements in veterinary technology, particularly with the use of the Tono-pen Vet® and TonoVet® , these highly valued animals may be diagnosed and treated before the onset of severe ocular disease. If we know the normal IOP, we can evaluate captive sea lions early in life and chart their progression. If an animal's IOP starts to elevate over the normal range, veterinarians can begin with aggressive medical therapy.

Hypothesis and objectives

The initial objective was to evaluate whether there was a difference in IOP in clinically normal eyes when comparing the Tono-pen Vet® and TonoVet® in California sea lions. Ten animals were going to be evaluated on a daily basis for 1 year using both instruments to determine whether there was a difference in IOP values between instruments.

Note: Three months into study some of these animals passed away after hurricane Katrina hit the coast of Mississippi and both instruments were destroyed in the hurricane. The Tono-pen Vet® proved to be difficult to use on this species and very invasive for the collection of consecutive measurements. New hypotheses were created after the study was halted and restarted again a few months later.

Hypothesis #1

H_0: IOP measurements will not fall within a consistent range in clinically normal eyes of California sea lions.

H_1: IOP measurements will fall within a consistent range in clinically normal eyes of California sea lions.

Objective #1: To evaluate 19 animals on a recurring basis (depending on animals' location) using the TonoVet® to determine the normal range of IOPs.

Hypothesis #2

H_0: There will be no difference in IOP measurements in clinically normal animals that are positioned in a nose up and nose down position.

H_1: There will be a difference in IOP measurements in clinically normal animals that are positioned in a nose up and nose down position.

Objective #2: To evaluate 19 animals on a recurring basis (depending on the animals' location) using the TonoVet® to determine if there is a difference between nose up and nose down position.

Hypothesis #3

H_0: There will be no difference in IOP measurements in clinically normal and abnormal animals between zone 1 and zone 2.

H_1: There will be a difference in IOP measurements in clinically normal and abnormal animals between zone 1 and zone 2.

Objective #3: To evaluate a group of normal and abnormal sea lions on a recurring basis (depending on the animals' location) using the TonoVet® to determine if there is a difference between zone 1 and zone 2 position of the corneal surface.

Hypothesis #4

H_0: There will be no difference in IOP measurements in clinically abnormal animals that are positioned in a nose up and nose down position.

H_1: There will be a difference in IOP measurements in clinically abnormal animals that are positioned in a nose up and nose down position.

Objective #4: To evaluate a group of abnormal sea lions on a recurring basis (depending on the animals' location) using the TonoVet® to determine if there is a difference between nose up and nose down position.

Hypothesis #5

H_0: There will be no differences in IOP measurements among animals with normal eyes and those with clinically diseased eyes using rebound tonometry.

H_1: There will be differences in IOP measurements among animals with normal eyes and those with clinically diseased eyes, using rebound tonometry, however, only those with glaucoma will have a significant increase in IOP compared with clinically normal eyes.

Objective #5: To measure IOP in a group of captive sea lions exhibiting a variety of ocular diseases using the TonoVet® and these values will be compared to clinically normal eyes.

Hypothesis #6

H_0: There will be not a difference in IOP measurements among animals with abnormal eyes.

H_1: There will be differences in IOP measurements among animals with abnormal eyes.

Objective #6: To measure IOP in a group of captive sea lions exhibiting a variety of ocular diseases, divided into 5 different groups, using the TonoVet®.

Hypothesis #7

H_0: There will be no difference in IOP measurements between eyes with clinical signs of glaucoma compared to normal eyes.

H_1: There will be a difference in IOP measurements between eyes with clinical signs of glaucoma compared to normal eyes.

Objective #7: To measure IOP in two sea lions demonstrating signs of glaucoma to evaluate if measurements are higher in these animals compared to normal eyes.

Hypothesis #8

H_0: There will be a difference in IOP measurements when one sea lion is measured at different locations (high and low platform).

H_1: There will be no difference in IOP measurements when one sea lion is measured at different locations (high and low platform).

Objective #8: To compare IOP measurements in one sea lion that had IOP's collected at 2 different locations (platform high and low).

CHAPTER IV

MATERIALS AND METHODS

Animals

At the Institute of Marine Mammal Studies, Gulfport, MS, ten sea lions with clinically normal eyes had been trained the year prior to start this study designed to collect IOP data in a weekly basis. Within three months of beginning the study, Hurricane Katrina hit the coast of Mississippi and demolished the entire facility. Some animals passed away and others were relocated to various aquariums in the United States and the Caribbean. The study was halted for a few months to rewrite IACUC protocols for numerous marine facilities prior to resumption of this study. Replacement facilities were located and included in this study.

Use of rebound tonometry on clinically normal sea lion eyes

Nineteen male California sea lions (*Zalophus californianus*), maintained at eight different facilities, were evaluated. These included: SeaWorld Orlando in Florida (n=6), Atlantis Marine World Riverhead in Riverhead, New York (n=2), SeaWorld San Diego, California (n=2), SeaWorld San Antonio in Texas (n=5), Gulf World Marine Park in Panama City Beach, Florida (n=1), Mystic Aquarium and Institute for Exploration in Mystic, Connecticut (n=1), Theater of the Sea in Islamorada, Florida (n=1), and New York Aquarium in Brooklyn, NY (n=1). All animals were kept outdoors except at Mystic

Aquarium. All sea lions had ophthalmic examinations performed by a board certified veterinary ophthalmologist (CMHC except at NY aquarium and Atlantis marine world riverhead), prior to the onset of this study, and were considered healthy with no pre-existing ocular pathology. Ophthalmic exams were evaluated under dimmed lighting allowing anterior segment evaluation including menace response, slit lamp examination with a Kowa SL-15 (Kowa, Tokyo, Japan), fluorescein staining, indirect ophthalmoscopy with a Keeler indirect ophthalmoscope (All Pupil, Keeler), digital photography using a Nikon D7000 with macro lens and a ring flash Nikon, and physiological dilation of the pupils. Ages of the sea lions ranged between 2 and 14 years, mean = 5.5 years. IOPs were collected and compared to evaluate differences between right and left eye and nose position (nose up versus nose down).

Use of rebound tonometry on abnormal sea lion eyes

Thirty-seven California sea lions (*Zalophus californianus*), 9 females, 28 males, maintained at twelve different facilities, were evaluated. These included: Dolphin Cay Atlantis Paradise Island, Bahamas (n=3), Dolphins Plus in Key Largo, Florida (n=2), Gulf World Marine Park in Panama City Beach, Florida (n=1), Miami Seaquarium in Miami Florida (n=6), Mystic Aquarium and Institute for Exploration (n=1), SeaWorld Orlando (n=6), SeaWorld San Antonio (n=3), SeaWorld San Diego (n=6), Theater of the Sea in Islamorada, Florida (n=3), New York Aquarium in Brooklyn, New Jersey (n=2), Six Flags Discovery Kingdom in Vallejo, California (n=2), and Six Flags Great Adventure in Jackson, New Jersey (n=2). All animals were kept outdoors except at Mystic Aquarium. All sea lions had ophthalmic examinations performed by a board certified veterinary ophthalmologist (CMHC except at NY aquarium), prior to the onset

of this study, and were placed into groups based on the ocular disease state within each eye. Corneal damage and overall ocular disease state was rated on a 0-4+ scale with 0 being normal i.e. lacking clinical pathologic abnormalities (Table 1). Ages of the sea lions ranged between 4 and 28 years, mean= 17 years.

Table 1 37 Sea lions (72 eyes) were included in this study. Two animals had OS eye that was normal. Two animals had OS eye with glaucoma. Each animal, sex, age, and group is depicted.

	Facility Animal	Age	Sex	OD eye	OS Eye
1	Dolphin Cay Atlantis	23	male	1A	1A
2	Dolphins Plus	23	female	1A	Glaucoma
3	Dolphins Plus	17	female	1A	1A
4	Gulf World	20	male	1A	1A
5	Miami Seaquarium	12	female	1A	1A
6	Miami Seaquarium	32	female	1A	1A
7	Miami Seaquarium	23	male	1A	1A
8	Mystic Aquarium	21	male	1A	1A
9	SeaWorld-Fl	14	male	1A	1A
10	Sea World-Fl	18	male	1A	1A
11	SeaWorld-Fl	21	male	1A	1A
12	SeaWorld-Tx	21	male	1B	1A
13	SeaWorld-Tx	5	male	1A	Normal
14	SeaWorld-Ca	21	male	1A	1B
15	SeaWorld-Ca	15	male	1A	1A
16	SeaWorld-Ca	20	male	1A	1A
17	SeaWorld-Ca	16	male	1A	1A
18	SeaWorld-Ca	14	male	1A	1B

Table 1 (Continued)

19	Theater of the Sea	24	female	1A	1A
20	Theater of the Sea	20	female	1A	1A
21	New York Aquarium	16	male	1B	Normal
22	Sea World-Fl	18	male	1B	2
23	Sea World-Fl	24	male	3B	1B
24	Sea World-Fl	18	male	1B	1B
25	Sea World-Tx	31	male	1B	1B
26	Dolphin Cay Atlantis	18	female	2	2
27	Dolphin Cay Atlantis	14	female	2	2
28	New York Aquarium	21	male	2	2
29	Theater of the Sea	18	male	2	2
30	Miami Seaquarium	20	female	3A	3A
31	Six Flags	25	male	3A	3A
32	Six Flags	27	male	3A	3A
33	Miami Seaquarium	26	male	3B	Glaucoma
34	Miami Seaquarium	24	male	3B	3B
35	SeaWorld-Fl	18	male	3B	3B
36	Six Flags	27	male	3B	3B
37	Six Flags	25	male	3B	3B

Experimental design

Animals were trained for collection of IOP measurements under behavioral control. In the initial training process, trainers approximated an object that resembles the

tonometer. Objects used included a dark colored toothbrush, black remote control, a black two-way radio, and a paper towel roll with two cotton tip swabs glued to one end to mimic the tip of the tonometer (Figure 6).

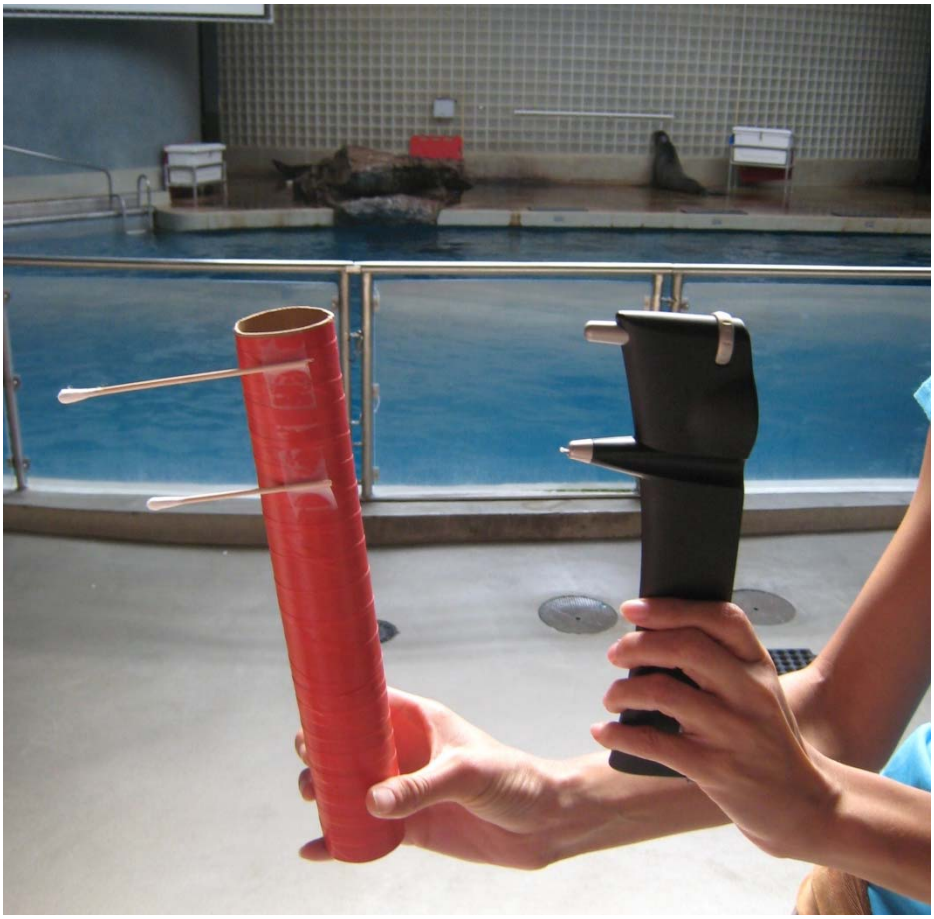

Figure 6 TonoVet® Rebound tonometer positioned next to a mock instrument used for training IOP (paper towel roll with two q tips used for desensitization).

These sessions were repeated over the course of a few days until the animal felt comfortable with the mock instrument coming close to his or her eye. After each reading, the instrument made a soft beep. To desensitize to the noise, trainers either pronounced a

beeping noise when they approximated the instrument or used a 2-way radio to both approximate and make a beeping noise. This was performed 1 week prior to using the real instrument. Animals were trained to hold a target for 10-30 seconds to allow the instrument close to the eye (but not touching the cornea yet). The instrument was pulled away following this approximation. Sea lions were trained to hold the target completely still and become comfortable with the instrument coming close to his or her eye. Readings were taken, which required the animal to hold a target for about 30-60 seconds. The ultimate goal was to get three consecutive readings from each eye and then take the average of the three readings. Animals usually sat in an upright position with the nose targeted horizontally parallel to ground below or in a nose up position. The instrument needed to contact the cornea at a 90 degree angle. Although each facility trained the 'open eye' behavior a bit differently, the underlying premise involved, targeting to a pole, giving the sea lion the 'eye' verbal cue and initially training to giving saline eye drops. When the sea lion opened the eye for the drops the behavior would be captured and positively reinforced with fish. After a few sessions, the animal started to associate 'eye' with holding eye open. Some trainers after targeting the animal to a pole, placed their thumb above the sea lion eye in the air. This behavior allowed the sea lion to follow the trainer's finger while opening their eye (Figure 7).

Probes were disposed of between patients to prevent contamination. Some facilities had no disinfectants while others had either chlorine or ozone or both. Some animals lived in natural sea water, while others were in fresh water pools in which salt was added. The tonometer was directed perpendicularly to the corneal surface. In order to record an accurate IOP, the central groove was held in a horizontal position and the

distance was 4-8 mm (0.2- 0.3 inches) from the tip of the probe to the corneal surface.[59] The use of the horse setting was based on equine corneal thickness (1.0–1.5 mm in the center and 0.8 mm at the periphery)[59] which is similar to that of sea lions in the axial area (1.5-2.0 mm in the center and 2.5-3 mm at the periphery).[20] The tonometer with probe installed was held still in front of the eye and the probe touched the cornea six times without error. The IOP value was digitally displayed on the screen. After the sixth touch, the previous six measurements were internally averaged to show one mean value after the instrument cancelled the highest and lowest values. Three mean value measurements were collected per training session per eye, but some situations allowed for only one or two mean value measurements per session. An error code appeared when there was probe motion, excessive deviation between measurements, or misalignment with the central portion of the cornea. The error readings were not included in the data set. IOP was collected on the clear corneal surface when possible, as some animals had pathology in 100% of the corneal surface, not leaving sufficient healthy cornea for a comparative measurement. One observer (JMF) traveled to each facility and collected all of the measurements recorded. The variability in numbers of training sessions depended on how well the animal was trained for repeat sessions and whether the observer was able to travel to the facility again. To minimize stress-associated increases in IOP, measurements were collected in the training location where the animal felt most comfortable and least stressed.

Statistical Analysis

Use of rebound tonometery on clinically normal sea lion eyes

Descriptive analysis was performed for all California sea lions (age, sex, and average rebound tonometer measurements). A restricted maximum likelihood-based mixed model was utilized for the analysis due to its ability to account for both a fixed effect as well as a random effect. For this analysis, the sea lion is the random effect while the eye (OD or OS), nose position, zone and age all represent fixed effects. For independent categorical variables, the mixed model was run to determine if there was a significant difference between groups. Values of $p<0.05$ were considered significant.

A linear mixed model was also performed to determine if age had a significant impact on IOP in each animal. Values of $p<0.05$ were considered significant. The analyses were performed using commercially available software.[73,74,a,b]

Use of rebound tonometry on abnormal sea lions

Descriptive analysis was performed for all California sea lions (age, sex, and average rebound tonometer measurements). Sea lions with various ocular conditions were analyzed via a linear mixed model to best account for the varying sample sizes from each animal (Table 2). The linear mixed model was run again to exclude animals that had glaucoma to evaluate if this made a difference (Table 3). There was still no statistical significance between abnormal groups.

Table 2 A linear mixed effects model showing a significant difference in intraocular pressure of left vs. right eye, no difference between groups of sea lions (1A, 1B, 2, 3A, 3B), and lower IOP in nose up position vs. nose down position. Glaucomatous eyes have been included in this table.

Linear Mixed Effects Model Across Groups				
	Value	Std.Error	T-value	P-value
(Intercept)	33.90	1.50	22.70	<.01
EyeOS	1.90	0.40	5.50	<.01
Group1B	3.20	2.80	1.10	0.26
Group2	1.20	3.30	0.40	0.73
Group3A	-3.30	4.10	-0.80	0.42
Group3B	-0.20	3.00	-0.10	0.95
Position	-3.40	0.40	-7.90	<.01

Table 3 A linear mixed effects model showing a significant difference in intraocular pressure of left vs. right eye, no difference between groups of sea lions (1A, 1B, 2, 3A, 3B), and lower IOP in nose up position vs. nose down position. Glaucomatous eyes have been removed in this table.

