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In Sickness and in Health: Parasites of Stranded Pacific Harbor Seals (*Phoca vitulina richardii*) in Northern Oregon and Southern Washington

Cecily Douglas Bronson
Portland State University

In Sickness and in Health: Parasites of Stranded Pacific Harbor Seals (*Phoca vitulina richardii*) in Northern Oregon and Southern Washington

by

Cecily Douglas Bronson

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Thesis Committee:
Deborah Duffield, Chair
Annie Lindgren
Erin Shortlidge

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Abstract

Parasites have the capability to infect virtually every living organism on the planet and have adapted to infiltrate every trophic level. Many species have complex indirect life cycles and rely upon hosts at different levels of the food web for growth and reproduction. In the marine environment, having a high level of parasite diversity is thought to indicate a more stable ecosystem than an environment with low parasite diversity. As one of the top predators in their environment and because of their amphibious behaviors, pinnipeds (seals and sea lions) are exposed to a wide variety of parasites, making them ideal for parasite research. One of the most common and widely distributed pinnipeds is the harbor seal, *Phoca vitulina.* While parasitic infections are common in harbor seals, they are often overlooked unless they have a direct impact on human health or the fisheries industry.

Although there have been recent studies conducted on the parasites of Pacific harbor seals, *P. vitulina richardii*, along the coasts of California, Washington, and Alaska, there have been no reports for Oregon since the 1970's. Earlier studies in Oregon looked at parasite presence and diversity, but lacked any in-depth analyses on parasite prevalence with host characteristics like sex, age, health status, season, or over time. The Northern Oregon/Southern Washington Marine Mammal Stranding Program (NOSWSP; Portland State University, Department of Biology) responds to stranded marine mammals from Tillamook, OR through Long Beach, WA. These are routinely necropsied and all are examined for parasites. Pacific harbor seals are one of the most commonly stranded

pinnipeds in the NOSWSP area. We examined and collected parasites from 53 stranded Pacific harbor seals between the years of 2018-2019.

Parasites were collected from the heart, lungs, stomach, and intestines of each seal and found in 51 of the 53 processed seals (96% overall parasite prevalence). Nematodes were found in 43 seals (81% prevalence) and in each organ examined (heart, lungs, stomach, and intestines). The nematodes from the stomach (72% prevalence) were all from the family Anisakidae. Nematodes from the heart (21% prevalence) were from the family Onchocercidae, and strongly suspected to be *Acanthocheilonema spirocauda.* Nematodes from the lungs (28% prevalence) were from the order Strongylida with the possibility of being either *Parafilaroides sps.* or *Otostrongylus circumlitus.* Cestodes were found only in the intestines and in a total of 4 seals (8% prevalence) and were most likely from the family Diphyllobothriidae. Acanthocephalans, all from the genus *Corynosoma*, were also found in the intestines of 50 seals (94% prevalence) and were the most frequent parasites.

The aim of this work was to: 1) update the diversity of parasites in stranded Pacific harbor seals along the coast of Northern Oregon and Southern Washington, and 2) evaluate potential correlations between parasite diversity, prevalence, and intensity with host sex, age, health status, season, and stranding year.

Parasites in the lungs were found to have significantly higher rates of prevalence in yearlings when compared to other age classes ($p<0.001$), and had a significant relationship to season ($p<0.01$) with winter having the highest prevalence (100%). No other parasite had any significant findings with host sex, age, health status, season, or

year. However, we did observe that compromised seal hosts had a higher prevalence of parasites in the heart (31%) than healthy hosts (8%) and that the stomach and intestines had consistently high parasite prevalence regardless of sex, age, health status, season, or year suggesting that the intermediate hosts for these parasites are present year-round.

The intestines of 50 seals were used for parasite intensity analyses against host sex, age, health status, season, and year. The acanthocephalan, *Corynosoma sp.*, was the most dominant parasite in the intestines with a prevalence of 96% (48/50) and an intensity range of 0-851 parasites per host. While *Corynosoma* was the dominant genus and used for all analyses, *C. strumosum* was also found in this study. Our findings indicate that there is a significant relationship between *Corynosoma sp.* intensity and host age, with the pups having significantly lower intensities than the subadults and adults ($p<0.001$). This was also supported by a strong positive correlation between *Corynosoma sp.* intensity and host body length ($p<0.001$). No other variables were found to have statistically significant relationships with parasite intensity. The distribution of *Corynosoma sp.* along the intestinal tract was evaluated and we found that the colon had statistically lower intensity than any other section of the intestines ($p<0.001$), and that the second section (10m anterior to the colon) had significantly higher intensities than the intestinal section which was the closest to the stomach ($p<0.05$). This suggests that *Corynosoma sp.*, while inhabiting the length of the small intestine, may prefer the microhabitat found in the posterior section of the intestines.

This study demonstrated that the parasites of stranded Pacific harbor seals are common and consistently present in our area. Efforts should be made to continue

monitoring their diversity and prevalence, using them as bioindicators to assess any potential changes to the marine ecosystem. As a result of this work, a parasitology CURE was developed and implemented at Westfield State University in Westfield, Massachusetts. Undergraduate students used the *Corynosoma sp.* specimens collected in this study to conduct a variety of research projects including trophic web analyses, heavy metal analyses, and confirmed species identification of *Corynosoma strumosum* through DNA sequencing. This was a very exciting opportunity to involve undergraduates in real research experiences, proving that these parasites are a rich resource of experimental data.

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Table of Contents

Abstract……i

Acknowledgements……...……...v

List of Tables……..……. viii

List of Figures……..……..… ix

Chapter One
The Natural History of the Pacific Northwest Harbor Seal, *Phoca vitulina richardii*, and its Parasites…….. 1

Chapter Two
Parasites of Stranded Pacific Harbor Seals, *Phoca vitulina richardii*, Along the Northern Oregon and Southern Washington Coasts from 2018-2019……..… 13

Chapter Three
Acanthocephalans of the Pacific Harbor Seal, *Phoca vitulina richardii*……...……... 29

Chapter Four
Summary and Broader Impacts…… 45

References…… 71

Appendix A. Pinniped Intestinal Tract Analysis Form …… 79

Appendix B. Staining Protocol…..…….. 81

Appendix C. Parasite Taxonomy List……... 84

Appendix D. Stranded Pacific Harbor Seal Data……..…… 86

List of Tables

Chapter One
Table 1.1 Pacific Harbor Seal Strandings and Necropsies……..……..... 53

Chapter Two
Table 2.1. Parasite Diversity and Prevalence by Anatomical Location…… 53

Table 2.2. Prevalence of Parasites by Host Sex…… 53

Table 2.3. Prevalence of Parasites by Host Age……... 53

Table 2.4. Prevalence of Parasites by Host Health…….…….. 54

Table 2.5. Prevalence of Parasites by Season …….……. 54

Table 2.6. Prevalence of Parasites by Year…….……. 54

Chapter Three
Table 3.1. Acanthocephalan Intensity Summary Table…… 55

Table 3.2. Post-hoc Analysis for Host Age and Acanthocephalan Intensity…… 55

Table 3.3. Intestinal Distribution of Acanthocephalans Summary Table……. 56

Table 3.4. Post-hoc Analysis for Host Age and Acanthocephalan Intensity…… 56

List of Figures

Chapter One

Figure 1.1. Pacific harbor seals fully processed for parasites..…… 57

Figure 1.2. Stranded Pacific harbor seals……..…… 58

Chapter Two

Figure 2.1: Cestode specimens from the intestines...…… 59

Figure 2.2: Nematode specimens from the stomach.…… 59

Figure 2.3: Acanthocephalan from the intestine……60

Figure 2.4: Processed Pacific harbor seals by sex and age 2018-2019……. 60

Figure 2.5: Processed Pacific harbor seals by season 2018-2019…….. 61

Chapter Three

Figure 3.1: Proboscis of stained Corynosoma specimen magnified 20x……..…… 62

Figure 3.2: Intestinal parasite prevalence and polyparasitism by year……. 63

Figure 3.3: Acanthocephalan intensity and host health status.……. 64

Figure 3.4: ANOVA boxplot of acanthocephalan intensity and host age class…… 65

Figure 3.5: Boxplot of acanthocephalan intensity and year……..…… 66

Figure 3.6: Linear regression of body length and acanthocephalan intensity……..…….. 67

Figure 3.7: Linear regression of body length and intestinal length……. 68

Figure 3.8: Distribution of acanthocephalan intensity along the intestinal tract…….…. 69

Figure 3.9: ANOVA of the distribution of acanthocephalan intensity throughout the intestinal tract……70

Chapter One: The Natural History of the Pacific Northwest Harbor Seal, *Phoca vitulina richardii*, and its Parasites

Introduction

Harbor seals, *Phoca vitulina*, are an extremely common and charismatic marine mammal found all around the world. They are one of the most well-studied pinnipeds because of their widespread coastal distribution and high site fidelity (Shaughnessy and Fay 1977, Teilmann and Galatius 2018). Because of their abundance, high position in the trophic web, coastal habitat, site fidelity, and relatively long lifespan, they make excellent study organisms for a wide variety of research topics including parasitology. Harbor seals belong to the family Phocidae and are commonly called true or earless seals as they lack external pinnae unlike sea lions and fur seals which belong to the Otariidae family and are known as eared seals. Previously, harbor seals consisted of five subspecies defined by geographical range: Eastern Pacific harbor seal (*P. vitulina richardii*), Western Pacific harbor seal (*P. vitulina stejnegeri*), Eastern Atlantic harbor seal (*P. vitulina vitulina*), Western Atlantic harbor seal (*P. vitulina concolor*), and the Ungava harbor seal (*P. vitulina mellonae*). However, recent genetic analyses (Westlake and O'Corry-Crowe 2002, Berta and Churchill 2012) suggests just three subspecies: Pacific harbor seal (*P. vitulina richardii*), Atlantic harbor seal (*P. vitulina vitulina*), and the Ungava harbor seal (*P. vitulina mellonae*). The Pacific harbor seal, *P. vitulina richardii*, was the focus of this thesis due to the location of this study.

Pacific harbor seals have a range from the west coast of North America to Japan. Along the west coast of North America, they occur as far north as the Aleutian Islands in Alaska and as far south as Baja, California (Orr et al. 2018). While they typically stick

closer to the shore, there have been reports of individuals traveling over 100 miles both out to sea and inland through sounds and rivers (Peterson et al. 2012). For management and monitoring purposes, Pacific harbor seals are divided into stocks. The west coast of the United States is made up of 15 stocks, 12 in Alaska, two in Oregon and Washington, and one in California (NOAA). Washington and Oregon stocks are divided into a coastal and an inland water stock, the Oregon and Washington Coastal Waters Stock, and the Washington Inland Waters Stock (Jefferies et al. 2003). The harbor seals discussed in this work are from the Oregon and Washington Coastal Waters stock.

During the early 1900's, state sanctioned hunting activities severely reduced the population of Pacific harbor seals. In some cases, the estimates for the Oregon and Washington Coastal stock were just a few hundred individuals (Pearson and Verts 1970). The seals were seen as a significant threat to the fisheries industry and were routinely hunted in an effort to protect fish populations. However, since the enactment of the Marine Mammal Protection Act in 1972, Pacific harbor seal populations have fully recovered and the populations in both Oregon and Washington are believed to be at carrying capacity with an estimated size of 40,000 individuals (Jefferies et al. 2003, Brown et al. 2005). Pacific harbor seals have a lifespan of approximately 25 to 35 years (Reeves et al. 2002). The causes of death of Pacific harbor seals range from natural causes like shark attacks and bacterial infections, to human caused deaths due to fisheries interactions and gunshot wounds (Stroud and Roffe 1979, Duffield pers. comm. 2022).

Unlike some pinnipeds, Pacific harbor seals exhibit high site fidelity (Shaughnessy and Fay 1977, Teilmann and Galatius 2018), and typically do not venture

too far for food. They utilize the continental shelf off the coast of Oregon and Washington to hunt and have a highly varied diet consisting of crustaceans, demersal fish, pelagic schooling fish, octopus, and squid (Reeves et al. 2002, Teilmann and Galatius 2018). When foraging for food at sea, the seals are solitary. However, when hauled out during molting and pupping seasons, they can occur in larger groups (Reeves et al. 2002, Teilmann and Galatius 2018), but they are still not nearly as gregarious as other pinnipeds, preferring to keep a distance from one another (except for mothers and pups). Breeding occurs shortly after pups are weaned in the spring and summer and females exhibit delayed implantation (Reeves et al. 2002).