Linear Mixed Effects Model Across Groups				
	Value	Std.Error	T-value	P-value
(Intercept)	33.9	1.5	22.7	<,01
EyeOS	1.9	0.4	5.5	<.01
Group1B	3.2	2.8	1.1	0.26
Group2	1.2	3.3	0.4	0.72
Group3A	-3.3	4.1	-0.8	0.41
Group3B	-0.2	3	-0.1	0.94
Position(Nose Up)	-3.4	0.4	-7.9	<.01

The rationale for this was two-fold: 1) there is no statistically acceptable method for omitting observations to ensure all animals had the same number of samples, and, 2) if a simple linear regression was run with all samples then those animals with larger sample sizes would skew the model incorrectly. In the model, IOP is the dependent variable while eye (either OS or OD), group association and nose position (either up or

down) were independent variables. Values of $p<0.05$ were considered significant. The analyses were performed using commercially available software.[73,74,a,b]

IOPs were also compared among sea lions that had IOP's collected at two different locations (platform high and low). Two variables were examined to gain a greater understanding of the variance in IOP. IOPs were subjected to a two-way ANOVA, where the first variable (or factor) being platform height and the second variable (or factor) being eye (OS and OD).

CHAPTER V

EVALUATION OF HEAD AND EYE POSITION IN ORDER TO ESTABLISH NORMAL INTRAOCULAR PRESSURE USING REBOUND TONOMETRY

Abstract

- **Objective**- To determine the range of normal intraocular pressures (IOP) in California sea lions without ocular pathology and document selected variables that can affect IOP measurements in this species.
- **Design**- Prospective Study
- **Procedures-** Individual IOPs were based on measurements of 1-3 readings per eye. Two head positions (nose-up, nose-down) and 2 zones per eye (zone 1- flattened plateau, zone 2-temporal area) were recorded per measurement.
- **Results-** 624 measurements were collected. The mean IOP +/- SD were: OD 32.8 +/-5.31 mm Hg at a 95% CI of 31.85 to 33.75; OS 34.3 +/-4.86 mm Hg at a 95% CI of 33.34 to 35.25. The range of the means for each eye included OD (24-39 mm Hg) and OS (25.5-38.0 mm Hg). A linear mixed model was used with fixed and random effects. Based on a restricted maximum likelihood-based mixed model, a significant difference between OD and OS IOP was found with OS having higher

IOPs. Head position also had a significant effect on IOP with higher pressures detected in the head down position. There was no significant difference in pressures between zones collaterally.

- **Conclusion and Clinical Relevance**- Though OS had a significantly higher IOP than OD, it is unlikely to be clinically important. Nose position has an effect on IOP measurement in sea lions, therefore, the nose up position is recommended based on the resulting data as the nose down position may provide aberrantly higher numbers.
- Abbreviation- IOP= intraocular pressure

Introduction

The prevalence of ocular disease in senior, captive California sea lions *(Zalophus californianus)* is estimated at approximately fifty percent or higher.[1,3,20,75,76] Early detection of keratopathy and cataracts, along with medical and/or surgical management are critical to maintaining vision and comfort. Keratopathy associated with cataract development can diminish sight and have the potential to initiate chronic inflammation and luxate anteriorly, causing pain and secondary glaucoma.[67,77,78] Glaucoma is a progressive disease that may lead to blindness if not diagnosed and treated early following the onset of clinical signs. Due to the advancements in veterinary technology, intraocular pressure (IOP) abnormalities in these highly valued animals, may be identified and addressed before the onset of severe ocular disease. Once the normal range of IOP is established in this species, animals under human care can be trained for this technique and evaluated periodically for changes in IOP. If an animal's IOP begins to

increase beyond the normal range, the underlying cause can be identified and appropriately managed.

The objective of this study was two-fold: (1) to establish reference ranges for normal IOP measurements using the TonoVet®[a] in California sea lions under human care with clinically normal eyes and (2) to compare IOP in two different regions or zones of the cornea and in two different head positions and to evaluate the possibility for age-related differences. We hypothesized that differences in IOP would not be significant between the two areas of the cornea evaluated, nor between eyes, head position or age. By establishing a normal range of IOPs in this species, we will be able to more rapidly identify changes in intraocular health and address them immediately.

Materials and Methods

Animals

Nineteen male California sea lions (*Zalophus californianus*), maintained at eight different facilities, were evaluated. These included: SeaWorld Orlando (n=6), Atlantis Marine World Riverhead (n=2), SeaWorld San Diego (n=2), SeaWorld San Antonio (n=5), Gulf World Marine Park Panama City Beach (n=1), Mystic Aquarium and Institute for Exploration (n=1), Theater of the Sea Islamorada (n=1), and New York Aquarium (n=1). All animals were kept outdoors except at Mystic Aquarium. All sea lions had ophthalmic examinations performed by a board certified veterinary ophthalmologist (CMHC except at NY Aquarium and Atlantis Marine World Riverhead), prior to the onset of this study, and were considered healthy with no ocular pathology. Ages of the sea lions ranged between 2 and 14 years, mean= 5.5 years.

Experimental design

Animals were trained for collection of IOP measurements under behavioral control. Since the TonoVet® instrument makes audible beeps, the animals were desensitized to a few beeping noises prior to the onset of the study by using a 2-way radio sound near the eye for 1 week prior to using the real instrument. Each sea lion was trained to hold the eye open for 30 seconds. Although each facility trained the 'open eye' behavior a bit differently, the underlying premise involved, targeting to a pole, giving the sea lion the 'eye' verbal cue and initially training to giving saline eye drops. When the sea lion opened the eye for the drops the behavior would be captured and positively reinforced with fish. After a few sessions, the animal started to associate 'eye' with holding eye open. Some trainers after targeting the animal to a pole, placed their thumb above the sea lion eye in the air (Figure 7). This behavior allowed the sea lion to follow the trainer's finger while opening their eye.

Probes were disposed of between patients to prevent contamination. The tonometer was directed perpendicularly to the corneal surface. In order to record an accurate IOP, the central groove was held in a horizontal position and the distance was 4-8 mm (0.2- 0.3 inches) from the tip of the probe to the corneal surface.[59] The use of the horse calibration was based on equine corneal thickness (1.0–1.5 mm in the center and 0.8 mm at the periphery)[59] which is similar to that of sea lions, axially, not peripherally (1.5-2.0 mm in the center and 2.5-3 mm at the periphery).[20] The tonometer with probe installed was held still in front of the eye and the probe touched the cornea six times. The IOP value was digitally displayed on the screen. After the sixth touch, the previous six measurements were internally averaged to show one mean value after cancelling the

highest and lowest values. Three mean value measurements were collected per training session per eye, but some situations allowed for only one or two mean value measurements per session. Each animal had at least one session per day. An error code appeared when there was probe motion, excessive deviation between measurements, or misalignment with the central portion of the cornea. One observer (JMF) traveled to each facility and collected all of the measurements recorded. Each animal had between one and 19 ocular trainings sessions. The variability in numbers of training sessions depended on how well the animal was trained for repeat sessions and whether the observer was able to travel to the facility again.

Intraocular pressures were collected and compared in both nose up (Figure 7) and nose down positions (Figure 8) and in both zone 1 and zone 2 (Figure 9).

Figure 7 Nose up position used to collect an intraocular pressure measurement.

Notice the trainer using her thumb to train the ‘eye open’ behavior. Courtesy of Theater of the Sea, Islamorada, Florida.

Figure 8 Nose down position used to collect an intraocular pressure measurement. Courtesy of Theater of the Sea, Islamorada, Florida.

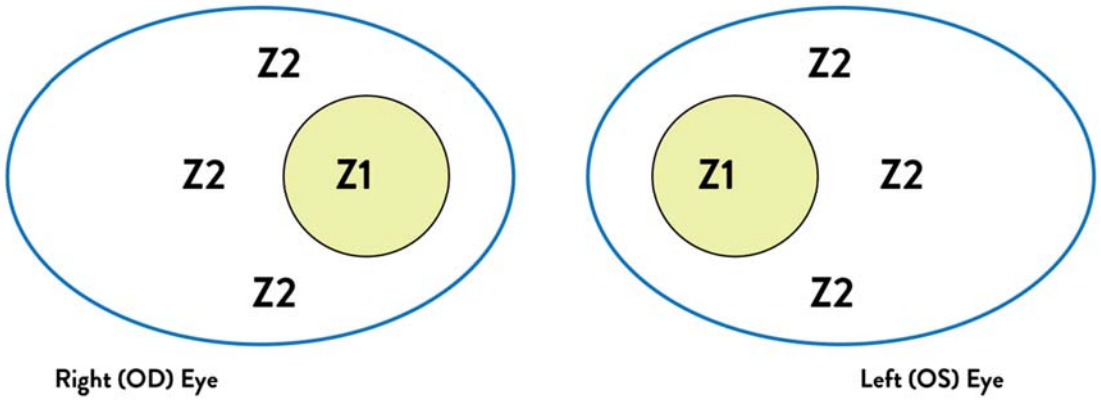

Figure 9 Z1= Zone 1 (medial paraxial flattened plateau), Z2= temporal area of the sea lion cornea.

Due to the small sample size and imbalanced nature of the data, a restricted maximum likelihood (REML) model was appropriate.[73] To minimize stress-associated increases in IOP, measurements were collected in the training location where the animal felt most comfortable and least stressed.

Statistical Analysis

Descriptive analysis was performed for all California sea lions (age, sex, and average rebound tonometer measurements). A restricted maximum likelihood-based mixed model was utilized for the analysis due to its ability to account for both a fixed effect as well as a random effect and repeated measurements. For this analysis, the sea lion was the random effect while the eye (OD or OS), nose position, zone and age all represent fixed effects. For independent categorical variables, the mixed model was run to determine whether a significant difference between groups occurred. Values of $p<0.05$ were considered significant.

A linear mixed model was also performed to determine if age had a significant impact on IOP in each animal. Values of $p<0.05$ were considered significant. The analyses were performed using commercially available software.[73,74,b,c]

Results

Sea lion #10 would not allow a measurement to be collected on the right eye, resulting in 37 clinically normal eyes for analysis. Measurements per ocular training sessions were averaged together per eye so that each of the 37 eyes contributed one mean value (Table 4).

Table 4 Nineteen sea lions, 37 eyes, are shown with total number measurements of IOPs.

Animal	Total No. of Measurements	Mean OD, +/-SD	Mean OS, +/-SD
1-GulfWorld	5	32, +/-1.4	35.3, +/-1.2
2-Atlantis Marine World	14	32.5, +/-2.8	33.3, +/-2.14
3-Atlantis Marine World	98	33.8, +/- 4.8	35.9, +/-4.7
4-Mystic Aquarium	2	24	33
5-New York Aquarium	68	29.5, +/-4.5	31.8, +/-4.5
6-SeaWorld, Ca	2	35	39
7-SeaWorld, Ca	9	30.6, +/-4.1	30.7, +/-2.5
8-Theater of the Sea	6	39, +/- 8.5	38, +/-6.4
9-SeaWorld, Tx	12	33, +/-6.1	34.3, +/-2.4
10-SeaWorld, Tx	3	n/a	26, +/-2.6
11-SeaWorld, Tx	5	23.7, +/- 2.9	25.5, +/-6.4
12- SeaWorld, Tx	12	35.5, +/-6.2	331, +/-2.8
13-SeaWorld, Tx	12	28.3, +/-1.8	30.1, +/-3.2
14-SeaWorld, Fl	12	39, +/-2.1	32.7, +/-4.7
15-SeaWorld, Fl	108	32.1, +/-4.5	36.5, +/-4.7
16-SeaWorld, Fl	75	34.5, +/-8.3	32.1, +/-4.3
17-SeaWorld, Fl	23	32.7, +/-5.1	32.9, +/-4.3
18-SeaWorld, Fl	75	33.3, +/-3.9	34.4, +/-3.3
19-SeaWorld, Fl	83	33.9, +/-4.0	36.2, +/-5.2

Nineteen sea lions were examined and the results are included in the histogram (Figure 10).

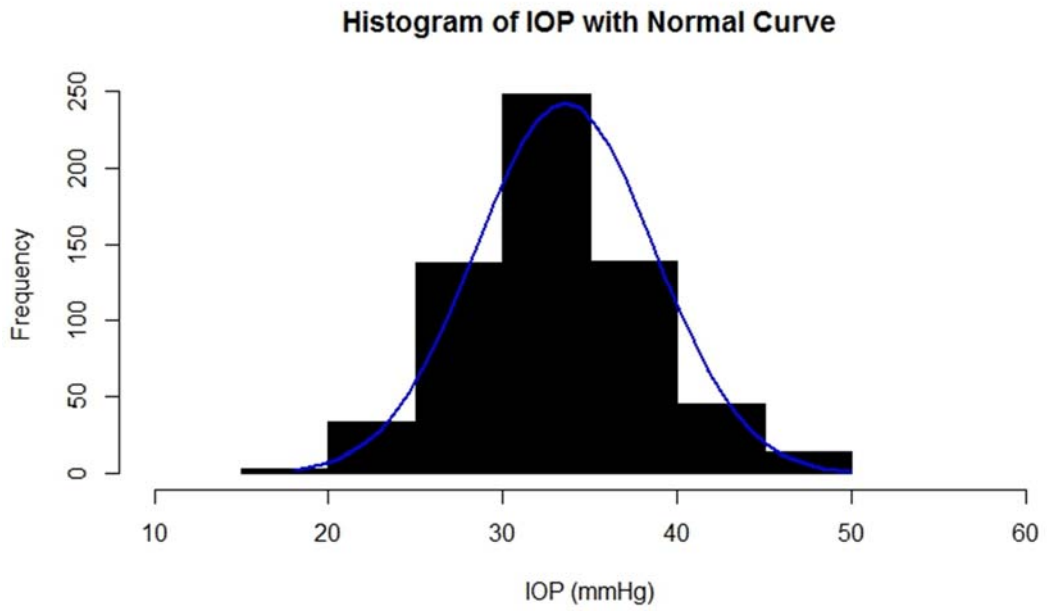

Figure 10 Frequency of IOP (mm Hg) for the measurements collected for nineteen California sea lions

Findings revealed that mean IOP +/- SD for both nose up and nose down position obtained with the rebound tonometer were OD 32.8 +/-5.31 mm Hg (95% CI:31.85 - 33.75) and OS 34.3 +/-4.86 mm Hg(95% CI: 33.34 - 35.25) The range of the means for each eye included OD (24-39 mm Hg) and OS (25.5-38 mm Hg). The results of this study show both significant variables and variables found to be not significant. First, age was not statistically associated with intraocular pressure, p=0.49. There was also no significant difference associated with eye zone (p = 0.995) (Table 5).

A restricted maximum likelihood-based mixed model with associated p values demonstrated significantly higher IOP in OS than in OD, the nose-up position was significantly lower than the nose-down position, and eye zone and age have no significant effects (Table 5).

Table 5 Restricted maximum likelihood-based mixed model with associated p values demonstrated significantly higher IOP in OS than in OD, the nose-up position was significantly lower than the nose-down position, and eye zone and age have no significant effects.

Linear Mixed Model Results for Intraocular Eye Pressure

Variable	Model #1	Model #2	Model #3	Model #4
(intercept)	33.69 (1.65)*	33.24 (1.57)*	34.56 (1.02)*	34.25 (.86)*
Eye (Left Eye)	1.82 (.60)*	1.64 (.55)*	1.88 (.58)*	1.75 (.52)*
Head Position (Up)	-3.74 (.61)*	-3.76 (.61)*	-3.80 (.57)*	-3.81 (.57)*
Eye Zone (Zone 2)	.60 (.76)	.59 (.76)		
Age	-.09 (.12)		-.06 (.11)	
AIC	1476.29	1472.37	1521.54	1517.3
BIC	1500.86	1493.45	1542.84	1535.06

Standard errors are reported in parantheses.

* indicates significance at the 95% level

Left eyes had significantly higher IOPs than right eyes (Figure 11) ($p<0.01$).

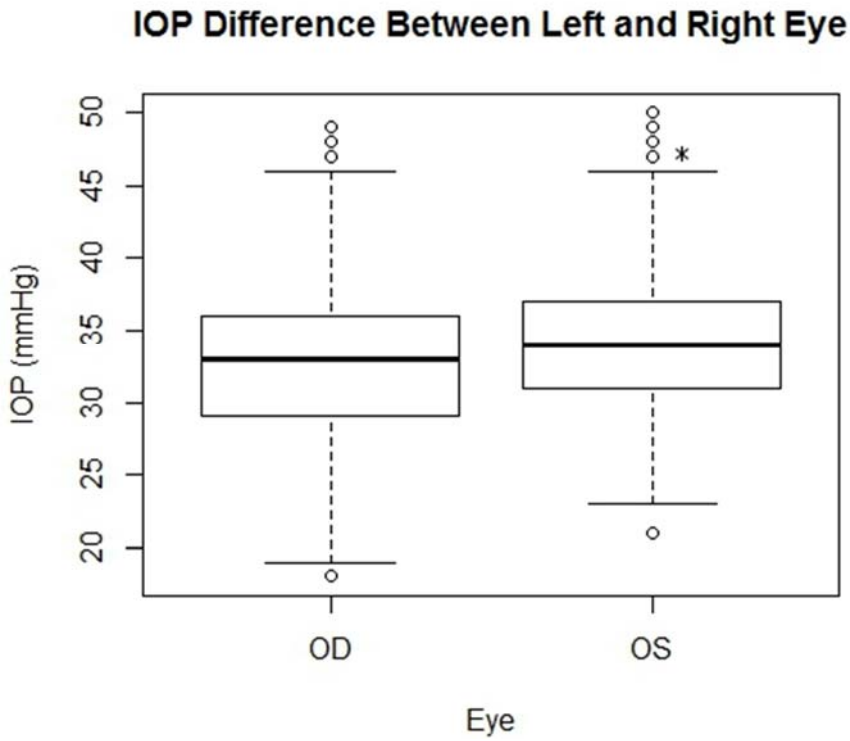

Figure 11 Boxplot of intraocular pressure (mm Hg) for sea lions comparing left and right eyes.

Likewise, the mean measurements for the nose down position was significantly greater than the nose up position (Figure 12) ($p<0.01$).

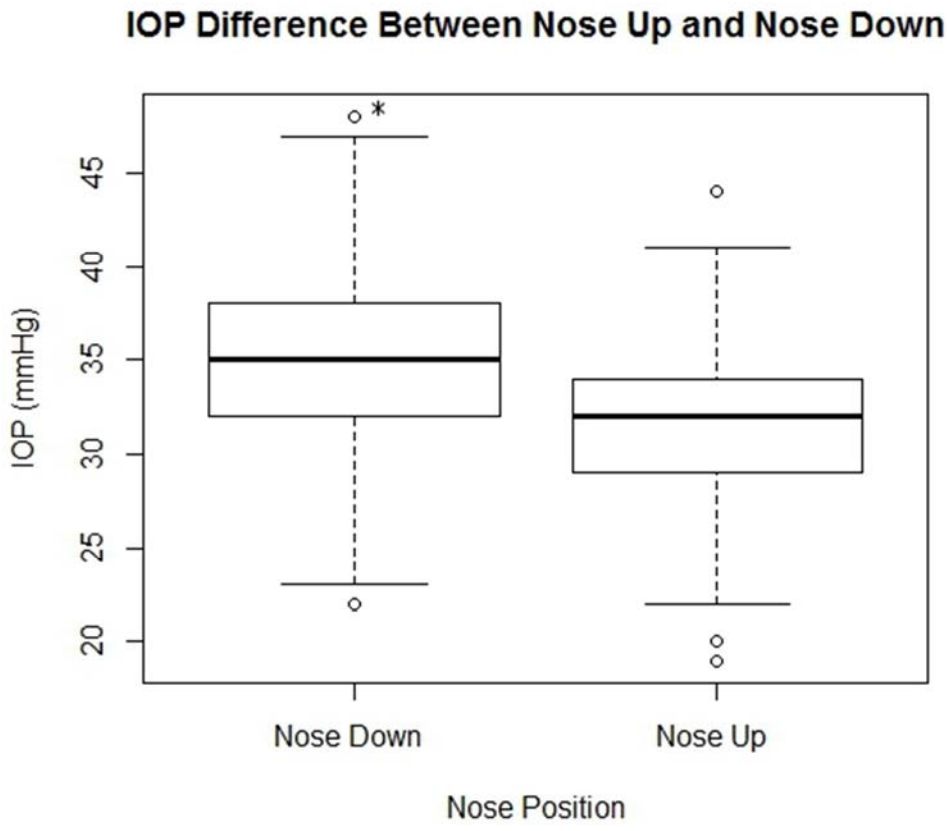

Figure 12 Boxplot of intraocular pressure (mm Hg) for sea lions comparing nose up and nose down position.

After analyzing the variables associated with IOP individually, the next step was to understand the relationship among the variables. To accomplish this, a regression model was utilized.

Several iterations of the REML model, using IOP as a dependent variable, were run to capture multiple combinations of the independent variable. Table 5 shows the results from the top four models based on two information criteria, Akaike's (AIC) and Schwarz's Bayesian (BIC). AIC and BIC measure the "goodness of fit" of a statistical model. The lower the AIC and BIC value, the greater the "goodness of fit." Multiple models were presented to show all possible combinations of the non-significant variables with the two significant variables (head position and between OD and OS eye).[13] We found the AIC and BIC to be in agreement that Model #2 was the best fitting model for IOP.

Discussion

The aim of this study was to evaluate the use of the TonoVet® rebound tonometer in California sea lions with clinically normal eyes. These animals had their IOPs measured numerous times in some cases over time to estimate a normal range of IOPs and to evaluate if IOPs changed with variation of nose-position, region of the cornea, and age.

In a study conducted by Hirst et al.,[67] seven pinnipeds were anesthetized with halothane gas and applanation tonography was conducted on seven eyes revealing IOPs between 8-18 mm Hg. The applanation tonometer used was not specified and was not adjusted for scleral rigidity and corneal curvature in that species.[67] Our range of IOP's was higher for both eyes (24-39 mm Hg). Halothane anesthesia has been shown to decrease IOP in anesthetized humans, therefore, it is possible that these IOPs may have been lower due to anesthetic effects.[79] To the authors' knowledge, ours study is the first

to collect tonometric measurements in awake pinnipeds with neither manual nor chemical restraint.

Sea lions have higher IOPs when comparing them to other terrestrial species such as the equine and avian species. The IOP range (24-39 mm Hg) was slightly higher in comparison to the horse (10 to 34 mm Hg).[59] Bayón et al.,[80] found mean IOP values to be 10.0 mm Hg in common kestrels, 9.31 mm Hg in little owls (Athene noctua), and 11.96 mm Hg in Eurasian eagle owls (Bubo bubo). Other species of raptors including eagles (booted eagle [*Aquila pennata*], Bonelli's eagle [*Aquila fasciata*], and short-toed snake-eagle [*Circaetus gallicus*]) had higher IOP values (up to 40.26 mm Hg) associated with thicker corneas.[80-82] Eagles are a high flying species and when they dive to catch prey, the dive can reach incredible speeds up to 150 miles (250km) per hour.[83] Eagles may be able to withstand quick changes in air pressure due to the protection of increased corneal thicknesses and biomechanics of the cornea.[84,85] The mean IOP in aquatic birds such as the black footed penguin varied between 31 (OD-D) to 27 (OS-D) and 25 (OD-H and OS-H).[86] Mean IOP in a semi-aquatic species such as the American alligator (*Alligator mississippiensis*) ranged between 5-35 mm Hg.[86] In marine mammals, manatee IOPs have been recorded to be lower than the sea lion, mean (± SD) IOP was 8.5 (± 2.7) mm Hg and the median was 9.5 mmHg, perhaps this is because these mammals are shallow divers.[87] Dolphins, which dive to depths similar to sea lions, have a similar range of IOPs. Mean IOP in a group of six dolphins in natural horizontal position was 33.56 mmHg OD-H and 33.32 mmHg OS-H.[88]

Differences in IOP measurements can be the result of a variety of factors including: diurnal variation, corneal thickness, corneal diameter, tear film viscosity,

corneal rigidity, and the animal's susceptibility to stress.[9] When comparing IOP measurements, species-specific differences should always be considered. Diurnal variations in IOP were not measured in this study as sessions could only be performed during daylight working hours. IOP can become increased in men that wear a necktie, in dogs with tight collars,[89] or with the Valsalva maneuver.[90] This occurs when air is forced against a closed windpipe and pressure goes up within the chest. This can occur during coughing, vomiting, playing, and weightlifting due to greater intrathoracic pressure caused by the air retained in the lungs when holding one's breath.[90,91] All of the sea lions in this study held their breath when targeting their nose to hold still for the IOP reading. Therefore, IOPs in these animals may be slightly elevated when holding their breath.[91] Animals should always be positioned in the training area where they feel most comfortable. The area where they normally eat and station for training is a good location. Sea lions possess well-developed extraocular muscles that can push the eyes forward making them appear mistakenly buphthalmic or exophthalmic. When an individual animal performs this action during a session, one should not collect IOP measurements with the eye protruding as falsely elevated readings are common (Colitz, 2014, personal communication). It is best to stop the session and resume later when the animal exhibits a calmer demeanor. In our study, animals were not restrained; although they did hold their breath so this may have been a factor in changing eye pressures.

Rebound tonometry offers a stress-minimizing, well-tolerated tool for ophthalmic diagnostic testing in sea lions. Species-specific differences should always be considered. Evolutionary ocular changes including size of the eye, corneal thickness, and extensive development of the tapetum lucidum which covers the majority of the posterior aspect of

the fundus, allowing pinnipeds to have more effective vision for light dim and enhanced motion detection.[2,20] Anatomically, the California sea lion's corneal stroma is thickest peripherally and thinnest centrally. The sclera is thinnest equatorially and thickest at the limbus and posterior pole.[20] IOP measurements using rebound tonometry are influenced by corneal thickness,[82] which may be another factor as to why sea lion IOP measurements are higher than in other animals. In this study, a difference in IOP was not observed when comparing corneal zones which have different thicknesses.

A study performed in horses showed that head position below the heart level had a significant increase in IOP in comparison to head position above the heart.[92] Similarly, sea lions appear to have higher pressures when the nose is positioned down. This may be due to pressure being exerted on the neck leading to slight jugular occlusion or increased episcleral venous pressure. Therefore, we recommend collection of IOP measurements in a nose-up position.

The intraocular pressures collected in OS were higher than in OD, although the difference is probably of little clinical significance as the difference in normal IOP between eyes can vary normally <4mm Hg.[93] One hypothesis why this may have occurred can be due to whether OS IOP measuring was done prior to OD. The trainer elected which eye of the sea lion had IOP collected first as this was based on the animal's behavior. A limitation in this study was that we did not record which eye had pressures taken first. In future studies, this data should be recorded as animals may have higher pressures in the second eye, the longer they wait, perhaps associated with a stress response of the autonomic nervous system.

It was not possible to include a larger population of California sea lions with clinically normal eyes in this study as other animals trained for the behavior were observed to have ocular disease. Nineteen sea lions participated in the study from eight marine mammal facilities. By the average age of 15 years, there is a high incidence of cataracts and lens luxations in captive California sea lions rendering many animals blind or requiring surgical lensectomy.[1,75,94] Cataracts are detrimental to sight and have the potential to induce anterior uveitis as well as to cause lens instability, each having the potential to cause secondary glaucoma.[95] Initial medical treatment is imperative, as glaucoma is painful and can impair vision by causing neural damage of the inner retina and impairment of rapid axoplasmic transport within the optic nerve axons and accumulation of cellular organelles in the scleral lamina cribrosa as described in the dog.[96] By establishing the normal range of IOPs in captive sea lions, animals can be trained and evaluated periodically through their lives to monitor individual IOP changes. If an animal's IOP becomes elevated, a cause can be identified and appropriate treatment can be initiated decreasing the likelihood of chronic changes and possible blindness.

Footnotes

List of products mentioned in text

- [a] Icare Finland Oy, Espoo, Finland
- [b] R Development Core Team (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

- [c]Jose Pinheiro, Douglas Bates, Saikat DebRoy, Deepayan Sarkar and the R Development Core Team (2012).nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-103.

CHAPTER VI

EVALUATION OF INTRAOCULAR PRESSURE TONOMETRY IN THE CAPTIVE CALIFORNIA SEA LION (*ZALOPHUS CALIFORNIANUS*) WITH OCULAR DISEASE

Abstract

- **Objective**- Sea lion intraocular pressure (IOP) was compared in five different groups diagnosed with various degrees of ocular conditions, including a comparison of 2 animals diagnosed with glaucoma, and one sea lion had IOP measured and compared at different platform heights.
- **Design**- Prospective Study
- **Animals**- 37 California sea lions [Zalophus californianus] (72 eyes) with clinical ocular pathology.
- **Procedures**- Individual IOPs were based on measurements of 1-3 readings per eye. Two head positions (nose-up, nose-down) and two zones per eye (zone 1- flattened plateau, zone 2-temporal area) were recorded per measurement. Platform height (high and low) was also evaluated for one animal.
- **Results**- Linear mixed model showed a significant difference between IOP of the left and right eye with eye OS significantly higher. There were

no significant differences among groups of varying degrees of ocular conditions. Nose up position was significantly less than the nose down position. Two-way ANOVA with sea lion and glaucoma status (with glaucoma and one without) demonstrated that eyes with glaucoma had significantly higher IOPs than normal eyes ($p< 0.05$). Two-way ANOVA with two factors (platform height) and sea lion eye (OS and OD) resulted in a significant difference in IOP when platform height was compared, $p<0.01$.

- **Conclusion and Clinical Relevance-** In our study, despite varying degrees of ocular pathology, glaucoma was diagnosed in 2 out of the 37 animals (5.4%). One animal consistently had higher pressure measurements when placed in a new area that she was not familiar with. This finding suggests that IOPs of California sea lions should be collected in the familiar training area.
- Abbreviation- IOP= intraocular pressure; OS=left eye; OD=right eye; OU=both eyes

Introduction

Understanding the variety of ophthalmological diseases that pinnipeds develop is important in their management and attempt to avoid progression of disease. Diseases commonly identified have included keratopathy and cataract with and without luxation.[1,20,75] By the age of 15 years, approximately 50% of captive pinnipeds may be diagnosed with varying stages of cataracts.[1,7] Cataract development causes inflammation, or uveitis, and this, can cause lens instability. Cataracts and lens luxation have the

potential to cause secondary glaucoma.[10] Glaucoma is a progressive and painful disease that may lead to blindness if not diagnosed and treated at the onset of clinical signs.

Tonometry is a non-invasive method of measuring intraocular pressure and is used to screen for glaucoma, which evaluates the balance between aqueous humor production and outflow within the eye. Normal captive sea lion IOP has been studied and it was determined to range between 24-39 mm Hg (see chapter V). The objectives of this study were three-fold: (1) to compare IOP in five different groups of sea lions diagnosed with various degrees of ocular pathology (early versus late cataract development and corneal disease) using the TonoVet®, (2) to compare IOP of two cases with clinical signs of glaucoma, and (3) to compare IOP from one sea lion where IOP was collected in different locations. We hypothesized that IOP would be significantly different among the five groups with varying ocular conditions, animals showing clinical signs of glaucoma such as buphthalmos, exophthalmos, and blepharospasm would have elevated IOPs, and that IOP may increase depending on location if pressure elevation is associated with stress. By evaluating IOPs in animals with varying degrees of ocular conditions, the prevalence of glaucoma in California sea lions can be identified.

To the authors' knowledge, glaucoma has never been reported in a California sea lion. Advances in medical technology and training have allowed marine facilities to perform ocular examination techniques and include measurement of IOPs, which will ultimately improve the animals' health care.

Materials and Methods

Animals

Thirty-seven California sea lions (*Zalophus californianus*), 9 females, 28 males, maintained at twelve different facilities, were evaluated. These included: Dolphin Cay Atlantis Paradise Island, Bahamas (n=3), Dolphins Plus in Key Largo, Florida (n=2), Gulf World Marine Park in Panama City Beach, Florida (n=1), Miami Seaquarium in Miami Florida (n=6), Mystic Aquarium and Institute for Exploration (n=1), SeaWorld Orlando (n=6), SeaWorld San Antonio (n=3), SeaWorld San Diego (n=6), Theater of the Sea in Islamorada, Florida (n=3), New York Aquarium in Brooklyn, New Jersey (n=2), Six Flags Discovery Kingdom in Vallejo, California (n=2), and Six Flags Great Adventure in Jackson, New Jersey (n=2) (Table 1). All animals were kept outdoors except at Mystic Aquarium. All sea lions had ophthalmic examinations performed by a board certified veterinary ophthalmologist (CMHC except at NY aquarium), prior to the onset of this study, and were placed into groups based on the degree of ocular disease state within each eye. Corneal damage/ ocular disease state was rated between 0 and 4+ with 0 having no clinical corneal pathology and 4+ having severe corneal pathology (Table 6) (Figure 13-17).

Table 6 Sea lions grouped based on varying ocular disease. Clear cornea=0, not visible though edema= 4+. A definition is provided of the ocular disease state in each group.

Group	Description of Ocular Disease
1A	Sea lions with cataract development with clear to mildly affected corneas (0-2+)
1B	Sea lions with cataract development with 3-4+ affected corneas
2	Sea lions with corneal disease including severe corneal edema/ corneal scarring with cataracts not apparent/diagnosed
3A	Sea lions that have had cataracts removed, clear corneas (0)
3B	Pre or post surgery sea lions for cataract removal in which lens may have already luxated or have had cataracts removed causing corneal damage and scarring (2-4+)

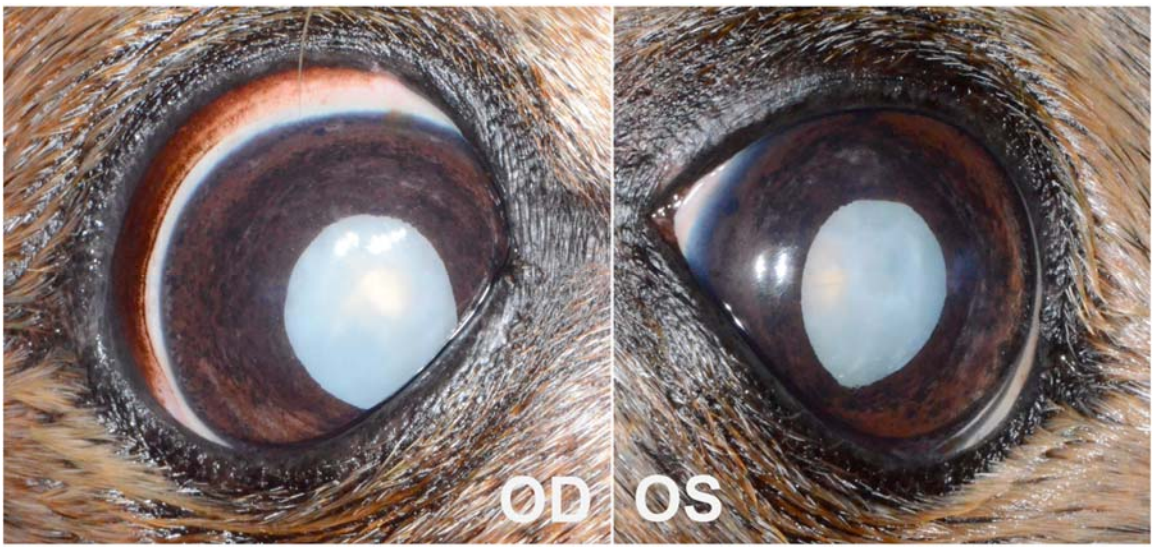

Figure 13 This animal is showing clinical signs of group 1A- Sea lion with cataract development with clear cornea.