Parasites of Pacific Harbor Seals

With their high placement in the trophic web, pinnipeds in general are excellent bioaccumulators of a wide variety of parasites (Raga et al. 2009). Despite a world-wide distribution and overwhelming abundance across trophic levels, the field of parasitology is relatively small, and unless it is directly tied to human health, an understudied science. Due to their amphibious nature and their broad diet, Pacific harbor seals expose themselves to a wider range of parasites (Leidenberger et al. 2007), making them a particularly interesting species to study. The most commonly found parasites in Pacific harbor seals are helminths consisting of nematodes (roundworms), cestodes (tapeworms), and acanthocephalans (thorny-headed worms) (Stroud and Dailey 1978, Dailey and Fallace 1989). Nematodes are found throughout the body including the heart, lungs, and stomach, whereas cestodes and acanthocephalans are typically found in the intestines.

The nematode typically found in the heart is *Acanthocheilonema spirocauda* and is believed to use the chewing louse, *Echinophthirius horridus* as a vector. The exact life cycle of *A. spirocauda* is still unknown, but transmission most likely occurs when the seals are hauled out and the lice passes from mothers to pups or to other individuals during physical contact (Leidenberger et al. 2007). *Acanthocheilonema spirocauda* has routinely been found in Pacific harbor seals (Stroud and Dailey 1978, Eley 1981, Dailey and Fallace 1989, Leidenberger et al. 2007) and if the heartworm infection is severe enough it can be fatal (Taylor et al. 1961). Younger animals seem to be more affected (Dunn and Wolke, 1976, Lunneryd, 1992), with the prevalence of the heartworm decreasing with age (Dunn and Wolke 1976, Borgsteede et al. 1991, Claussen et al. 1991, Lunneryd 1992), suggesting that the animals either succumb to or overcome their infections. Another species that has been found in hearts of harbor seals in Portugal, is the canine heartworm *Dirofilaria immitis* which uses a mosquito as a vector (Alho et al. 2017).

There have been three main species of parasites reported in the lungs: *Otostrongylus circumlitus* (Dailey and Fallace 1989, Gerber et al. 1993, Gulland et al. 1997, Elson-Riggins et al. 2001, Colegrove et al.2005, and Colón-Llavina et al. 2019); *Parafilaroides sps.* (Herreman et al. 2011, Rhyan et al. 2018); and *Parafilaroides gullandae* (Dailey 2006). The life cycle of *Parafilaroides sps.* and *O. circumlitus* are unknown. However, the lungworm of the California sea lion, *P. decorus,* is closely related to the *Parafilaroides* species that infect Pacific harbor seals, and it is believed that the first stage larvae of this species are eaten by a fish, such as the opaleye, as the

intermediate host before it can infect another sea lion (Dailey 1970). Once in the gastrointestinal tract of the definitive host, the lungworms burrow out of the stomach or intestines and migrate up into the lungs through an unknown pathway, where they mature and reproduce (Rhyan et al. 2018). While in the lungs, the pregnant female parasites release the first stage larvae into the bronchi of the definitive host. From here, the first stage larvae are coughed up and spit out or swallowed by the definitive host and released out into the ocean where the life cycle begins again (Dailey 1970). Similar to the heartworm, severe infections of lungworm have been known to cause fatalities in Pacific harbor seals (Stroud and Dailey 1978, Gulland et al. 1997) and are typically found in younger animals (Elson-Riggins et al. 2001).

Nematodes regularly found in the stomach of Pacific harbor seals belong to the family Anisakidae (Dailey and Fallace 1989). These parasites have a complex indirect life cycle that requires multiple hosts for the parasites to grow and mature before reaching their definitive host. Parasites with this kind of life cycle progressively move up the trophic web as they make their way through intermediate hosts to reach their definitive or final host which is often near or at the top of the trophic web. These anisakid nematodes begin as free-living larvae and enter the food web by being consumed by a crustacean as their first intermediate host, then to a fish like the Pacific Herring as a second intermediate host (Stroud and Dailey 1978), and finally entering the seals as their definitive host where they fully mature and reproduce (Mattiucci and Nascetti 2007). Some of the fish used as intermediate hosts are of commercial value and readily eaten by people who enjoy sushi and seafood including sardines, anchovies, and bluefin tuna

(Mladineo and Poljak 2014). This poses a potential human health concern as these parasites have been known to accidentally end up in humans causing anisakiasis (Mladineo and Poljak 2014), a painful gastrointestinal disease that requires medical attention and can vary in severity (Audicana and Kennedy 2008). Pacifc harbor seals have been reported to have a number of species from the anisakid family including; *Anisakis sp.* (Stroud and Dailey 1978, Herreman et al. 2011, Gerber et al. 1993), *Anisakis simplex* (Stroud and Roffe 1979, Dailey and Fallace 1989), *Contracaecum sp.* (Stroud and Roffe 1979, Gerber et al. 1993, Herreman et al. 2011, Colón-Llavina et al. 2019), *Contracaecum osculatum* (Margolis 1956, Stroud and Dailey 1978, Stroud and Roffe 1979, Dailey and Fallace 1989), *Pseudoterranova decipiens* (Margolis 1956, Stroud and Dailey 1978, Dailey and Fallace 1989, Herreman et al. 2011, Colón-Llavina et al. 2019). Once in their definitive host, the seal, they attach to the lining of the stomach and if present in high enough numbers can cause large ulcerations, perforations, and even death (Stroud and Dailey 1978, Stroud and Rouffe 1979). While in the stomach, the nematodes are able to reproduce and their eggs pass through the feces of the seal and back out into the ocean.

Cestodes have also been found in Pacific harbor seals, specifically in the gastrointestinal tract. These include; *Anophryocephalus sp.* (Herreman et al. 2011), *Diphyllobothrium sp.* (Gerber et al. 1993, Herreman et al. 2011), *Diphyllobothrium alascense* (Dailey and Fallace 1989), and *Diplogonoporus sp.* (Herreman et al. 2011). The latter species is no longer an accepted name, but is now considered synonymous with *Diphyllobothrium sp.* While the exact lifecycles of these cestodes are still unknown, it

has been assumed that the first intermediate host is a copepod, the second intermediate host is a fish, with seals being the final hosts (Hernandez-Orts et al. 2015, Kuzmina et al. 2015). Humans may also become infected by eating poorly prepared or raw fish, like the fine flounder (Kutcha et al. 2015), that are infected with *Diphyllobothrium sp.* Although *Adenocephalus pacificum (*formally *Diphyllobothrium pacificum*) which causes the most human infections, has not been reported in Pacific harbor seals (Hernandez-Orts et al. 2015).

Acanthocephalans, specifically from the genus *Corynosoma* are routinely found in the intestines of Pacific harbor seals. They are commonly called "thorny-headed" worms because the anterior body trunk and proboscis are covered in hooks or spines. Parasites in the genus *Corynosoma* also have hooks on the posterior body and a more hooded anterior body than other acanthocephalans (Van Cleave 1923, Van Cleave 1953a, Van Cleave 1953b, Dailey and Gilmartin 1980). Identification to species using morphological characteristics is challenging because it relies of the number of hooks and rows of hooks found on the proboscis (Van Cleave 1923, Van Cleave 1953a, Van Cleave 1953b, Dailey and Gilmartin 1980, Waindok et al. 2018) and there is morphological plasticity in the number of hooks and hook rows among individuals from the same acanthocephalan species (Aznar et al. 2016, Waindok et al. 2018). Pacific harbor seals have historically been found to be infected with *Corynosoma sp.* (Herreman et al. 2011), *C. strumosum* (Margolis 1956, Stroud and Dailey 1978, Dailey and Fallace 1989), *C. semerme* (Margolis and Dailey 1972, Stroud and Dailey 1978, Dailey and Fallace 1989, Herreman et al. 2011), and *C. cameroni* (Kuzmina et al. 2012). The first intermediate host of

Corynosoma sp. is believed to be an amphipod, which is then eaten by a fish or crustacean. It has been reported that *Corynosoma sp.* and *C. strumosum* can infect approximately 20 different species of fish including species like the sawtooth flounder, Arctic cod, Pacific herring, and Pacific halibut (Moles 2007, Kuzmina et al. 2012, Lisitsyna et al. 2018) and that they may prefer intermediate hosts that reside in cooler waters (Dailey and Fallace 1989). They also may move through multiple intermediate hosts before ending up in the Pacific harbor seal, their definitive host, where they make their way into the small intestine. There they use their hook covered proboscis to attach to the mucosal lining of the intestinal wall and passively absorb nutrients through their cuticle as they have no mouths or gastrointestinal tract and reproduce. Their eggs are carried out with the feces of the host and released into the ocean where the lifecycle begins again. It is suggested that these parasites may be relatively long lived (Fujita et al. 2016), but still unknown. Typically, these parasites do not cause the host much harm, but if the intensity of the infection is too high some species may perforate the intestinal lining (Waindok et al. 2018).

Understanding how parasites like these contribute to and interact within ecosystems, as well as in individual hosts and host populations, can reveal a wealth of information about the health and stability of their environment. Research efforts outside of human health or economically important fisheries species generally neglect the parasites and their complex role in ecosystems. This creates a large gap in knowledge concerning how parasites may be affecting various marine vertebrate species and how their prevalence may influence or reflect the health of an entire ecosystem. Parasites can

be effectively used as biological tags to indicate ecosystem health and biological diversity due to their complex and tropically linked life cycles (Horwitz and Wilcox 2005, Marcogliese 2005, Hudson et al. 2006, Sures et al. 2017a). For example, the presence of an adult parasite in their definitive host, like the Pacific harbor seal, indicates that the ecosystem was stable enough at each trophic level to allow that parasite to find all required intermediate hosts in order to mature and be able to infect the seal. Furthermore, the level of genetic diversity and total load of anisakid nematodes found in pinnipeds were confirmed to be good indicators of marine food web stability and overall biodiversity of the marine ecosystem in which their definitive hosts reside (Mattiucci and Nascetti 2007). In addition to indicating trophic stability, some parasites have been shown to be reliable bioindicators of environmental pollutants, like heavy metals and pesticides (Sures and Siddall 1999, Sures et al. 1999, Sures 2004, Nachev and Sures 2016, Sures et al. 2017b). Specifically, acanthocephalans have been shown to act as potential lead sinks for their hosts (Sures and Siddall 1999). Similar results have been found in pinnipeds with various species of acanthocephalans having higher concentrations of mercury than surrounding host tissues (McGrew et al. 2018). Therefore, understanding and continuing to study the diversity of parasites of sentinel species like marine mammals provides critical insight into the status of the changing marine ecosystem. This work contributes to updating the existing knowledge of parasite diversity found in stranded Pacific harbor seals in Chapter Two.

Marine Mammal Health and Stranding Response Program

Stranded dead marine mammals are excellent specimens to use for parasite research as they are routinely examined for pathologies and cause of death under the Marine Mammal Health and Stranding Program that is coordinated by the National Oceanic and Atmospheric Administration (NOAA). The Northern Oregon/Southern Washington Marine Mammal Stranding Program (NOSWSP) operates out of Portland State University (PSU Biology, Duffield) and responds to marine mammals that strand on the coast between Tillamook, OR and Long Beach, WA. The objective of the NOSWSP is to determine the cause of death of marine mammals and to document human interactions (i.e. boat strikes, bullet wounds, fisheries interactions, etc.). They respond to an average of 160 dead stranded mammals each year (Duffield et al. 2020). Marine mammals that strand on the coast are assessed and necropsied either in the field or in the Duffield lab at PSU. The vast majority are pinnipeds of which a good proportion are Pacific harbor seals. For each necropsy there are detailed measurements, photographs, tissue collection, and records of trauma or evidence of disease. Tissue samples, including grossly observed parasites are collected, frozen, and stored. Fresh tissue samples are sent for histopathological analysis. This extensive necropsy database of marine mammals serves as the primary source of data for Chapters Two and Three, and is critical to providing insight into the diversity and intensity of parasites found in this marine ecosystem.

Due to an unusual mass mortality event in 2018 that caused 94 harbor seals to strand in the NOSWSP area, this thesis focuses specifically on the parasites of stranded

Pacific harbor seals during the years of 2018 and 2019. These two years provided a unique opportunity to assess parasitic infections of harbor seals during the mass mortality event of 2018 compared with a more typical year like 2019 (Table 1.1, Figures 1.1 and 1.2).

Prior to this work, the only known report on the diversity of parasites that infect harbor seals along the Northern Oregon and Southern Washington coasts was conducted in the 1970's by Stroud and Dailey (1978) and Stroud and Roffe (1979). While there have been other reports on the parasites of harbor seals, they have been in other states or areas of the world. Additionally, investigating possible correlations of parasite diversity with a variety of host and environmental characteristics in harbor seals along our coast has never been done. This research project provides an updated evaluation of the diversity of parasites found in stranded Pacific harbor seals as well as a focus on the intestinal parasites and addresses these two gaps in knowledge by adding an evaluation of host health and parasite prevalence.