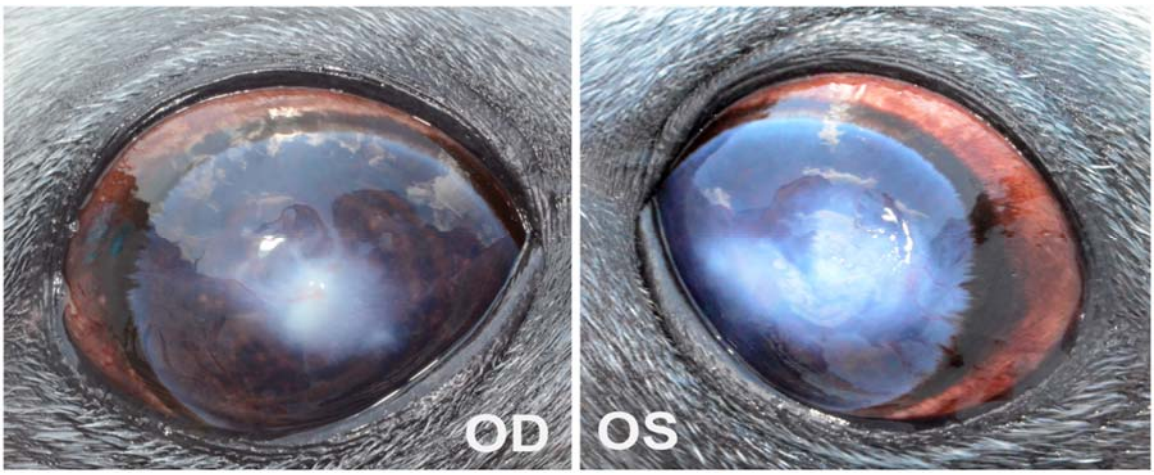

Figure 14 This image is depicting ocular clinical signs described in group 1B for OD eye (cataract development with affected corneal disease) and group 2 for OS eye (severe corneal edema/scarring where cataract may not be apparent).

Figure 15 This image is depicting ocular clinical signs described in group 2- sea lion with corneal disease, including severe edema/scarring where cataract may not be apparent.

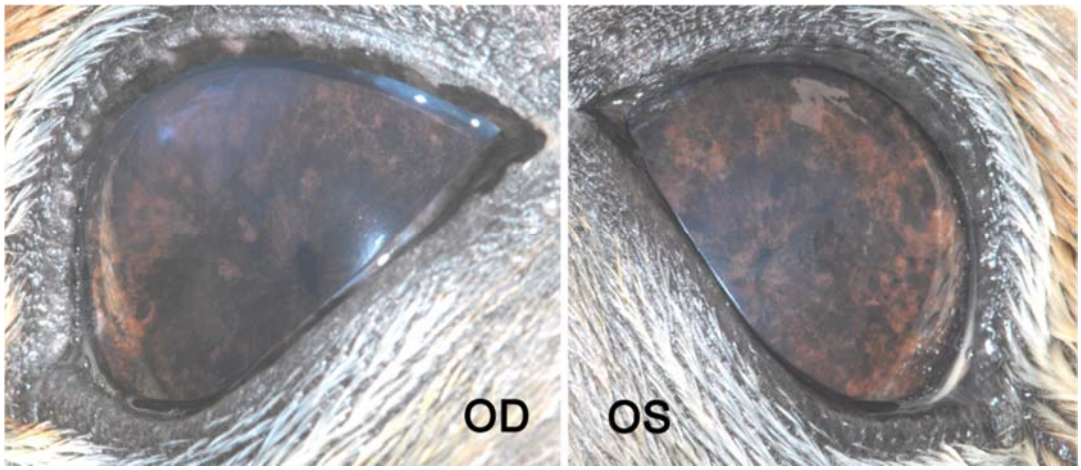

Figure 16 Both OD and OS eye are in group 3A, this sea lion has had bilateral lens extraction before luxation and the cornea is clear and healed with no scarring.

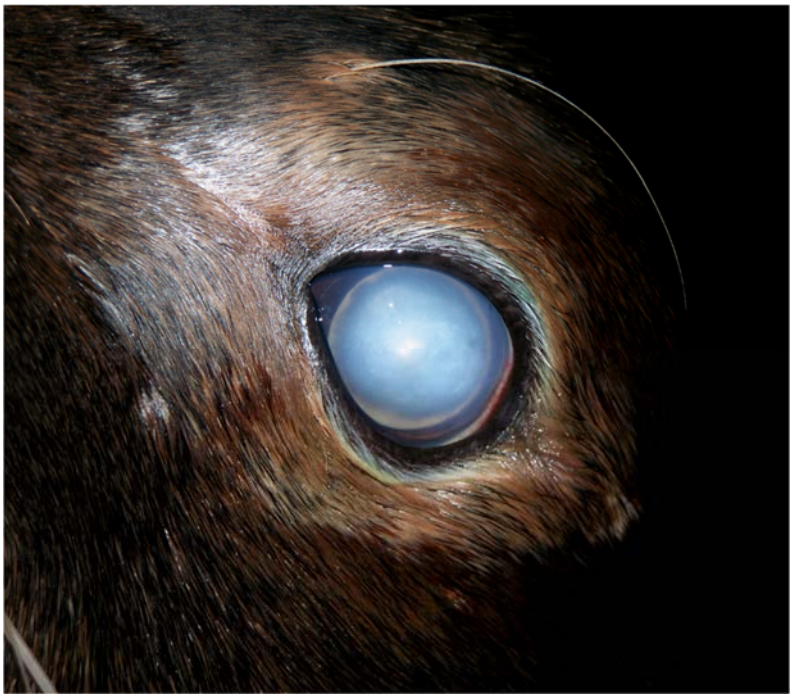

Figure 17 Sea lion OS eye in group 3B pre-operative eye, lens has luxated causing corneal disease.

One sea lion had measurements collected at two different levels of platform (high and low). We found out after the study that the high platform was a new area that was being trained and the sea lion was not very comfortable with this platform. The mean age was 17 years and ranged from 5 – 28 years. Median was 20 years, i.e. 28 males and 9 females (Table 1).

Experimental Design

Animals were trained for collection of IOP measurements under behavioral control.[97] Since the TonoVet® instrument makes audible beeps, the animals were desensitized to audible noises prior to the onset of the study by using a 2-way radio sound near the eye for 1 week prior to using the real instrument. Each sea lion was trained to hold the eye open for 30 seconds. Although each facility trained the 'open eye' behavior a bit differently, the underlying premise involved targeting to a pole, giving the sea lion the 'eye' verbal cue and initially training to give saline eye drops. When the sea lion opened the eye for the drops, the behavior would be captured and positively reinforced with fish. After a few sessions, the animal started to associate 'eye' with holding eye open. Some trainers place their thumb above the sea lion eye in the air which allows the sea lion to follow the finger and open the eye. Probes were disposed of between patients to prevent cross contamination. The tonometer was directed perpendicularly to the corneal surface. In order to record an accurate IOP, the central groove was held in a horizontal position and the distance was 4-8 mm (0.2- 0.3 inches) from the tip of the probe to the corneal surface.[59] The use of the horse calibration was based on equine corneal thickness (1.0–1.5 mm in the center and 0.8 mm at the periphery)[59] which is similar to that of sea lions, axially, not peripherally (1.5-2.0 mm in the center and 2.5-3

mm at the periphery).[20] The tonometer with probe installed was held still in front of the eye and the probe touched the cornea six times without error. The IOP value was digitally displayed on the screen. After the sixth touch, the previous six measurements were internally averaged to show one mean value after the instrument cancelled the highest and lowest values. (manufacturer's instructions). Three mean value measurements were collected per training session per eye, but some situations allowed for only one or two mean value measurements per session. An error code appeared when there was probe motion; probe was not parallel to the horizon, excessive deviation between measurements, or misalignment with the central portion of the cornea. One observer (JMF) collected all of the measurements recorded. Each animal had between 2 and 124 ocular training sessions as necessary. The variability in numbers of training sessions depended on how well the animal was trained for repeat sessions.

Intraocular pressures were collected and compared to evaluate differences between OS and OD and nose position (nose up versus nose down) and 2 zones per eye (zone 1- flattened plateau, zone 2-temporal area). Due to the imbalanced nature of the data and repeated measurements on each eye, a mixed linear model with restricted maximum likelihood (REML) estimation was appropriate.[73]

Statistical Analysis

Descriptive analysis was performed for all California sea lions (age, sex, and average rebound tonometer measurements). Sea lions with various ocular conditions were analyzed via a linear mixed model to best account for repeated measurements from each animal. Measurements per ocular training session were averaged together by group and segmented by eye, nose position, and zone. The rationale for this was two-fold: 1) there is

no statistically acceptable method for omitting observations to ensure all animals had the same number of samples, and, 2) if a simple linear regression was run with all samples then those animals with larger sample sizes would skew the model incorrectly. In the model, IOP is the dependent variable while eye (either OS or OD), group association and nose position (either up or down) were independent variables. Values of $p<0.05$ were considered significant. The analyses were performed using commercially available software.[73,74,a,b]

IOPs were also compared among sea lions that had IOPs collected at two different locations (platform high and low). Two variables were examined to gain a greater understanding of the variance in IOP. IOPs were subjected to a two-way ANOVA, where the first variable (or factor) being platform height and the second variable (or factor) being eye (OS and OD).

Results

Every animal, except 2, had clinical ocular pathology OU (Table 1), but only 2 animals were diagnosed with glaucoma OS.

Table 7 IOP measurements per ocular training session averaged by group and sorted by eye, nose position, and zone resulting in 72 abnormal eyes for analysis.

Table of Means						
Group	Eye (OD/OS)	Zone	Position	No. of Eyes	Mean Pressure	St. Deviation
1A	OD	1	0	15	35.7	9.67
1A	OS	1	0	16	38.2	10.30
1A	OD	2	0	17	36.0	8.52
1A	OS	2	0	17	36.8	8.31
1A	OD	1	1	6	25.9	6.27
1A	OS	1	1	5	29.0	6.29
1A	OD	2	1	7	27.2	6.42
1A	OS	2	1	9	31.4	6.90
1B	OD	1	0	5	37.6	5.94
1B	OS	1	0	3	33.7	3.51
1B	OD	2	0	5	38.2	6.53
1B	OS	2	0	5	38.5	6.79
1B	OD	2	1	3	32.8	5.64
1B	OS	2	1	2	37.5	10.77
2	OD	1	0	2	27.7	4.51
2	OS	1	0	2	42.3	8.94
2	OD	2	0	4	35.6	4.51
2	OS	2	0	5	35.6	11.20
2	OD	2	1	2	35.3	4.50
2	OS	2	1	2	45.3	17.15
3A	OD	1	0	3	34.3	5.10
3A	OS	1	0	2	29.9	3.57
3A	OD	2	0	3	28.8	3.93
3A	OS	2	0	3	30.5	4.93
3A	OD	1	1	1	29.9	4.27
3A	OS	1	1	1	26.4	5.30
3A	OD	2	1	2	23.0	1.41
3A	OS	2	1	3	28.0	4.00
3B	OD	1	0	5	37.5	8.90
3B	OS	1	0	2	45.1	9.37
3B	OD	2	0	6	36.9	10.77
3B	OS	2	0	5	38.7	14.80
3B	OD	1	1	1	50.0	0.00
3B	OS	1	1	2	34.0	13.00
3B	OD	2	1	4	32.8	6.39
3B	OS	2	1	3	38.8	11.60

Seventy-one abnormal eyes (i.e. 1169 measurements) were analysed. Measurements per ocular training sessions (which varied between 2 and 124 session) were averaged together by facility per OS and OD (Table 8).

Table 8 IOP measurements per ocular training sessions were averaged by facility per left and right eye so that each of the 72 abnormal eyes contributed one mean value.

Facility	Total Number of Measurements	OD Eye	Mean OD, +/- SD	OS Eye	Mean OS, +/- SD
Dolphin Cay Atlantis	18	3	32.7, +/- 5.79	3	27.4, +/- 2.92
Dolphins Plus	262	2	36.4, +/- 5.53	1	39.8, +/- 6.77
Gulf World	6	1	26.0, +/- 1.41	1	25.7, +/- 1.53
Miami Seaquarium	1278	6	29.9, +/- 7.33	5	27.3, +/- 5.00
Mystic Aquarium	78	1	27.2, +/- 3.93	1	26.2, +/- 4.15
New York Aquarium	92	2	34.4, +/- 5.80	1	26.8, +/- 6.96
Sea World-Ca	65	6	36.7, +/- 6.34	6	39.5, +/- 8.21
Sea World-Fl	954	6	36.0, +/- 6.80	6	37.1, +/- 7.71
Sea World-Tx	84	3	34.8, +/- 6.17	2	32.8, +/- 5.95
Six Flags-Ca	16	2	27.5, +/- 5.42	2	33.4, +/- 6.30
Six Flags-Nj	158	2	40.8, +/- 9.57	2	43.8, +/- 12.09
Theater of the Sea	353	3	38.6, +/- 7.65	3	40.6, +/- 9.02

From the linear mixed model, three interesting dynamics surfaced (Table 2). First, there was a significant difference between IOPs of the OS and OD, with OS being significantly higher; this has been previously reported in sea lions with no clinical signs of ocular disease (see chapter V). Second, as seen by p-values greater than 0.05, there

were no significant differences between groups, which is also depicted in a box plot (Figure 18).

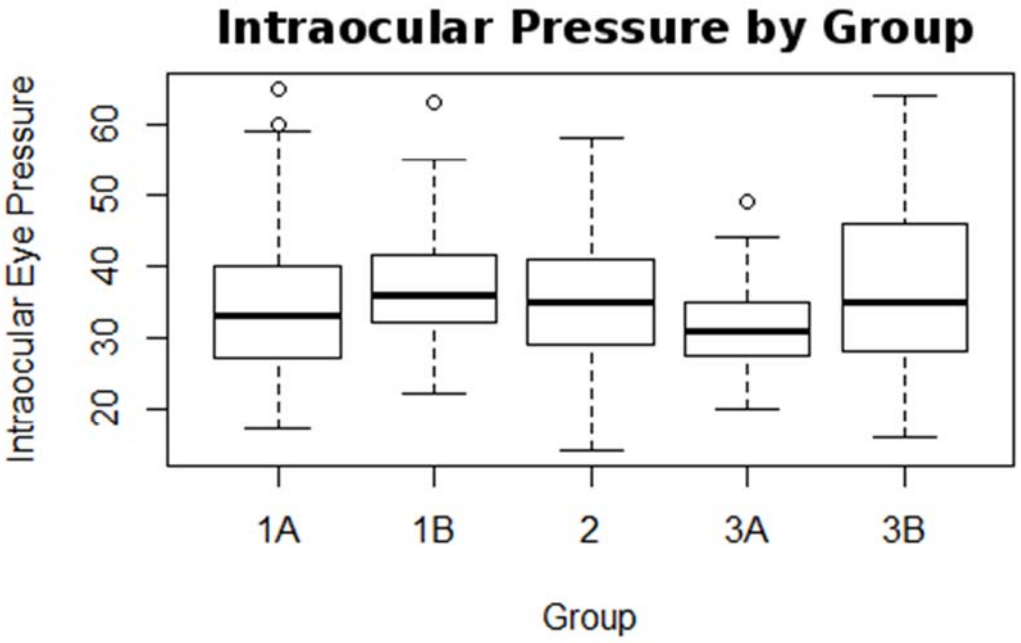

Figure 18 Boxplot of intraocular pressure (mmHg) for sea lions comparing various groups of ocular conditions (1A, 1B, 2, 3A, 3B).

Median is represented by the bold line and the 'o' represent outliers. Legend: 1A: Clear cornea = 0 with cataract, 1B: cataract with 3-4+ severe corneal disease, 2: Severe corneal edema/scarring, cataract not apparent, 3A: cataract removed, clear cornea, 3B: pre and post cataract removal where lens has already luxated, 3-4+ scarring.

Finally, in the nose up position, the IOP was significantly lower than the nose down position, which has also previously been reported in clinically normal sea lion eyes (see chapter V) (Table 2).

Intraocular pressures were subjected to a two-way ANOVA by examining two sea lions both possessing one eye with glaucoma and one without. Both effects, comparing both sea lions as well as comparing glaucoma against non-glaucoma eye, were found to be statistically significant with p-values less than 0.05. The main effect of sea lion produced a F-value of $F(1, 378)=8.17$, $p<0.01$. This indicates that the mean IOP scores were significantly greater for Sea Lion 1 (mean=41.43 mm Hg) when compared to Sea Lion 2 (mean=39.4 mm Hg) irrespective of eye. The main effect of having glaucoma produced an F-value of $F(1,378)=224.12$, $p<0.01$ (Table 9).

Table 9 Intraocular eye pressures were subjected to a two-way ANOVA with two levels of sea lion (sea lion 1 and sea lion 2) as well as two levels of eyes (one with glaucoma and one without).

Comparison by Eye and Sea Lion for Glaucoma and non-Glaucoma Eyes

Variable	DF	Sum Sq	Mean Sq	F-value	P-value
Sea Lion	1	362	362	8.17	<.01
Eye	1	10830	10830	244.12	<.01
Residuals	378	16770	44		

This indicates that the mean IOP for eyes with glaucoma (mean=46.2 mm Hg) was significantly higher than IOP in eyes without glaucoma (mean=35.5 mm Hg). (Figure 19).