Specific Objectives

This work updates and builds upon the foundation of knowledge of the parasites available from stranded Pacific harbor seals on the coasts of Northern Oregon and Southern Washington. Using existing data from the marine mammal necropsy database provided by Dr. Duffield of NOSWSP and new data from parasite examination of the intestines of stranded harbor seals from 2018-2019, this work aims to answer two main questions, 1) What is the current parasite diversity of stranded Pacific harbor seals on the Northern Oregon and Southern Washington coasts, and 2) Does the diversity or intensity

of parasites vary depending on host or environmental characteristics? These questions have been broken into three aims:

Aim 1: Establish a new and more current baseline of the parasites that are present in our stranded Pacific harbor seals (Chapter Two).

Aim 2: Analyze patterns of parasite prevalence and diversity with respect to the host characteristics sex, age class, health status, or temporally by seasonality or year (Chapter Two).

Aim 3: Determine if acanthocephalan intensity has any correlations to the host characteristics sex, age class, health status, or temporally by seasonality or year (Chapter Three).

Chapter Two: Parasites of Stranded Pacific Harbor Seals, *Phoca vitulina richardii*, Along the Northern Oregon and Southern Washington Coasts from 2018-2019

Introduction

Pacific harbor seals can be found along the Oregon coast year-round and are one of the top predators in their ecosystem. They are often laden with parasites making them an ideal study organism for parasite research and a descriptive analysis of the parasites found in stranded Pacific harbor seals off the coast of Oregon has not been done since Stroud and Dailey (1978). Although, Stroud and Roffe (1979) reported on the causes of death for stranded marine mammals along the Oregon coast which also included reports of parasitism. These two studies reported solely on the diversity of parasites found in stranded Pacific harbor seals, but neither included any further analysis comparing parasite diversity with host characteristics like sex, age, or the season or year of stranding. Studies conducting these more in-depth analyses have not occurred in Oregon, but they have been done in free-ranging Pacific harbor seals in Washington by Dailey and Fallace (1989), and Herreman et. al (2011). Therefore, the purpose of this chapter was to first update current understanding of the diversity of parasites in stranded Pacific harbor seals along the northern Oregon and southern Washington coasts (Aim 1) and second, to expand upon the previous work and evaluate parasite prevalence with host sex, age, health status, stranding season and year (Aim 2).

Methods

Specimen Acquisition

All Pacific harbor seals used in this study were stranded dead along the Northern Oregon and Southern Washington coasts, between Tillamook, Oregon and Long Beach,

Washington (Figure 1.2), and obtained through the normal operation of the Northern Oregon/Southern Washington Marine Mammal Stranding Program (NOSWSP). For this study, specimen collection covered January 2018 through December 2019. During this time period, an unusual mass mortality event (UME) affected the Pacific harbor seals leading to a large increase in the number of dead seals in 2018 (Figure 1.1). The cause of this UME has yet to be established (Duffield et al. 2018). Collection continued through 2019 as the UME had concluded and we were interested in evaluating the differences in parasite prevalence between the two years.

As part of normal NOSWSP operating procedures, when a seal was reported as stranded, it was photographed, given a unique identification number, and a GPS coordinate was taken at or near the stranding site. The identification number included the month, day, and year the animal stranded, and these data were used to determine the proper season for the seasonality analyses. The seasons were assigned as follows: Spring (March–May), Summer (June–August), Fall (September–November), and Winter (December–February). Either on the beach or in the necropsy lab, a full standardized necropsy was performed on each seal. External examination included photographs, documentation of signs of trauma, illness, scavenging, and detailed body measurements. Sex and reproductive status were determined by external and internal anatomical characteristics. Age was determined by the body length and reproductive status of the individual and categorized into one of the four age classes required by the National Oceanic and Atmospheric Administration (NOAA) for level A reporting: pup, yearling, subadult, and adult. Each organ was photographed, measured, weighed, and sampled. If

the specimen was fresh enough (code 2 or 3), tissue samples were placed in 10% formalin and sent to the Oregon State University Veterinary Pathology Laboratory for histopathological analysis. Body condition was assessed by blubber thickness. Hosts were classified as “healthy”, “compromised”, or “suspected ill” after thorough evaluation of necropsy records and available histology reports. “Healthy” hosts were animals that had no discernable chronic pathologies, although they could have acute signs of illness or presence of non-pathogenic parasitic infection. “Compromised” hosts were those that had suspected or confirmed chronic illnesses or parasitic infections associated with pathologies. Unique to 2018, some seals had distinctly yellow-tinged blubber believed to be associated with the UME and therefore these animals were classified as “compromised”. “Suspected ill” was used to classify seals that did not appear healthy, but had no concrete evidence of chronic illness. Since all specimens were stranded dead, no Institutional Animal Care and Use protocols were needed. All work was conducted under NOAA Permit 18786-06.

Parasite Collection

As part of normal necropsy procedures, the examination for the presence of parasites in the heart, lungs, and stomach were conducted. This study expanded parasite collection to also include the intestines. The heart and lungs were carefully dissected, paying close attention to the bronchi and pulmonary artery, to find and collect any grossly observable parasites. The stomach was removed with the esophagus intact and the contents were washed and passed through a sieve to collect any parasites or bones. Parasites embedded in the stomach were recorded as well as any ulcerations. The

intestines were removed and examined as a whole with careful documentation of their color, texture, whether there was twisting (a sign of trauma), or any possible obstructions or foreign bodies. Contents of the small intestines were passed through a sieve to collect grossly visible parasites followed by a visual examination of the interior to collect attached parasites. The large intestine was analyzed in the same manner as the small intestines and feces were collected if present. All parasites were collected carefully by hand, cleaned in tap water, separated by general morphological characteristics and anatomical location, and then frozen in water for future identification. Microscopic parasites were not addressed in this study.