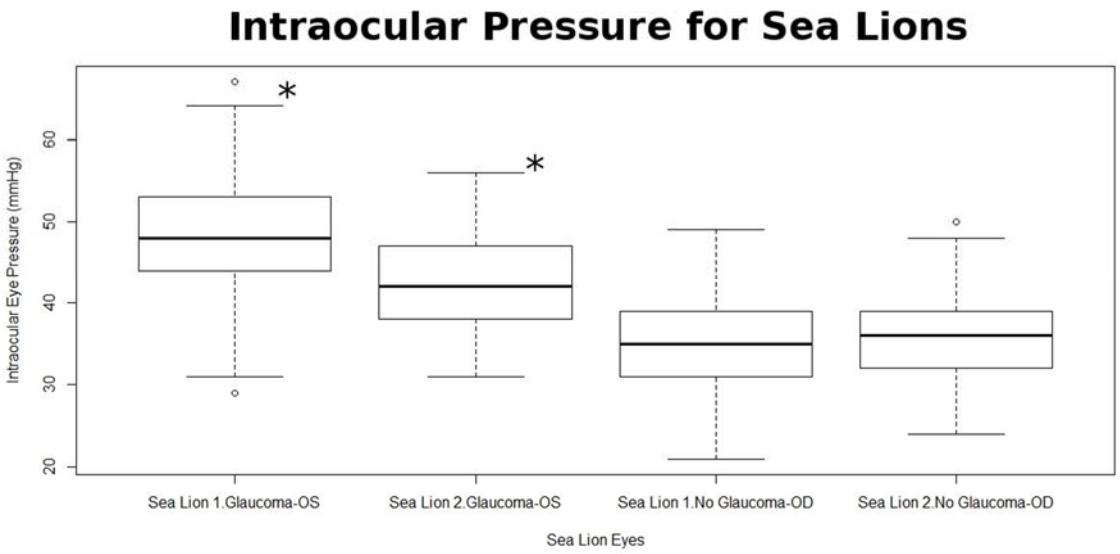

Figure 19 Boxplot of intraocular pressure (mmHg) comparing 2 sea lions (each animal having one glaucomatous eye versus non-glaucomatous eye). Median is represented by the bold line and the 'o' represent outliers.

Intraocular pressures were subjected to a two-way ANOVA with two groups (platform height) and sea lion eye (OS and OD).The results from a two-way ANOVA showed that there is a significant difference in IOP when comparing platform heights as seen by $F(1,130)=43.47$, $p<0.01$. There was not a statistically significant difference between eyes with $F(1,30)=3.03$, $p=0.08$. (Table 10, 11, Figure 20**).**

Table 10 Table of mean and standard deviation of intraocular pressure (mm Hg) evaluating 1 sea lion (platform height, high and low).

Platform Height	Eye	No. of Observations	Mean	St Dev
High	OD	27	45.44	6.65
High	OS	48	48.23	7.36
Low	OD	20	38.4	6.20
Low	OS	38	39.76	6.78

Table 11 Intraocular pressures were subjected to a two-way ANOVA with two levels (platform height) and sea lion eye (left and right).

Comparison by Platform Height and Eye for One Sea Lion

Variable	DF	Sum Sq	Mean Sq	F-value	P-value
Platform Height	1	2059	2058.6	43.573	<.01
Eye	1	143	143.3	3.033	0.084
Residuals	130	6142	47.2		

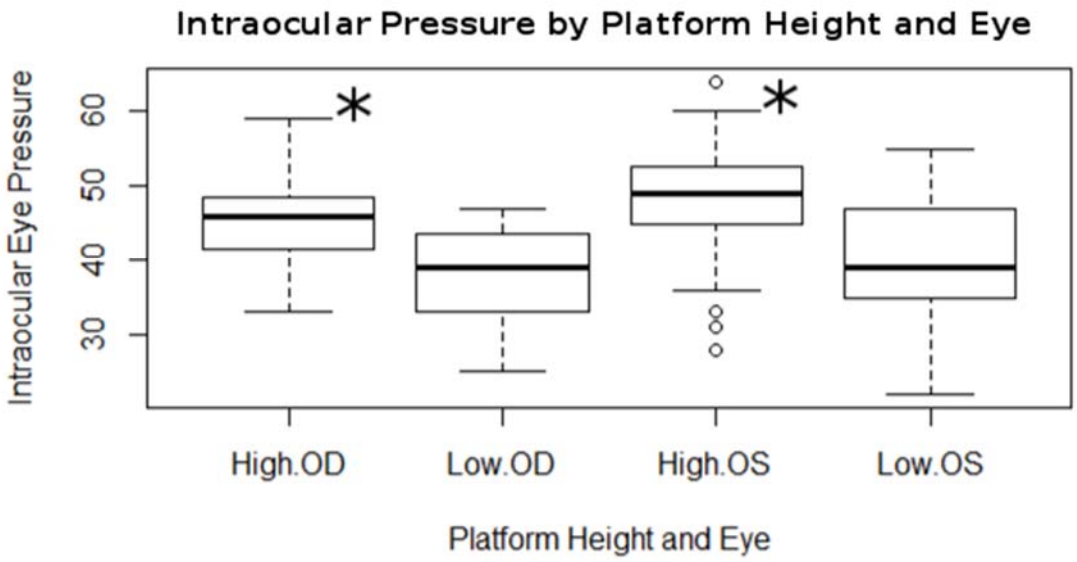

Figure 20 Boxplot of intraocular pressure (mmHg) comparing 1 sea lion (intraocular eye pressure by platform height and eye).

Median is represented by the bold line and the 'o' represent outliers.

The eye (left vs. right) was included in the results because it approached significance and the thought is that if there had been more animals studied, there would have been a significant difference between eyes.

Discussion

The results of this study showed a significant difference between intraocular pressures of the left and right eyes with OS being significantly higher in a group of sea lions that had been previously diagnosed with ocular disease. Second, as seen by p-values greater than 0.05, there were no significant differences in IOPs among groups of varying degrees of ocular conditions. Finally, nose position produced a significant effect, as the intraocular pressure in the nose up position was significantly less than the nose down

position. Eyes with clinical signs of glaucoma had significantly higher IOPs than normal eyes ($p < 0.05$). There was a significant difference in IOP when platform height was compared, $p<0.01$.

Other authors have cited numerous reasons why California sea lions develop ocular disease including age, lack of shade, higher ultraviolet index, water quality imbalances, by-products of disinfection, trauma, viral and bacterial infections, and others.[21,72] Four risk factors were associated with cataracts and lens luxation. Insufficient access to shade was the most important risk factor as this increased the likelihood of cataracts and lens instability by 10-fold.[1] Other risk factors included age ≥ 15 years, history of fighting, and history of any ocular disease.[1] Animals living under human care are living longer than their wild conspecifics manifesting age-related diseases including cataracts and lens instability. Cataracts are detrimental to sight and have the potential to luxate causing acute pain, corneal fibrosis, and secondary glaucoma.

This study evaluated IOP using the TonoVet® rebound tonometer in California sea lions with varying degrees of ocular pathology. A high incidence of cataracts and lens instability has been reported in captive California sea lions, and, this study evaluated the potential for these diseases to be associated with secondary glaucoma.[1,75,94] To the authors' knowledge, glaucoma is rare in California sea lions with fewer than five cases definitively diagnosed (Colitz, 2014, personal observation). In our study, despite varying degrees of ocular pathology, glaucoma was diagnosed in 2 out of the 37 animals (5.4%).

Glaucoma is characterized as an ocular syndrome that manifests as a result of decreased outflow of aqueous humor resulting in a rise in intraocular pressure. As IOP increases above that which is compatible with the health of the globe, the retina and optic

nerve degenerate leading to blindness.[3] Glaucoma causes inner retinal damage and impairment of rapid axoplasmic transport within the optic nerve axons as well as accumulation of cellular organelles in the scleral lamina cribrosa.[9] The lamina cribrosa is the sieve-like connective tissue through which the optic nerve exits that helps maintain pressure gradient between the intraocular space and the retrobulbar space.[98] This pressure gradient is the IOP minus the pressure in the retrobulbar cerebral spinal fluid space. These collagen fibers bridge into the scleral canal, though which ganglion cells cell axons run through pores formed by these collagen beams.[98] The weakest part of the sclera is considered to be the lamina cribrosa. With chronicity, as IOP rises and the optic nerve degenerates irreversibly, it bulges outward and becomes visibly cupped.[99] As nerve fibers degenerate, there is a loss of retinal ganglion cells and thinning of the outer layers of the retina to the point where they disappear and are replaced by glial scar formation.[9] With glaucoma, displacement of the lamina cribrosa causes the pores to deform, pinching the transversing nerve fibers and blood vessels.[100] Sea lions have a very delicate lamina cribrosa, while various fish species that have been studied lack this mesh-like structure completely.[20,98] It is unknown why fish have no lamina cribrosa in the relatively thick optic nerve. In horses, the lamina cribrosa has a very resilient structure that may provide some protection to the optic nerve axons during episodes of elevated intraocular pressures.[101] Perhaps sea lions during submerged activity, use the lamina cribrosa as a mechanism to maintain normal hydrostatic pressure, especially when intracranial pressure may rise during a dive as they hold their breath.[15,98]

Glaucoma can be primary or secondary in origin. In our study, 2 out of the 37 sea lions were diagnosed with secondary glaucoma. Both sea lions showed clinical signs of buphthalmos, blepharospasm and epiphora in the left eye of each animal (Figure 21).

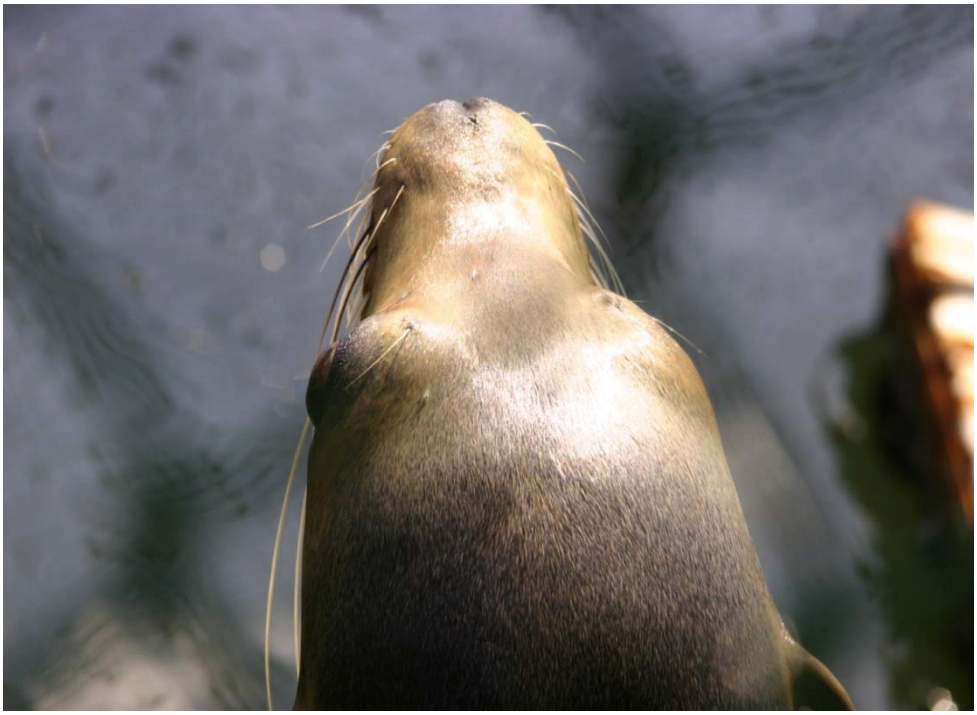

Figure 21 OS appears buphthalmos compared to OD of this sea lion which was diagnosed with glaucoma.

These clinical signs were suggestive of glaucoma when compared to the right eye. Elevated IOP confirmed the clinical signs of glaucoma.

Due to the advancements in diagnostic technology, the TonoVet® has been used to establish the normal IOP range of the means for each eye, OD (24-39 mm Hg) and OS (25.5-38 mm Hg) in captive California sea lions without clinical evidence of ocular disease (see chapter V). In sea lions with asymptomatic eyes, a statistical, though not clinical, difference was found between the IOP of OD and OS with OS having a higher IOP. Head position also had a significant effect on IOP with higher IOP detected in the

head down position. There was no significant difference in IOP between zones (axial and paraxial regions) among eyes of different sea lions or between zones between eyes of the same sea lion (see chapter V).

The normal IOP between eyes of the same sea lion can vary by <4mm Hg.[93] Two animals were diagnosed with glaucoma in this study. Both animals displayed statistically significant increases in IOP between their glaucomatous eye and their non-glaucomatous eye. One showed a mean 6 mm Hg higher while the other showed a mean 12 mm Hg higher. Because cataracts are so common, it has been suggested that secondary glaucoma would be more likely in sea lions since glaucoma typically occurs following the presence of long standing cataracts with or without luxation.[102,103] (Colitz, 2014, personal communication). In our study, although some animals had intermittently higher IOPs than the published normal sea lion range, glaucoma was rarely diagnosed.

By comparing sea lions to various other species, anatomical similarities may provide insight as to why glaucoma is rarely seen in this species. Sea lions evolved from the superfamily Arctoidea within the suborder Caniformia that gave rise to the Ursidae and Mustelidae. Interestingly, bears, being very close in evolution to sea lions, are not reported in the literature to be commonly afflicted with glaucoma.[104] One common aspect that they both share is the relatively large size of their eyes. Sea lions have large eyes in comparison to the size of their skulls allowing better vision at depths where light is dim.[2] Evolutionary anatomical changes allow pinnipeds to be able to dive to depths of ~450-900 feet.[2] The sea lion has a thick sclera, it is thinnest equatorial and thickest at the limbus and posterior pole.[20] The sea lion is exposed to continuous external water pressure changes that occur on a daily basis. As they dive they may rely on increasing IOP which

may facilitate repositioning of the lens. It may not be a coincidence that the equator of the eye is the thinnest part of the eye in most species signifying its potential to change length (Samuelson, 2014, personal communication).

Sea lions have thick pectinate ligaments which are visible on ophthalmic exam, and a prominent ciliary cleft, similar to the horse.[105] In addition, some aquatic and terrestrial species also have a uveoscleral or unconventional pathway for outflow. This allows for another potential avenue for rapid removal of aqueous humor in response to certain underwater conditions, such as accommodation.[58] We do not know if sea lions have this outflow mechanism. The hippopotamus is a semiaquatic animal that has a uveoscleral route which may help with pressure changes as they remain underwater for extended periods of time.[58] The hippopotamus may contribute more of a percentage of aqueous humor outflow because in animals such as herbivores, they have a large anterior chamber and larger volume of aqueous humor than other species.[58] Specific anatomical features of the outflow pathways allow for the incidence of glaucoma to be rare in horses, hippopotamus', and marine mammals. Marine animals have developed anatomical mechanisms to have better control of intraocular pressure changes when exposed to continuous external water pressure associated with diving.[58]

Pinnipeds should be positioned in the training area where they feel most comfortable. The area where they normally eat and station for training is a good location. One sea lion in this study had IOPs collected in two different locations. One location was the normal training and feeding area and a second, alternate location was an area the trainer wanted the animal to become more acclimated. Previous history revealed that this location was a new area that the animal was very hesitant in entering. IOPs in the new

area were consistently higher during the same session in comparison to the area where the animal was normally trained. This particular animal would become intermittently exophthalmic when pressures were collected in the new area. Sea lions possess well-developed extraocular muscles that contract, pushing the eyes anteriorly, making them appear mistakenly buphthalmic or exophthalmic. These findings suggest that one should not collect IOP measurements when the eye is protruding, as falsely elevated, or aberrant, readings are common (Colitz, 2014, personal communication). If suspected aberrantly high readings are measured, the sea lion should be moved to another location, as animals may be calmer when not in the presence of other animals. Another suggestion to collect more accurate measurements includes stopping the training session and coming back when the animal has a calmer demeanor. A limitation in this study was that we only studied platform location change in one animal because the trainer wanted to evaluate how the animal did in a new setting. Normally, eye pressures were always collected in the area in which the animal was most familiar.

The expected hypothesis was not found in which there would be a difference among 5 groups with varying degrees of ocular conditions. The animals in group 2, although they had a severe keratopathy with fibrosis, cataracts were not apparent or diagnosed, which makes cataract subluxation less likely decreasing the chances of elevated IOPs. Since glaucoma may be caused by acute and chronic anterior lens luxation, it was hypothesized that group 3B, presurgical animals with lens luxation, would have higher IOPs than animals with normal eyes. These results were not found in any animal suggesting that sea lion eyes are resistant to secondary glaucoma. Reasons for this may include very large iridocorneal angles or differences in sequelae to chronic

uveitis such as lack of pre-iridal fibrovascular membranes (PIFM) formation. Groups 1A and 1B had cataracts, but not luxation, and IOPs were not elevated. Cataracts have been removed in animals in group 3A and corneas appeared clear upon ophthalmic exam. Cataract surgery prior to anterior lens luxation removed the risk of a lens luxation also diminishing the potential of secondary glaucoma.

Interestingly, position of the nose when measuring IOPs in sea lions appeared to be important with higher pressures measured when the nose was positioned down in both normal and abnormal eyes. This may be due to contraction of neck muscles causing jugular occlusion or increased episcleral venous pressure.[106] Therefore, we recommend collection of IOP measurements in a nose-up position. IOPs of OS were significantly higher than the IOPs of OD in both normal and abnormal eyes. One hypothesis why this may have occurred can be due to whether OS IOP measuring was done prior to OD. The trainer elected which eye of the sea lion had IOP collected first as this was based on the animal's behavior. A limitation in this study was that we did not record which eye had pressures taken first. In future studies, this data should be recorded as animals may have higher pressures in the second eye, the longer they wait, perhaps associated with a stress response of the autonomic nervous system.

Most marine DVM's agree that the cause of ocular disease in sea lions is mutifactorial and a combination of changes in their environmental physiological habitat is essential. Four risk factors have been associated with cataracts and lens luxation. Insufficient access to shade is reported to be the most important risk factor as this increases the likelihood of cataracts and lens instability by 10-fold.[1] Light colored paint should be avoided and colors that are not considered UV or light-reflective such as earth

tones are recommended. UV light meters, such as Solarmeter model 6.5 UV index meter, should be used to quantify the amount of UV light reflected from the pool.[107] Facilities can be viewed from "Google Earth" to subjectively assess the amount of visible light reflected from the animal pools.[19] Shade structures should be used to reduce the amount of UV light reflected into the eyes. Trainers and keepers are becoming very aware of how, when, and where they should feed their animals to reduce sunlight exposure. Training and feeding should occur in a shaded area, never allowing the animal to look directly towards the sun. Feeding pools are usually designed where the sea lion has to look up to see out of the exhibit or catch fish (Figure 22).

Figure 22 Sea lions in a feeder pool staring up straight into the sun as they feed.[19]

The public should be asked to feed the animals later in the day and not during the bright sunny hours as well a shade structure should be positioned behind the public. Other risk factors included age ≥ 15 years, history of fighting, and history of any ocular disease.[1] In this study, one animal was 5 years old and the others were ≥12 years. Light colored paint should be avoided and colors that are not considered UV or light-reflective such as earth tones are recommended. Life support and filtration systems should be carefully monitored, as these oxidant spikes can be very detrimental to eyes. Antioxidants can be supplemented to manage these damaging intermediates.[19] The earlier we start these animals on ocular support supplementation, the better protection they will have as they age. A wholesome varied diet supplemented with protective antioxidants is integral. If facilities follow all of these recommendations, ocular lesions in the future generation of pinnipeds may be preventable or at least delay the onset of premature disease and make these animals more comfortable.

CHAPTER VII

DISCUSSION

Importance of ophthalmic exams

It is very important to schedule weekly or regularly scheduled ophthalmic exams on sea lions. Training is extremely critical in order for these ophthalmic exams to be performed. Animals need to be acclimated to being touched all over the head, especially the eyes. This behavior is called tactile training. Next, they need to feel comfortable with instruments such as an ophthalmoscope and tonometer, near the eye. Using an ophthalmoscope or a transilluminator in a trained animal will allow visualization of the pupillary light response, corneal integrity, and obvious opacities or abnormalities. In dim light, direct or indirect ophthalmoscopy may, occasionally, allow for evaluation of the fundus (Colitz, 2014, personal communication). The menace response may be complicated, by the sensitive vibrissae. Therefore, care must be taken to avoid moving air near the vibrissae which will result in movement of the eye or head which may be perceived as a positive menace response (Colitz, 2014, personal communication). An ophthalmic exam is not a simple task; usually the animal's head is moving and the narrow pupil makes it difficult to see the lens and retina. Physically restraining these animals can be difficult and stressful for them, or may be dangerous for the examiner or trainer. Therefore, most procedures are trained. Topical mydriatic agents do not tend to dilate the pupils.[20] In order to dilate sea lion eyes, animals need to be moved into a dark

enclosure or outside during night fall. To dilate the eye for cataract surgeries, intraacameral injections of epinephrine have been used.[3,94] A new study is evaluating the use of ultrasonography in sea lions. Images of internal eye structures and measurements will help diagnose cataracts, anterior lens luxation, retinal detachment, foreign bodies, swelling/inflammation, tumors and other disorders that are sometimes difficult to identify through lack of pupillary dilatation and/ or direct ophthalmoscopy.[40]

Unfortunately, by the time sea lions manifest chronic ocular lesions, most of them have lost their sight. The importance of tactile and target training, voice commands, and acoustic cues come into play. A blind animal will accommodate very quickly to familiar surroundings, but when moved to a new area, the animal may find it hard to avoid obstacles.[108] Sea lions have sensitive vibrissae which use nerve fibers to help in navigation and to detect vibrations from prey in water. When draining a pool to clean it, blind animals should be enclosed in a separate pool area. Sea lions are prone to develop cataracts by the average age 15 years.[1] Knowing that ocular disease is inevitable, marine mammal trainers have initiated training sea lions at an earlier age with verbal cues. Visual impairment is not always a dead end for sea lions. Through training and medical care, they can still have acceptable quality of life.

Comparison of IOP in normal versus abnormal sea lion

Both normal animals and those with varying ophthalmic diseases were used to evaluate the use of the TonoVet® rebound tonometer. IOP mean values in both normal and abnormal groups fell within similar ranges. The expected hypothesis of animals in group 2, which had severe keratopathy with fibrosis, would have elevated IOPs compared to normal eyes was not found. Though an elevated IOP was hypothesized in these eyes,

based on diffuse corneal opacity and vision impairment, they did not have glaucoma. A theory as to why these pressures were not elevated include that these animals may not have had cataract disease, therefore the lens had not luxated. Also, corneal edema was observed, which is a clinical sign seen in glaucomatous animals, but any injury to the corneal can cause this edema. Since glaucoma may be caused by acute and chronic anterior lens luxation, it was hypothesized that group 3B, presurgical animals with lens luxation, would have higher IOPs than animals with normal eyes. These results were not found in any animal suggesting that sea lion eyes are resistant to secondary glaucoma. Reasons for this may include very large iridocorneal angles supported in part by a prominent pectinate ligament, and differences in sequelae to chronic uveitis such as lack of pre-iridal fibrovascular membranes (PIFM) formation. Other reasons would be that the lens anteriorly luxated, but the vitreous was not degenerated and did not detach from the retina and therefore did not cause a pupillary block when the lens entered the anterior chamber. This can occasionally happen with anterior lens luxation (sometimes the pressure is elevated, but not always).[109]

Groups 1A and 1B had cataracts, but not luxation, and IOPs were not elevated. Animals in Group 3A had had their cataracts removed and corneas appeared clear upon ophthalmic exam. Cataract surgery prior to anterior lens luxation removed the risk of a lens luxation also diminishing the potential of secondary glaucoma.

Interestingly, position of the nose when measuring IOPs in sea lions appeared to be important with higher pressures measured when the nose was positioned down in both normal and abnormal eyes. This may be due to contraction of neck muscles causing jugular occlusion or increased episcleral venous pressure.[106] Therefore, we recommend

collection of IOP measurements in a nose-up position. IOPs of OS were significantly higher than the IOPs of OD in both normal and abnormal eyes.

Contrast of IOP in normal versus abnormal sea lion

We hypothesized that there would be a significant difference in IOPs between eyes with clinical signs of glaucoma compared to normal eyes. In this study, of the 37 animals evaluated, two animals were diagnosed with glaucoma. Both animals displayed statistically significant increases in IOP between their glaucomatous eye and their non-glaucomatous eye. One showed a mean 6 mm Hg higher while the other showed a mean 12 mm Hg higher. Each of the 2 sea lions had one normal looking eye and one eye with glaucomatous signs (buphthalmos, corneal edema, or blepharospasm). One of the animals had bilateral cataracts and the other had bilateral cataracts removed with central corneal scarring. Because cataracts are so common in these animals, it had been suggested that secondary glaucoma may be seen more often in sea lions as glaucoma typically occurs following the presence of long standing cataracts with or without luxation in other species (Colitz, 2014, personal communication). In this study, glaucoma was found to be rare in sea lions with ocular disease.

We also hypothesized that there would not be a difference in IOP when a single sea lion was measured in different locations (i.e. high and low platform). One animal consistently had higher IOP measurements when placed in a new area that she was not familiar with and was uncomfortable with her surroundings. This particular animal would have intermittent exophthalmia when IOPs were collected in the new area. When moved to her normal training area, the IOPs would measure in the normal range. These findings suggest that animals IOP's should be collected in a familiar area.

Limitations of the study

This study was limited to animals that were trained for the behavior of IOP measurements. Therefore, the population of sea lions with normal and ocular problems at each facility is not a true representation. Also, some animals are in protective contact and these animals were not included in the study. The number of sea lions in zoos and aquariums that have clinically normal eyes is low so it was difficult to increase our sample size to evaluate normal range. Animals were not randomly selected as we included all sea lions in which IOP behavior could be trained. Animals had three values measured per training session per eye, but some situations allowed for only one or two mean value measurements per session. The animal has an allotted amount of food per session so if the trainer runs out of food or if the animal breaks away from the session, the session would end. Measurements in the nose up and down position and zone 1 and 2, were not always collected in an even manner as some animals allowed more consistent measurements in a certain positions and depending on how the animal was positioned would depend on what zone the pressure could be collected. The animal's positioning determined on which zone the IOP could be measured. The variability in numbers of training sessions depended on how well the animal was trained for repeat sessions and whether the observer was able to travel to the facility again. Each animal had at least one session per day. After a measurement was collected, the instrument would display a line (high, middle, or low position) next to the value. The line shows the standard deviation of the measurements being collected. In our study, the line in the high position was generally observed which demonstrates the greatest standard deviation. Any movement or blinking of the eye can cause this and because these are wild animals there was no way

to stop the eye from moving. Differences in IOP measurements can be the result of a variety of factors including: diurnal variation, corneal thickness, corneal diameter, tear film viscosity, corneal rigidity, and the animal's susceptibility to stress.[9] When comparing IOP measurements, species-specific differences should always be considered. Cyclic fluctuations can vary throughout the day with highest IOPs reported at awakening.[110] Diurnal variations in IOP were not measured in this study as sessions could only be performed during daylight working hours. Therefore, pressure elevation may be missed with only collecting IOPs at one point in the day.

IOP can become increased in men that wear a necktie, in dogs with tight collars,[89] or with the Valsalva maneuver.[90] This temporary elevation occurs when air is forced against a closed windpipe and pressure goes up within the chest. This can occur during coughing, vomiting, playing, and weightlifting due to greater intrathoracic pressure caused by the air retained in the lungs when holding one's breath.[90,91] All of the sea lions in this study held their breath when targeting their nose to hold still for the IOP reading. Therefore, IOPs in these animals may be slightly elevated when holding their breath.[91]

Animals should always be positioned in the training area where they feel most comfortable. At times, animal training sessions may not be done in the normal enclosure that the sea lions are familiar with, in which case caution in recording should be taken if elevated eye pressures occur. One animal in our study had statistically significant higher IOPs when collected on an elevated platform. The area where they normally eat and station for training is a good location. Sea lions possess well-developed extraocular muscles that can push the eyes forward making them appear mistakenly buphthalmic or exophthalmic. This ocular action can also be stress-associated which can falsely elevate

IOPs. When an individual animal performs this action during a session, one should not collect IOP measurements with the eye protruding as falsely elevated readings are common (Colitz, 2014, personal communication). It is best to stop the session and resume later when the animal exhibits a calmer demeanor. In our study, animals were not restrained. Although they did hold their breath, so this may have been a factor in changing eye pressures.

Evolutionary anatomical changes including size of the eye, corneal thickness, and a tapetum covering the majority of the posterior aspect of the fundus, allow pinnipeds to have better vision where light is dim.[20] Histologically, the California sea lion's corneal stroma is thickest peripherally and thinnest centrally. The sclera is thinnest equatorially and thickest at the limbus and posterior pole.[20] IOP measurements using rebound tonometry are influenced by corneal thickness,[82] which may be another factor as to why sea lion IOP measurements are higher than in other animals. In this study, a difference in IOP was not observed when comparing corneal zone, which have different thicknesses.

The most evident limitation of this study was that although these animals are kept under human care, they are essentially wild animals, unless born at the facility. We are limited by the data that can be collected, the time it takes to collect an IOP, the positions we use, the variation that can occur in IOP results (e.g. movement, blinking of eyes), and overall whether they are trained for this behavior.

Areas of future research

Another tonometer called, Tono-pen Vet®, is an applanation tonometer that functions by measuring the force required to flatten, or applanate, a specific area on the surface of the cornea.[92] When this force is converted into an estimate of IOP, there are a

few assumptions that must be made about various physical factors including corneal thickness and curvature, corneal and scleral rigidity, tear film viscosity, and the effects of any topical medications that might be present.[92] Proparacaine, a topical anesthetic, should be applied to the corneal surface prior to using the instrument. The Tono-pen Vet® measures "normal" IOP at a range between 15-25 mm Hg in the dog, cat, and horse.[81] Most veterinary ophthalmologists are suspicious of glaucoma if IOP is greater than 25 mm Hg. An IOP greater than 30 mm Hg indicates glaucoma.[93] The Tono-pen Vet® (Dan Scott and Associates) was initially used in our study, but was found to have a more extensive learning and training curve for the person collecting IOPs as well as the sea lions learning the behavior (Mejia-Fava, 2014, personal observation and Colitz, personal communication). Future studies may include comparing Tono-pen Vet® measurements with using the TonoVet® in the same session. The training session when using the Tono-pen Vet® is different and may require more time as there is a larger tip and different force touching the corneal surface.

IOPs can increase depending on head and body position. A study performed in horses showed that head position below the heart level had a significant increase in IOP in comparison to head position above the heart.[92] Similarly, sea lions appear to have higher pressures when the nose is positioned down. This may be due to pressure being exerted on the neck leading to slight jugular occlusion or increased episcleral venous pressure. Therefore, we recommend collection of IOP measurements in a nose-up position. Future studies may compare sea lions in a lying down position to evaluate if eye pressures increase. Cyclic variations of IOP can occur throughout the day with IOPs

being highest in the early morning. Research evaluating diurnal changes from early morning to late evening would be an area of investigation in the future.

Anesthetic agents can change intraocular pressures. Several anesthetic inhalants such as, isoflurane, halothane, and sevoflurane, have been shown to lower IOP during anesthesia.[111,112] In a study conducted by Hirst et. al.,[67] 7 pinnipeds were anesthetized with halothane gas and applanation tonography was conducted on 7 eyes revealing IOPs between 8-18 mm Hg. The tonometer used was not specified and was not adjusted for scleral rigidity and corneal curvature in that species.[67] Our range of IOP's was higher for both eyes (24-39 mm Hg). Halothane anesthesia has been shown to decrease IOP in anesthetized humans, therefore, it is possible that these IOPs may have been lower due to anesthetic effects.[112] Tonography is different than tonometry in that continuous measurements are recorded, as opposed to collecting pressure at one instance in time. The tonometer is placed on the cornea, acutely elevating the IOP, and the rate at which the pressure declines with time is related to the facility of AH outflow.[113] Therefore this technique may be recording lower IOPs than pressures collected in awake animals using tonometry and the two methods should not be compared.

Future studies may include comparing IOP with various types of anesthetic drugs before and after drug administration to evaluate the effect in sea lions. This can prove to be very important, as this may be the only way to collect IOP for animals that are in protective contact.

Manometry is used to calibrate tonometers for a specific species. The TonoVet® used in this study is calibrated for a horse, dog and mouse. Ocular manometry uses the principle in which a known predetermined volume of fluid is injected into the anterior

chamber of the eye in ascending or descending pressure steps to compare pressures observed on the manometer to the tonometer being used.[114] This procedure is invasive and therefore should be done in fresh enucleated eyes. Special permits would be needed to collect these types of samples from sea lions. This study should be done in the future in order to accurately calibrate tonometric instruments in sea lions.

Topical parasympatholytic or sympathomimetic agents do not have a pharmacological response in sea lions suggesting poor penetration and/or too few cholinergic and adrenergic receptors.[20] More research in marine mammal medicine is needed to understand the pharmacokinetics of topical ophthalmic drugs, especially for treatment of glaucoma. Ultrasonography studies in these animals may also help to diagnose ocular diseases, which are difficult to identify through lack of pupillary dilation and/ or direct ophthalmoscopy. Recording images and measurements such as the iridocorneal angle will be important information when evaluating a glaucomatous patient. Ultrasonography, especially above and below water, will allow for a better understanding of the physiology of the lens movement used for accommodation.

Wild sea lions dive at great depths as part of their normal feeding habits. In order to find food they use various ocular muscles to help with accommodation. Miller et. al,[20] also suggests that the lens moves anteriorly during diving via contraction of the well developed ciliary body musculature, thus physically dilating the pupil aperture. The position of the uveal muscles and the suspension of the lens suggest that these structures may be involved in accommodation. Extraocular muscles and retractor bulbi muscles also contract displacing the lens forward. If these muscles are continually used for dives and sea lions under human care stop diving to these depths, perhaps there may be subtle

atrophy of these muscles. This equilibrium of pressure change may be needed on daily basis to prevent future ocular diseases. (Samuelson, 2014, personal communication). Future research can evaluate animals under human care that are trained in the wild to dive and follow these animals with ocular examinations to elucidate if diving a few times a day to great depths makes a difference long term.

Significance of this work

Cataracts are detrimental to sight and have the potential to induce anterior uveitis as well as to cause lens instability, each having the potential to cause secondary glaucoma.[95] Subluxation or luxation of the lens can lead to glaucoma by blocking the outflow of aqueous humor. Initial medical treatment is imperative, as glaucoma is painful and can impair vision by causing neural damage of the inner retina and impairment of rapid axoplasmic transport within the optic nerve axons and accumulation of cellular organelles in the scleral lamina cribrosa as described in the dog.[96] By establishing the normal range of IOPs in captive sea lions, animals can be trained and evaluated periodically through their lives to monitor individual IOP changes. Glaucoma in this study was reported to be rare with only 2 animals showing both elevated IOPs and clinical glaucomatous signs. Since we know that glaucoma, although not common, can occur in sea lions, the significance of this work allows for future cases with ocular conditions to have IOP monitored. If an animal's IOP become elevated, a cause can be identified and appropriate treatment can be initiated decreasing the likelihood of chronic changes and possible blindness.

Treatment options for glaucoma and antioxidants

When considering treatment for glaucoma, it is important to understand that each patient is different, even if they are the same species. And, understanding the pathogenesis of the particular glaucoma and state of the iridocorneal angle is ideal, though not always possible.[11] In the canine, acute glaucoma is an emergency, and medical treatment must be initiated immediately if the goal is to save vision.[11] The typical causes for secondary glaucoma include chronic inflammation, i.e. uveitis, and chronic retinal detachment. These cause release of vascular endothelial growth factor (VEGF) which leads to pre-iridal fibrovascular membrane formation which grows over the iris and iridocorneal angle.[70] Lens instability can be caused by chronic uveitis and can cause uveitis.[95] Chronic uveitis causes matrix metalloproteinase 9 (MMP-9) production which degrades fibrillin, the primary protein that comprises lens zonules. When the lens begins to shift anteriorly, whether partially (subluxation) or entirely (luxation), it leans against the iris from its posterior aspect, which then narrows the iridocorneal angle. Lastly, when the lens or cataract luxates anteriorly into the anterior chamber, it may block the pupil completely trapping aqueous humor behind in the posterior chamber; and this is called pupillary block glaucoma. There is a high incidence of cataracts and lens luxations in captive California sea lions rendering many animals blind and painful.[1] Ideally, the luxated lens should be removed surgically. If this procedure is not performed, lens luxation can lead to secondary glaucoma, which is the most common pathogenesis of glaucoma seen in sea lions.