Parasite Identification

Parasites of the heart, lungs, and stomach were primarily identified by necropsy descriptions, anatomical location, and reference to previous studies (as noted in Discussion). Parasites collected from the intestines were identified to phylum using general morphological characteristics (body shape, flatness, and the presence or absence of body segments). Cestodes were confirmed by having flat and segmented bodies (Figure 2.1.a & 2.1b). Nematodes were all round bodied, with varying lengths and thicknesses (Figure 2.2a & 2.2b). Acanthocephalans were very distinct with a short bulbous body, and spine covered anterior and posterior ends (Figure 2.3a & 2.3b). In one case, a histopathology report by the Oregon State University Veterinary Pathology Lab, provided a preliminary identification of lung and heart parasites to family and genus. Classification to the lowest level of taxonomy was completed using a dissection and/or

compound microscope, the marine mammal identification key developed by Dailey & Gilmartin (1980), and previous research findings.

Statistical Analyses

All statistical analyses were done in RStudio (version 1.4.1717). Chi-square analysis was used to correlate prevalence of parasite type (determined by anatomical location) with host sex, age, health status, season, and year collected. Findings were considered significant when $p<0.05$. To prevent small sample sizes, statistical analyses were conducted on pooled data from both years for prevalence of parasite type with the exception of comparing overall parasite prevalence between the two years. All other findings are reported as prevalence percentages with no statistical interpretation.

Results

During the collection period of 2018-2019, a total of 134 Pacific harbor seals stranded along the territory of the NOSWP. Of the 134 seals, 53 were suitable for necropsies and intestinal examination for parasites (Table 1.1). The UME of 2018 lead to 94 stranded seals, of which 36 were necropsied and examined for parasites. Of the 36 seals necropsied, the distribution of sex was equal (18 female, 18 male), and there were 7 pups, 2 yearlings, 9 subadults, 18 adults (Figure 2.4). There were 27 healthy, 7 compromised, and 2 suspected ill. Seasonally, 1 was from the spring, 23 from the summer, 11 from fall, and 1 from the winter (Figure 2.5). For 2019, there were a total of 40 reported stranded seals, of which 17 were necropsied and examined for parasites and used in this study. The distribution of sex was not equal (10 female, 7 male), and there were 4 pups, 6 yearlings, 2 subadults, 5 adults (Figure 2.5). There were 5 healthy, 12 compromised, and none

suspected ill. Seasonally, 3 were from the spring, 5 from the summer, 7 from the fall, and 2 from the winter (Figure 2.5).

Parasite Prevalence, Diversity, and Anatomical Location

Parasites were found in 51 of the 53 processed seals (96% overall parasite prevalence). The two seals with no parasites were both pups, one female from the summer of 2018 and one male from the summer of 2019. Parasites were found in all four of the organs examined; heart, lungs, stomach, and intestines.

Nematodes were found in 43 seals (81% prevalence) and in each organ examined (heart, lungs, stomach, and intestines). The nematodes from the stomach were all from the family Anisakidae and in 38 seals (72% prevalence). Nematodes from the family Onchocercidae were found in the hearts of 11 seals (21% prevalence) and strongly suspected to be *Acanthocheilonema spirocauda,* or possibly *Dirofilaria immitis* as was suspected in a histopathology report, however this was not confirmed. Nematodes were found in the lungs of 15 seals (28% prevalence) and belonged to order Strongylida with the possibility of being either Filaroididae (*Parafilaroides sps.)* or Crenosomatidae (*Otostrongylus circumlitus)* according to a histopathology report. Cestodes were found exclusively in the intestines and in 4 seals (8% prevalence) and were most likely from the family Diphyllobothriidae. Acanthocephalans were found in the intestines of 50 seals (94% prevalence), and the sole genus identified was *Corynosoma* (see Chapter Three for further analysis and intensity data). See Table 2.1 for a complete summary of parasite diversity and prevalence by anatomical location and suspected taxonomy.

The organ with the greatest prevalence of parasites was the intestine with an overall prevalence of 94% (97% prevalence in 2018 and 88% in 2019). The stomach was the second most infected organ with an overall prevalence of 72% (75% prevalence in 2018 and 65% in 2019). The lungs had an overall prevalence of 28% (19% in 2018 and 47% in 2019). The heart had the lowest overall prevalence rate of 21% (25% in 2018 and 12% in 2019).

Host Sex

In 2018, parasites were found in 17 of the females (94% prevalence), and in all of the males (100% prevalence). In 2019, parasites were found in 9 of the females (90% prevalence), and 6 of the males (86% prevalence). Overall, parasite prevalence in males appeared to be slightly higher for all organs when compared to females, however there was no statistical difference (Table 2.2).

Host Age Class

Parasites were found in all organs for all age classes, but statistically only the yearlings had a significantly higher prevalence of parasites in the lungs ($p<0.001$; Table 2.3). Pups appeared to have the lowest total parasite prevalence of 82% (86% in 2018 and 75% in 2019). Yearlings, subadults, and adults all had 100% total prevalence rates for both years. Parasite prevalence in the heart appear to be highest in younger animals (pups 27% and yearlings 38%), but there was no statistical difference. The stomach and intestines had consistently high parasite prevalence regardless of age class.

Host Health

Hosts from all three health status categories had parasites. Overall, the compromised hosts appeared to have the highest rates of parasite prevalence for all organs (heart 31%, lung 31%, stomach 78%, intestines 97%) compared to healthy hosts (heart 8%, lung 26%, stomach 58%, intestines 89%), however this was not found to be statistically significant (Table 2.4). In 2018, there were 27 compromised hosts (12 female, 15 male), 7 healthy hosts (5 female, 2 male), and 2 suspected ill hosts (1 female and 1 male). In 2019, there were 5 compromised (2 female, 3 male), 12 healthy (8 female, 4 male), and no suspected ill hosts.

Season and Year

For both years, overall parasite prevalence was 100% for spring, fall, and winter. The summer of 2018 had an overall parasite prevalence of 96% and 80% for 2019. Parasites were found in all organs during all seasons for both years. Parasites in the lungs were found to have a significantly higher prevalence in the winter ($p<0.01$) when compared to the other seasons and appear to steadily increase from spring to winter (0% in spring, 18% in summer, 39% in fall, and 100% in winter) as seen in Table 2.5. Parasites in the heart had a similar pattern (21% in spring, 17% in summer, and 67% in fall) but no significance was seen. While parasites were seen in each organ both years, it appeared that parasites in the lungs were more prevalent in 2019, however there was no statistical significance to support this (Table 2.6).

Polyparasitism

Polyparasitism, infection with more than one species of parasite, was noted in a total of 42 seals (84%). There was a slightly higher rate of polyparasitism in 2018 than in 2019 (81% and 76% respectively), however there was no statistical significance between the years. There were also no statistical differences found with host sex, age, health status, or season with polyparasitism.