There is no cure for glaucoma. Medical and surgical intervention is the gold standard to control glaucoma. Aggressive medical therapy is the treatment of choice in

sea lions as they typically respond to this approach (Colitz, 2014, personal communication). Sea lions with glaucoma have been treated with various drugs including carbonic anhydrase inhibitors, beta-blockers, and steroids. Cosopt® (dorzolamide hydrochloride and timolol maleate) is a combination of a topical carbonic anhydrase inhibitor and beta-adrenergic antagonist. Carbonic anhydrase is the main enzyme transport system that catalyzes the formation of carbonic acid (H_2CO_3) from CO_2 and water.[11] Carbonic acid dissociates and results in the secretion of bicarbonate ions into the aqueous. Sodium also accompanies bicarbonate.[11] Inhibition of carbonic anhydrase, can decrease bicarbonate to enter the posterior chamber which results in less water following and an overall decrease in aqueous production. [11] Other carbonic anhydrase inhibitors that have been used in sea lions include brinzolamide (Azopt™), dichlorphenamide, and methazolamide. Adrenergic antagonist or beta-blockers reduce the blood flow of the ciliary processes, which decreases the rate of aqueous humor production.[115] Corticosteroids, both oral and topical, are also used for glaucoma to minimize underlying inflammation. They act by inhibiting phospholipase, which alters the arachidonic acid metabolic pathway.[115]

Training behavior is extremely important when considering medical treatment in the sea lion, especially for glaucoma. Strict compliance for the scheduling of medication is needed when treating glaucoma. Unless the animal is trained to stay out of the water after medication, the likelihood that all of the treatments will penetrate the eye is low. Buphthalmia results from chronic and untreated glaucoma.[9] Corneal degeneration and ulceration with rupture may occur if buphthalmia results in ulceration.[11] The goal of treatment at this stage of the disease is based primarily to relieve the patient's pain.

Surgical treatment has not been successful in sea lions using endolaser cycloablation. In end stage glaucoma, the placement of an intrascleral prosthesis has been used in one sea lion (Miller, 2005, personal communication). The surgical record reported that the conjunctiva was dissected ~6 mm posterior to the lens. Then, an incision was made through the sclera at approximately ~4 mm posterior to the limbus. The globe was eviscerated by removing the intraocular structures. Next, a silicone sphere of appropriate size (30mm) was inserted into the scleral shell. The sclera was then closed in a simple interrupted manner using 5-0 Vicryl®. The conjunctiva was closed in a simple interrupted manner using 5-0 Vicryl®. The sea lion received a subconjuctival, gentamicin injection and, post-operatively, was administered oral non-steroidal medication. The two typical non-steroidal medications used in sea lions include meloxicam and carprofen (Colitz, 2014, personal communication). Therefore, surgical management of chronic end stage glaucoma in a sea lion using evisceration and intrascleral prosthesis is a useful and cosmetic approach.

Antioxidant supplementation in marine mammals, which is being studied as prophylactic ocular support for these animals, is important.[116,117] Carotenoids include lutein, zeaxanthin and lycopene. Lutein and its isomer zeaxanthin are oxygenated carotenoids synthesized by plants and microbes and found in dark leafy vegetables, egg yolks and colored fruits.[117,118] Lutein is known to be deposited in various parts of the eye, including the lens, retina, and in the uveal tract with trace amounts found in the cornea and sclera.[119] Detectable lutein levels have been found in the retina of the dolphin and harp seal. Supplementation with lutein beadlet form provided greater blood lutein levels in cetaceans than lutein ester form. Lutein and zeaxanthin may be particularly effective in

preventing or delaying the progression of cataract formation.[118] These antioxidants also have protective effects on the retina against blue light and oxidative stress by limiting the degree by which oxygen penetrates the ocular membranes.[117] Flavonoids, such as bilberry and grapeseed extract (GSE), are antioxidant phytochemicals have been supplemented safely to marine animals. (Meija-Fava, 2014, personal observation) Bilberry and GSE help to reduce oxidative damage and GSE has been shown to inhibit formation of certain types of cataracts in animal models by increasing reduced glutathione, the predominant antioxidant system in the lens.[118]

Ocular supplements are being studied for use in glaucoma in both human and animal models. Prior to clinical onset of glaucoma, there is ongoing oxidative stress occurring within the eye, which may contribute to the pathogenesis of some types of glaucoma. Oxidative stress is responsible for cellular damage by stimulating the production of unstable and highly reactive oxygen species (ROS). Antioxidants may help to diminish lipid peroxidation in a mouse glaucoma model in which retinal immunohistochemistry was used for 2 markers of oxidative stress, malondialdehyde (MDA) and nrf2.[120] High levels of ROS causes lipid peroxidation leading to liberation of reactive aldehydes, such as malondialdehyde (MDA). Nrf2 is a trascription factor that regulates celluar defense against oxidative stress.[121-123] This cellular study performed in mice using a vision antioxidant supplement showed that they had statistically significant lower IOP and decreased changes in oxidative stress compared to animals not taking the supplement.[120]

Antioxidants have different ocular protectant mechanisms against oxidative stress. Two key antioxidants are found in the eye, ascorbic acid is found in highest concentration in the corneal epithelium and reduced glutathione (GSH) is found in the lens. The ciliary body functions to provide aqueous humor with antioxidants such as ascorbic acid and glutathione. The mechanism by which ascorbic acid is concentrated in the aqueous humor from the plasma is via SVCT2 located in the pigmented epithelial (PE) cell layer.[119] GSH secretion into the aqueous humor has a mechanism via the location of GSH efflux transporters to the basolateral membrane of the non-pigmented epithelial layer (NPE).[119] Aqueous humor has 25 times more ascorbic acid than in blood. A human study showed that increased intake of vitamin C lowered IOP with various mechanisms proposed. Intraocular pressure may be decreased with an opening of the many channels of the Schlemm's canal, any swelling in the area would be removed by the antihistaminic effect of vitamin C, and T4 lymphocyte cells are also increased with vitamin C intake removing debris from Schlemm's canal such that iris pigment and inflammatory cells are unable to plug the outflow of the eye.[124] Alpha lipoic acid(ALA) is a universal antioxidant that replenishes glutathione and has been used after ischemic reperfusion injury. ALA has been shown to protect the lens from oxidative damage and an imbalance of the glutothione-redox cycle leads to cataractogenesis.[116] A novel LCMS/MS analysis to quantify ALA levels in sea lion serum has been developed to test alpha lipoic levels.[116] In human primary open angle glaucoma, studies have shown a down-regulation in glutathione S-transferases, suggesting that oxidative stress may play a role in glaucoma.

Proanthocyanidins have been shown to reduce oxidative stress within lens epithelial cells during cataractogenesis and in trabecular meshwork cells.[125] Since

cataract development can lead to secondary glaucoma, via chronic lens induced uveitis, this supplement may have important protective properties. Epigallocatechin gallate (Green Tea Extract) EGCG, administered to rats orally, attenuates injury to the retina caused by ischemia/reperfusion.[125] This study supports the use of EGCG in the complementary treatment of glaucoma contributing as a potential neuroprotective agent. Both omega-6 and omega-3 fatty acids have been shown, in one study, to decrease IOP in rats and produce metabolites that may increase aqueous humor outflow, through the trabecular and uveoscleral pathway.[126]

Human patients with glaucoma develop retinal ganglion cell damage from excitotoxicity. Studies support that intraocular Coenzyme Q_{10} (ubiquinol), an essential cofactor of the electron transport chain, provides neuroprotection which prevents the formation of the mitochondrial permeability transition pores. CoQ_{10} minimizes glutamate increases suggesting neuroprotective capabilities against oxidative stress.[127] Other therapeutic potentials include protecting optic nerve head astrocytes against oxidative stress-mediated mitochondrial dysfunction or alteration in glaucoma and other optic neuropathies. Quercetin may provide a neuroprotective mechanism against the initiation of glaucomatous damage.[127] Studies have shown that this supplement prevents oxidative injury in rat cortical cell cultures, inhibiting lipid peroxidation and scavenging free radicals, and has hepatoprotective properties against ischemia-reperfusion injury, when given orally.[128] In mammals, quercetin inhibits lipid peroxidation and slows the progression of selenite-induce cataract in a rat model.[128] The major active components of ginseng are ginsenosides, a diverse group of steroidal saponins which demonstrate an array of pharmacological properties. Ginsenoside saponins Rb1 and Rg3 attenuate the

apoptotic cascade, including glutamate-induced neurotoxicity, calcium influx into cells in the presence of excess glutamate, and lipid peroxidation.[129] *Lycium barbarum* has long been used in Chinese pharmacopoeia for centuries. In humans, *L. barbarum* helped to restore vision after experimental light induced photoxicity and macular degeneration study. [129]This shows that *L. barbarum* may help in protecting retinal ganglion cells from glutamate and nitric oxide induced neuronal apoptosis in the retina.[129] *L. barbarum* in humans has been shown to have cytoprotective effect on neurons.[130] In humans, the supplement Neuo-plex combines both Neuroprevin™ and PolicosanolPlus®. [131] Evidence shows neuronal growth in PC12 neuronal cells. The combination of the two supplements has shown positive growth promoting effects in PC12 neuronal cells demonstrating neurite growth at 9 hours.[131] This supplement might be valuable for glaucoma with its nerve regenerative properties as well as its neuroprotective effects.

An increased danger of Ultraviolet (UV) rays in the eye has been linked to a decrease in stratospheric ozone.[132] Detrimental UV radiation can be more of a health risk as we age and antioxidant levels decrease in the eye, as well as the body. This can predispose to photokeratitis, cataracts, and glaucoma.[132] UV light and disinfectant byproducts create reactive free oxygen species (e.g. hydrogen peroxide, singlet oxygen, and oxygen free radicals such as superoxide anions and hydroxyl radicals) which can be dangerous to the eye.[132] Low molecular weight antioxidants (alpha-tochopherol, glutothione, asorbic acid) and high molecular weight antioxidants such as catalase, superoxide dismutase, glutathione peroxidase and reductase) have been found in ocular tissues and fluids playing a role to absorb and detoxify UV B radiation.[132] In mammalian tissue, three different forms of superoxide dismutase have been described including

mitochondiral superoxide dismutase, and extracellular superoxide dismutase, and cystolic copper-zinc superoxide dismutase. The latter may have a nutritional component since recent studies have shown that some types of frozen fish contain negligible levels of copper and may be important to evaluate copper levels in these animals with ocular disease.[132] In humans , the cornea is most sensitive to UV damage, absorbing 92% of UV B and 60% of UV A. One study showed that the anterior part of the cornea (the epithelium and Bowman layer) was more important for absorption than the posterior layers of the cornea.[132] Perhaps in the sea lion, that has a very thin Bowman layer, UV absorption is more likely making them prone to otartid keratitis (Mejia-Fava, 2014, personal obervation). Sea lions may benefit from these antioxidants as they can provide a natural oral form of physiological sunscreen preventing the penetration of UV light and protecting tissues from photo-induced oxidative damage.[117,132]

Since aging is associated with a decrease in cellular antioxidants, degenerative diseases are more likely due to poor healing and lack of regeneration. Not only do sea lions under human care live longer, but they are exposed to exogenous oxidative stressors including sunlight and water quality imbalances for their entire lifetimes. Therefore, the addition of high doses of a variety of natural antioxidants is important in order to protect their eyes and bodies against the cumulative damage of these oxidants.

Why glaucoma may be a rare disease in sea lions

Sea lions have one of the largest iridocorneal angles, compared to other marine mammals.[20] As extraocular muscles contract, the lens moves forward, dilating the eye mechanically, and possibly raising the IOP, as the aqueous fluid moves with increasing force into the prominent iridocorneal angle. The sea lion pectinate ligament extends from

the base of the iris toward the limbus with intermittent open channels between the anterior chamber and the ciliary cleft. The ciliary cleft shows a strong reticular pattern supporting stromal columns.[20] A corneoscleral and uveal trrabecular meshwork was observed similar to other caniforms.[20]

The horse also has a thick pectinate ligament that is visible on ophthalmic exam and a prominent ciliary cleft.[133] The large ciliary cleft of the horse is supported by the strong trabeculae of both the pectinate ligament and the inner part of the trabecular meshwork, making collapse of the ciliary cleft practically impossible.[105] The equine iridocorneal angle morphology helps to explain the rarity of glaucoma in the horse.[105] The goat also has a very large ciliary cleft in contrast to the pig and buffalo.[134] The large cleft facilitates for best drainage, whereas animals such as primates and carnivores have a smaller iridocorneal angle, they tend to have enlargement of the ciliary musculature.[134] Increased numbers and prominence of iridal and ciliary body blood vessels have also been found in animals such as the West Indian manatee, pilot whale, beluga, and narwal.[58] These aquatic species may have developed increased vascular supply and venous outflow mechanisms for greater control over the blood supply to the ciliary body, thereby allowing more rapid control over the production of aqueous humor.[58] In addition some aquatic and terrestrial species also have a uveoscleral pathway outflow which allows for another potential avenue for rapid removal of aqueous humor in response to certain underwater conditions.[58,134] Specific anatomical features of the outflow pathways allow for the incidence of glaucoma to be rare in some herbivorous and marine mammals.

ESCs have been found in various cetacean species and are believed to be mechanoreceptors.[58] These receptors found in proximity to vessels may detect vessel

volume and pressure due to diving.[58] Although ESCs have not been found in sea lions, they have one of the largest iridocorneal angles compared to other marine species and perhaps this is this mechanism allows them to handle increased ocular pressure as they dive. [20] IOP can become increased with the Valsalva maneuver.[90] This occurs when air is forced against a closed windpipe and pressure goes up within the chest. Greater intrathoracic pressure is caused by the air retained in the lungs when holding one's breath.[15] When marine mammals dive, it has been suggested that the trans-thoracic pressure gradient (ambient pressure-pressure inside the respiratory system) sharply increases during a dive, compressing air out of the alveoli, into the rigid upper airways.[15] The thought process is that as sea lions dive and hold their breath, the trans-thoracic pressure gradient increases which increases IOP. The prominent angular aqueous plexus in marine species may facilitate the rapid changes in intraocular pressure.[58]

Diving marine mammals have a special adaptation to help regulate changes in pressure in air filled cavities. The middle ear cavity is lined with an extensive venous plexus called the venous cavernous sinus, a type of corpus cavernosum which becomes engorged at depth and thus reduces the air space and prevents the development of the squeeze.[17] Sea lions also do not have paranasal sinuses, lacrimal bones (identified in domestic species as the ventromedial aspect of the orbit adjacent to the nasal cavity), and nasolacrimal ducts, which may be an anatomical adaptation to help decrease problems with equalizing pressures.[18] Surrounding the optic nerve, posterior to the globe, there is a prominent vascular plexus and although sea lions don't have ESCs, perhaps there may be a vascular hydrostatic mechanism in which ocular perfusion pressure is maintained (Samuelson, 2014, personal communication).

Sea lions have a very delicate lamina cribrosa.[20] In horses, the lamina cribosa has a very resilient structure that may provide some protection to the optic nerve axons during episodes of elevated intraocular pressures.[101] Perhaps in sea lions that live under water pressures use the lamina cribosa as a mechanism to maintain normal hydrostatic pressure, especially when intracranial pressure may rise during a dive as they hold their breath.[98,135] The sea lion has developed ocular anatomical structures, which allow them to be exposed to increased intraocular pressure changes without developing glaucoma.

CHAPTER VIII

CONCLUSION

The California sea lion is prone to a variety of ophthalmological diseases including keratopathy and cataracts with and without luxation.[1,20,75] In this study, we determined the range of normal intraocular pressures (IOP) in California sea lions without ocular pathology and documented selected variables that can affect IOP measurements in this species. Sea lion intraocular pressure (IOP) was also compared in five different groups diagnosed with various degrees of ocular conditions, including a comparison of 2 animals diagnosed with glaucoma, and one sea lion that had IOP measured at different platform heights to evaluate if these factors made a significant difference in changing IOP. Sea lions were examined (diseased=37 sea lions, 70 eyes), normal= 19 sea lions, 37 eyes).

Rebound tonometry offers a stress-minimizing, well-tolerated tool for ophthalmic diagnostic testing in sea lions. The IOPs collected in normal eyes, OS were higher than in OD, although the difference is probably of little clinical significance as the difference in normal IOP between eyes can vary normally <4mm Hg.[35] One hypothesis why this may have occurred can be due to whether OS IOP measuring was done prior to OD. The trainer elected which eye of the sea lion had IOP collected first as this was based on the animal's behavior. A limitation in this study was that we did not record which eye had pressures taken first. In future studies, this data should be recorded as animals may have

higher pressures in the second eye, the longer they wait, perhaps associated with a stress response of the autonomic nervous system.

Two animals were diagnosed with glaucoma in this study. Both animals displayed statistically significant increases in IOP between their glaucomatous eye and their non-glaucomatous eye. One showed a mean 6 mm Hg higher while the other showed a mean 12 mm Hg higher. One animal also had statistically higher pressures when placed in a feeding location in which the animal was not comfortable. A limitation in this study was that we only studied platform location change in one animal because the trainer wanted to evaluate how the animal did in a new setting. Normally, eye pressures were always collected in the area in which the animal was most familiar. Because this is one animal, we cannot draw a conclusion that location may increase IOPs for the general population, but further research is needed in this area as subjectively from a clinical aspect this has also been observed. (Colitz, 2014, personal communication).

Rebound tonometry offers a stress-minimizing, well-tolerated tool for ophthalmic diagnostic testing in sea lions. Species-specific differences should always be considered. The IOPs collected in normal eyes, OS were higher than in OD, although the difference is probably of little clinical significance as the difference in normal IOP between eyes can vary normally <4mm Hg in other species.[93] Two animals were diagnosed with glaucoma in this study. Both animals displayed statistically significant increases in IOP between their glaucomatous eye and their non-glaucomatous eye. One showed a mean 6 mm Hg higher while the other showed a mean 12 mm Hg higher. One animal also had statistically higher pressures when placed in a feeding location in which the animal was not comfortable. A limitation in this study was that we only studied platform location

change in one animal because the trainer wanted to evaluate how the animal did in a new setting. Normally, eye pressures were always collected in the area in which the animal was most familiar. Because this is one animal, we can't draw a conclusion that location may increase IOPs for the general population, but further research is needed in this area as subjectively from a clinical aspect this has also been observed. (Colitz, 2014, personal communication).

Most marine DVM's agree that the cause of ocular disease in sea lions is mutifactorial and a combination of changes in their environmental physiological habitat is essential. Four risk factors have been associated with cataracts and lens luxation. Insufficient access to shade is reported to be the most important risk factor as this increases the likelihood of cataracts and lens instability by 10-fold.[1] Light colored paint should be avoided and colors that are not considered UV or light-reflective such as earth tones are recommended. Underwater UV light meters should be used to quantify the amount of UV light reflected from the pool. Facilities can be viewed from "Google Earth" to subjectively assess the amount of visible light reflected from the animal pools.[19] Shade structures should be used to reduce the amount of UV light reflected into the eyes. Trainers and keepers are becoming very aware of how, when, and where they should feed their animals to reduce sunlight exposure. Training and feeding should occur in a shaded area, never allowing the animal to look directly towards the sun. Feeding pools are usually designed where the sea lion has to look up to see out of the exhibit or catch fish. The public should be asked to feed the animals later in the day and not during the bright sunny hours as well a shade structure should be positioned behind the public. Other risk factors included age ≥ 15 years, history of fighting, and history of any ocular disease.[1] In

this study, one animal was 5 years old and the others were ≥12 years. Light colored paint should be avoided and colors that are not considered UV or light-reflective such as earth tones are recommended. Life support and filtration systems should be carefully monitored, as these oxidant spikes can be very detrimental to eyes. Antioxidants can be supplemented to manage these damaging intermediates.[19] The earlier we start these animals on ocular support supplementation, the better protection they will have as they age. A wholesome varied diet supplemented with protective antioxidants is integral. If facilities follow all of these recommendations, ocular lesions in the future generation of pinnipeds may be preventable or at least delay the onset of premature disease and make these animals more comfortable.

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APPENDIX A

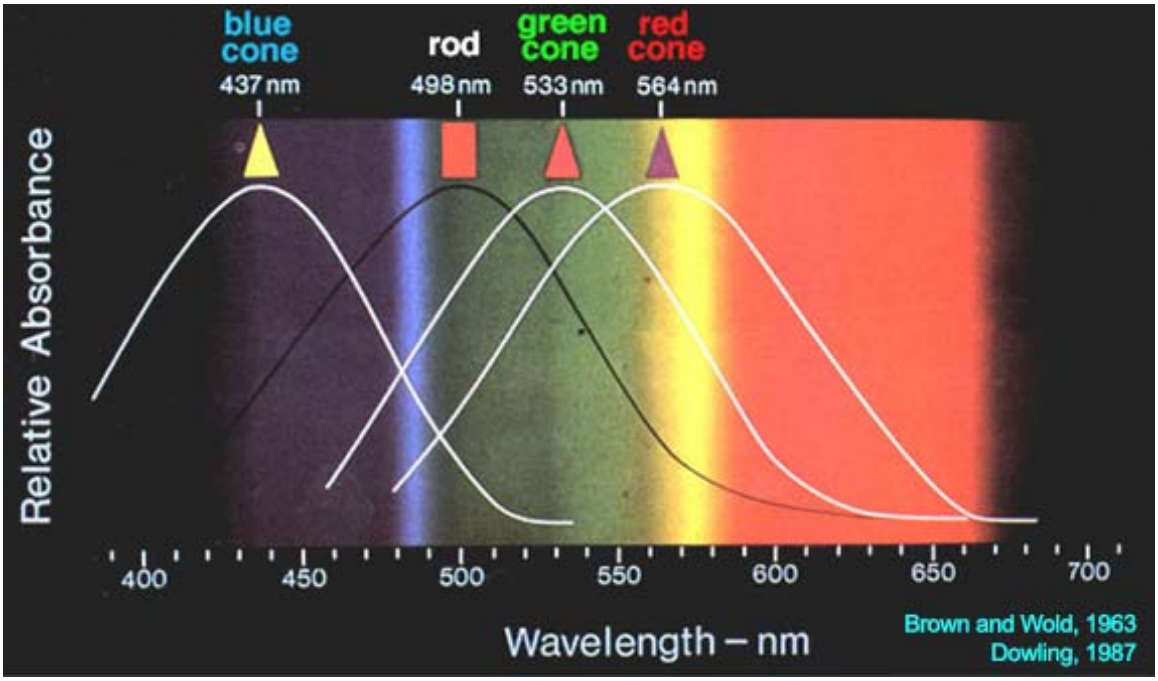
DEFINITIONS[10,22]

Neritic	In marine biology, the neritic zone, is considered the costal waters or shallow depths of the ocean where sunlight reaches the ocean floor.
Emmetropia	normal refractive system of the eye in which a focused image falls exactly on the retina
Multifocal	having more than one focal length
Bipolar and Amacrine	Amacrine cells are interneurons located in the inner plexiform layer that act as a neurotransmitters by using their dentrites to receive information from the bipolar neurons of the inner plexiform layer of the retina and sending information to the ganglion cells
Pericarya	cell body of a nerve cell containing the nucleus and organelles
Astigmatism	having blurred vision due to the inability of the eye to converge parallel rays of light on a single focal point on the retina
Mydriatic	dilation of the pupil
Corneal edema	swelling of the cornea that manifests as a blue translucency due to excess fluid within the outermost layer of the eye
Descemet's Membrane (Haab's Striae)	endothelialized ruptures of Descemet's Membrane which appear as horizontally orientated lines found in cases with buphthalmos
Lagophthalmos	condition in which the eyelids do not close the eyelids which can lead to corneal dryness and ulceration

APPENDIX B

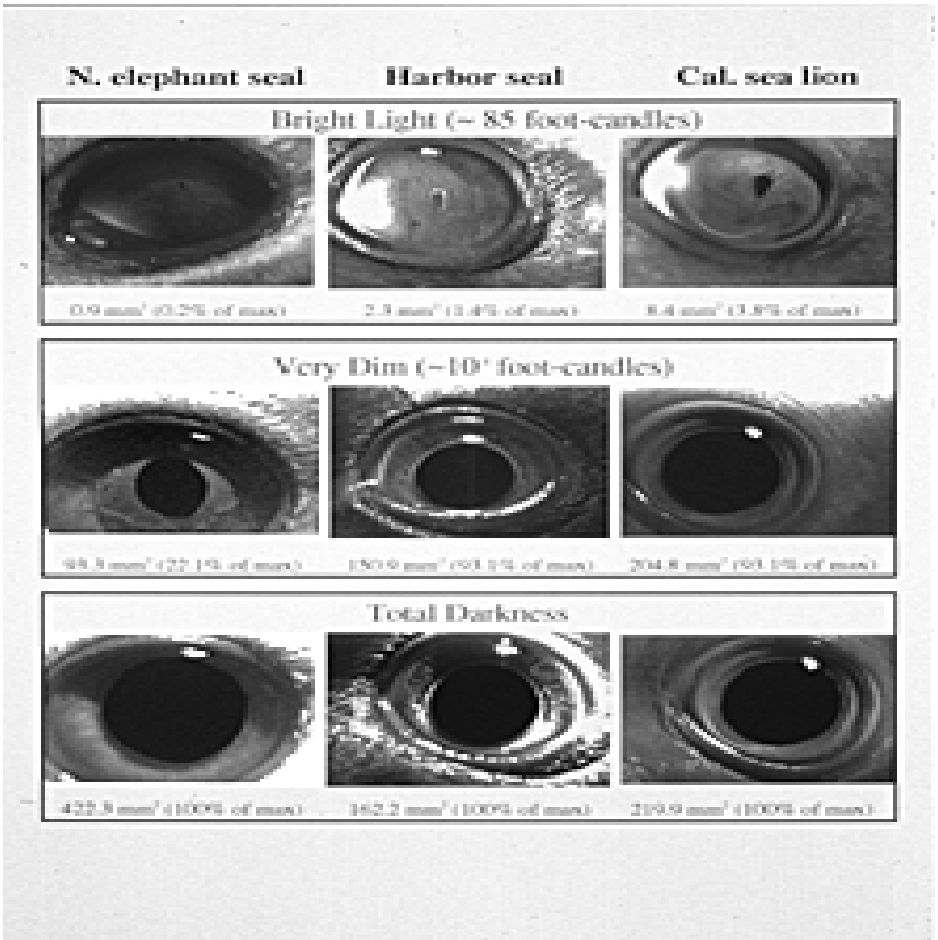
THE PEAK SPECTRAL SENSITIVITIES OF THE 3 CONE TYPES AND THE RODS

IN THE PRIMATE RETINA



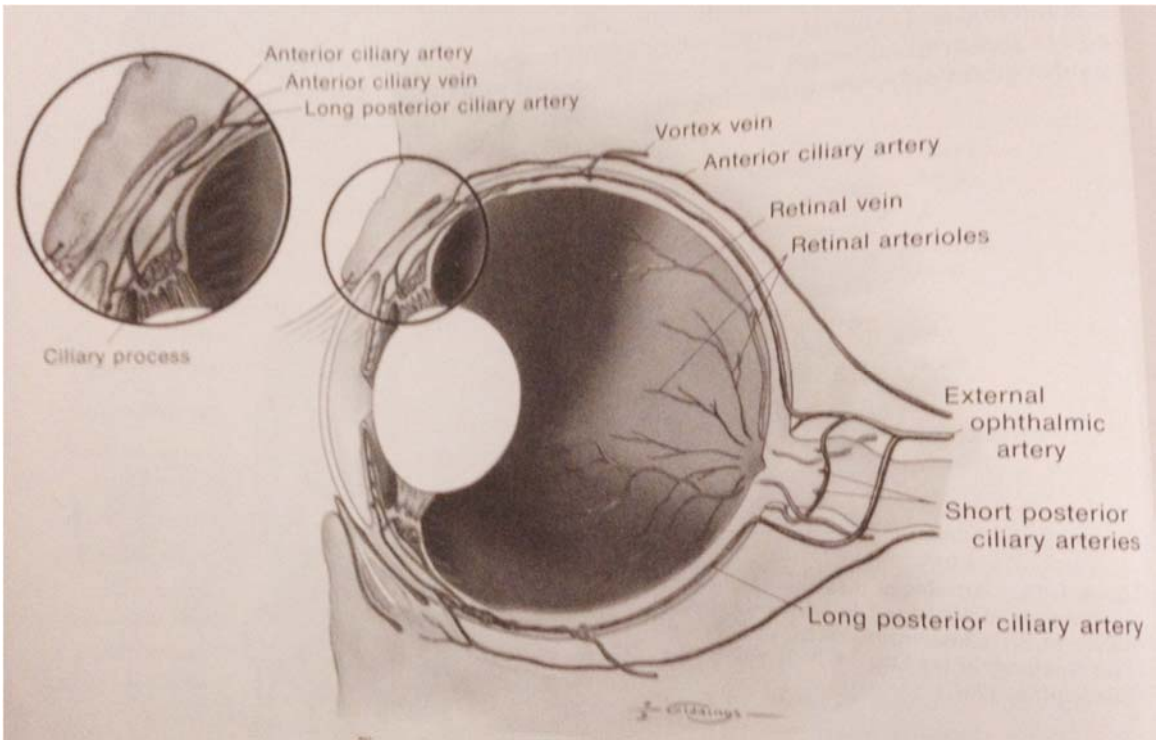
APPENDIX C

THE PUPIL MODEL OF A NORTHERN ELEPHANT SEAL, HARBOR SEAL, AND CALIFORNIA SEA LION[22]



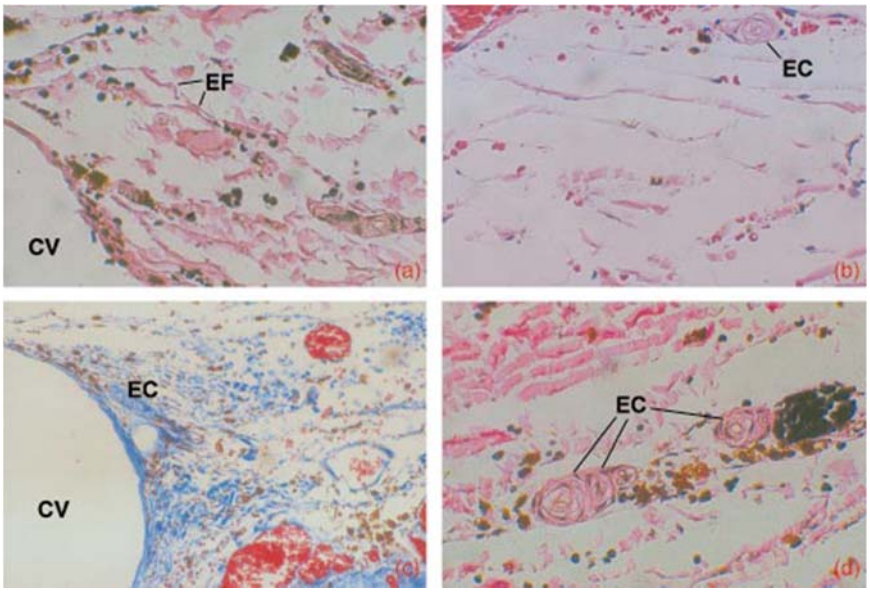
APPENDIX D

OCULAR VASCULATURE OF THE CANINE EYE[10]



APPENDIX E

ENCAPSULATED CORPUSCLE OF THE MARINE MAMMAL



a) Posterior uveal trabecular meshwork (UTM) of the short-finned pilot whale. Elastin fibers are more plentiful in the posterior UTM. CV: choroidal vessel. Elastin stain, Å~400. (b) Anterior coneoscleral trabecular meshwork) CSTM of the whale ICA. Encapsulated corpuscles within the CSTM are often found in close proximity to blood vessels. EC: encapsulated corpuscle. H&E, Å~400. (c) Posterior portion of the pilot whale ICA, including large choroidal vessels and encapsulated corpuscles. Trichrome stain, Å~200. (d) Outer CSTM of the pilot whale ICA, with encapsulated corpuscles. Elastin stain, Å~400. Courtesy and permission from author Don Samuelson.[58]

APPENDIX F

CLASSIFICATION OF GLAUCOMA, OPEN AND CLOSED ANGLE GLAUCOMA[10]

Open Angle Glaucoma: Normal, wide angle on gonioscopy

Primary no observable predisposing factors, angle normal on gonioscopy, bilateral, breed predisposition.

Secondary normal angle obstructed by aqueous contents or elevated episcleral venous pressure interferes with aqueous drainage:

- Inflammation-White blood count and fibrin obstruct outflow;
- Hyphema-Erythrocytes and fibrin obstruct flow;
- Pigment-Deposition or proliferation obstructs outflow;
- Lipids in anterior chamber;
- Anterior luxated lens-may obstruct angle or create pupil block;
- Elevated episcleral venous pressure-Arteriovenous fistula, orbital lesion, or increased blood pressure (rare).

Closed-angle glaucoma: Angle is collapsed or covered with peripheral iris or connective tissue

Primary:

Congenital Goniodysgenesis-maldeveloped angle covered with mesodermal tissue; usually bilateral; age of glaucoma onset varies.

Acquired closure associated with abnormal anterior chamber conformation.

- Forward displacement of lens presumably due to slack zonules; creates a relative pupillary blockage from increased adhesive forces between lens and iris.
- Shallow anterior chamber with small anterior segment; pupillary block may occur that results in peripheral anterior synechiae.
- Plateau iris- iris plane is flat, but peripheral iris has a recess adjacent to angle, which is susceptible to angle closure with pipillary blockage.

Secondary acquired lesions precipitate closure of previously normal angle

Associated with pupillary block:

- Intumescent lens;
- Posterior synechiae, iris bombe;
- Subluxated lens, luxated lens;
- Aphakic vitreous herniation;
- Increased volume in vitreous compartment, i.e., accumulation of vitreous, swelling of vitreous.

No pupillary block:

- Neoplasia with invasion of angle and/or pushing iris forward or thickening of iris;
- Inflammation with peripheral anterior synechiae;
- Subluxated lens pushing iris base forward;
- Epithelial downgrowth-perforating corneal wound with epithelium proliferating over angle.

APPENDIX G

CLINICAL SIGNS OF GLAUCOMA[10]

Clinical Signs of Glaucoma

Early	Moderate of Subacute	Advanced or Chronic
Pain	Pain	
Corneal Edema	Corneal Edema	Pain(variable)
Blepharospasm	Blepharospasm	Corneal vacularization and pigmentation
Cataract	Blindness	Lack of corneal aphakic crescent
Anorexia	Leukokoria	Lens opacity
Depression	Anorexia	Anorexia
Fixed, Dilated Pupil	Depression	Depression, timidity, or aggression
Episcleral Engorgement	Fixed, Dilated Pupil	Fixed, Dilated Pupil
(with conjunctival erythema)	Episcleral Engorgement	Episcleral Engorgement
Increased Intraocular Pressures	(with conjunctival erythema)	Increased Intraocular Pressures
Shallow anterior chamber	Increased Intraocular Pressures	Shallow anterior chamber
Impaired vision	Shallow anterior chamber	Impaired vision
Lens luxation(variable)	Impaired vision	Lens luxation(variable)
Direct pupillary reflex abolished	Lens luxation(variable)	Direct papillary reflex abolished
	Direct pupillary reflex abolished	Descemet's streaks (variable)
	Descemet's streaks (variable)	Iris atrophy
		Retinal and optic atrophy