Discussion

This study found a wide variety of parasites in stranded Pacific harbor seals off the Northern Oregon/Southern Washington coast. The overall prevalence of parasites was high (96%), however this was not abnormal for this area as Stroud and Roffe (1979) reported finding parasites or evidence of parasites in nearly all animals examined excluding the newborn pups. The diversity of parasites found in this study are also in line with previous work by Stroud and Dailey (1978) as well as studies conducted in Washington (Dailey and Fallace 1989, Herreman et al. 2011) and California (Gerber et al. 1993, Colón-Llavina et al. 2019).

The parasites found in the heart are strongly suspected of being *Acanthocheilonema spirocauda* based on morphological observation and previous findings (Stroud and Dailey 1978, Eley 1981, Dailey and Fallace 1989, Leidenberger et al. 2007). However, it is possible that they are *Dirofilaria immitis* (canine heartworm) based on the provided histopathology report and a previous report of this species in pinnipeds in Portugal (Alho et al. 2017). The prevalence of parasites in the heart found in this study (21%) was higher than that seen in other studies of Pacific harbor seals off the Pacific west coast; 11.1%

Stroud and Dailey (1978) and 17.2% Eley (1981), as well as in harbor seals in the Baltic sea (9.3%, Lehnert et al. 2016). However, Dailey and Fallace (1989) reported a prevalence of 47% in Gray's Harbor, Washington. With the life cycle of the heartworm *A. spirocauda* still unknown, it is hard to say exactly why there was such a large difference in prevalence rates between the Gray's Harbor study and the others including ours, but it may be due to the prevalence of the suspected vector the chewing louse, *Echinophthirius horridus.* On the other hand, the difference may be in the degree of exposure to this intermediate host, as Dailey and Fallace (1989) saw their highest prevalence of *A. spirocauda* in the summer which they believed to coincide with the life cycle and transmission of *E. horridus*. No lice were collected in this study, so no correlation with the prevalence of parasites in the heart could be made with this suspected vector. In regards to host age class and prevalence of parasites in the heart, the current study supports previous findings that these parasites are more common in younger animals (Stroud and Dailey 1978, Eley 1981, Dailey and Fallace 1989, Leidenberger et al. 2007). Although this was not statistically significant (Table 2.3), these findings suggest that younger animals may be more susceptible to heartworm infection and that they either overcome or succumb to the infection before adulthood (Leidenberger et al. 2007). Parasites were found in the hearts of stranded Pacific harbor seals regardless of season, sex, or year which is consistent with the work from Dailey and Fallace (1989) in Washington. Host health status was not found to have a statistically significant relationship with the prevalence of parasites in the heart, however, it was more common in the compromised hosts (31%) than the healthy hosts (8%), suggesting that parasites in

the heart could be correlated to other signs of chronic illnesses that were used to classify the health status of our hosts.

Parasites found in the lungs could be: *Otostrongylus circumlitus* according to the histopathology report and previous studies (Dailey and Fallace 1989, Gerber et al. 1993, Elson-Riggins et al. 2001, Colegrove et al. 2005, Colón-Llavina et al. 2019); or *Parafilaroides sps.* (Herreman et al. 2011, Rhyan et al. 2018); and/or *P. gullandae* (Dailey 2006). Parasites in the lungs in this study were found to have significantly higher rates of prevalence in yearlings when compared to other age classes ($p < 0.001$, Table 2.3), consistent with previous studies (Stroud and Dailey 1978, Stroud and Roffe 1979, Dailey and Fallace 1989). However, the prevalence of parasites in the lungs reported here (28%) is generally higher than seen in previous studies: for example, Dailey and Fallace (1989) found just one host with lungworm in their study (1% prevalence); Stroud and Dailey (1978) reported "light infections" of microscopic larviparous worms in the lungs of two Pacific harbor seals (11% prevalence, but did not report the age of the hosts), and Dailey (2006) reported a prevalence of 3%. On the other hand, a study in Glacier Bay and Prince William Sound in Alaska by Herreman et al. (2011) reported higher prevalence of lung parasites for their harbor seals, ranging from 46% to 73% between the two populations. In the current study, parasites in the lungs did have significantly higher prevalence's across seasons, with winter having the highest prevalence of 100% (Table 2.5). This was also reported in California by Gulland et al. (1997). Without knowing the lifecycle of these parasites, it is difficult to make concrete conclusions as to what is driving these differences and relationships. Although, with our overall prevalence of 28%

for parasites in the lungs, we suspect that the intermediate hosts for these parasites are reasonably well adapted to our environment as Dailey and Fallace (1989) concluded. There may be prey items that younger seals ingest more often than adult seals that serve as an intermediate host for these parasites. While fatalities can be caused by parasites in the lungs (Stroud and Dailey 1978, Gulland et al. 1997), in the two years of data collection for the current study, only two animals were reported to have died from parasitic infections in the lungs: a pup from the summer and a yearling from the fall of 2019 (PSU 19-08-18Pv, and 19-09-24Pv).

It should be noted that heartworms and lungworms can be found in both the heart and lungs (Gulland et al. 1997). Not all parasite specimens were available or suitable for identification. This is the reason that we have reported parasite prevalence and statistics based on anatomical location, not by species or suspected species.

The parasites found in the stomach were all nematodes and belonged to the family, Anisakidae, based on necropsy reports and collected specimens (Figure 2.2). Based on previous reports, the stomach nematodes could be one or more of the following species: *Anisakis sp.* (Stroud and Dailey 1978, Stroud and Roffe 1979, Herreman et al. 2011, Gerber et al. 1993), *Anisakis simplex* (Stroud and Roffe 1979, Dailey and Fallace 1989), *Contracaecum sp.* (Stroud and Roffe 1979, Gerber et al. 1993, Herreman et al. 2011, Colón-Llavina et al. 2019), *Contracaecum osculatum* (Margolis 1956, Stroud and Dailey 1978, Stroud and Roffe 1979, Dailey and Fallace 1989), and/or *Pseudoterranova decipiens* (Margolis 1956, Stroud and Dailey 1978, Stroud and Roffe 1979, Dailey and Fallace 1989, Herreman et al. 2011). The prevalence of stomach parasites was

consistently high for all variables in the current study (Tables 2.2-2.6) suggesting that the intermediate hosts (including but not limited to Pacific herring, sardines, and anchovies) for these parasites are commonly found in their diets year-round. The high prevalence of parasites in the stomachs from our study (78%) is also consistent with previous findings (Stroud and Roffe 1979, Dailey and Fallace 1989, Herreman et al. 2011). In the current study, no statistical significance was found when the prevalence of stomach parasites was analyzed against host sex, age, health status, season, or year, although Dailey and Fallace (1989) reported that older animals had higher prevalence rates of both *P. decipiens* and *C. osculatum* than younger animals.

The intestines had the highest diversity of parasites in this study, containing nematodes, cestodes, and acanthocephalans. Similar to the stomach, the intestines had consistently high parasite prevalence rates regardless of host sex, age, health status, season, or year (Tables 2.2-2.6), and no statistical significance was seen between these variables. It should be noted that two of the three animals sampled that did not have intestinal parasites were both small pups and the other was an adult that was missing the vast majority of its intestinal tract due to a shark attack. However, the consistent presence of intestinal parasites in all the remaining hosts suggests that it is very common for Pacific harbor seals to have intestinal parasites as soon as they are weaned and hunting on their own. The nematodes from the intestines are assumed to also be from Anisakidae, like those in the stomach, according to collected specimens, necropsy records, and previous reports on intestinal nematodes (Stroud and Dailey 1978), but it cannot be ruled out that they could possibly be lungworms that had been swallowed by the host.

Cestodes from the intestines were found in a total of 4 seals (8% prevalence). Only two specimens were recovered with intact scolexes; one fit the general morphological description of Diphyllobothriidae (Figure 2.1b), while the other (Figure 2.1a) has yet to be identified. The identified specimen had morphological characteristics consistent with other specimens from Diphyllobothriidae reported previously (Daily and Fallace 1989, Gerber et al. 1993, Herreman et al. 2011). The low prevalence of cestodes from our study (8%) seems to be consistent with other findings, as Dailey and Fallace (1989) found just one cestode in their study (1%) in Gray's Harbor, Washington, Gerber et al. (1993) found cestodes only in one seal (0.5%) in California, and Herreman et al. (2011) found cestodes in 4%-19% of their seals in Washington depending on the study site. Due to the single specimen found by Dailey and Fallace, no deductions could be determined in regards to cestode prevalence with host sex, age, seasonality or year. Gerber et al. (1993) and Herreman et al. (2011) did not evaluate cestode prevalence with host sex, age, season, or year. In this study, the four seals that had cestodes were all male, 3 from 2018 (1 adult from the summer, and a subadult and pup both from the fall) and 1 subadult from the summer of 2019. Their health statuses also varied (two healthy, one compromised, and one suspected ill). As the lifecycle of these cestodes remain unknown, we can only reason that the intermediate hosts for them are either not common in our area, or are not a favored prey item by our Pacific harbor seals.

Acanthocephalans were found in the intestines of 50 seals (94% prevalence, Table 2.1). The sole genus identified was *Corynosoma* based on morphological characteristics (Figure 2.3). The acanthocephalans had the highest prevalence of all parasite types

reported in this work (94%), similar to other studies (Delyamure et al. 1976, Dailey and Stroud 1978, Dailey and Fallace 1989, Herreman et al. 2011) suggesting that these parasites are well adapted to our area and are in regular prey items for the seals. Further identification of acanthocephalans, their prevalence, and intensity in regards to host sex, age, health status, season, and year will be discussed in Chapter Three.

This is the first report of the effect of host sex on parasite prevalence off the coast of Northern Oregon/Southern Washington, and there was no statistical difference seen, which is consistent with previous work in Washington by Dailey and Fallace (1989). While differences in diet have been reported to vary between the sexes of Pacific harbor seals in Washington (Herreman et al. 2011), the current findings suggest that the seals in Northern Oregon/Southern Washington share common dietary preferences regardless of sex, at least when it comes to parasite exposure. These findings also suggest that both sexes of the seals participate equally in the behaviors that may expose them to other parasites, like the heartworm, *A. spirocauda*.

This is also the first study to examine the effects of host health status and parasite prevalence off the Oregon coast. No statistical differences were seen between the health status categories and parasite prevalence regardless of the UME of 2018 that impacted the Pacific harbor seals.

The goal of this study was to create a new baseline for the common parasites found in the Pacific harbor seal to allow future work to address concerns of changing parasite populations due to issues like climate change. The findings here suggest that parasites are very common in stranded Pacific harbor seals off the coast of Northern Oregon/Southern

Washington, and specifically that the parasites of the stomach and intestines are ideal study organisms as they had the highest prevalence rates of all organs examined. A sudden drop in parasite prevalence in the stomach or intestines of the resident Pacific harbor seals could indicate a major disruption in diet, and consequently a change in the trophic web. It is my suggestion that parasite collection and identification continue to be monitored in the NOSWSP area to assess changes to the marine ecosystem using parasites as bioindicators.

Chapter Three: Acanthocephalans of the Pacific Harbor Seal, *Phoca vitulina richardii*

Introduction

As noted in Chapter Two, the intestinal tract had consistently high prevalence rates of parasites regardless of the host sex, age, health status, season, or year (Tables 2.1–2.6). The intestines also had the highest diversity of parasites consisting of acanthocephalans, nematodes, and cestodes, with the acanthocephalans having the highest prevalence of all parasites (94%). Acanthocephalans, specifically the genus *Corynosoma*, are extremely common in the intestines of harbor seals (Margolis 1956, Margolis and Dailey 1972, Stroud and Dailey 1978, Dailey and Fallace 1989, Herreman et al. 2011, Kaimoto et al. 2018, Waindok et al. 2018), and appear to be especially well established in the Pacific Northwest (Dailey and Fallace 1989).

The genus *Corynosoma* has a worldwide distribution and has species that infect both marine mammals and fish-eating birds (Van Cleave 1953a, Van Cleave 1953b). The three species that most commonly infect Pacific harbor seals, *C. strumosum*, *C. semerme*, and *C. cameroni*, are restricted to the northern hemisphere (Van Cleave 1953a, Van Cleave 1953b, Leidenberger et al. 2020). The first intermediate host for *Corynosoma* is assumed to be an arthropod, with a wide range of fish serving as additional intermediate or paratenic hosts including: starry flounder, Pacific staghorn sculpin, Pacific cod, yellowfin croaker (Van Cleave 1953a, Van Cleave 1953b), white sturgeon, Pacific herring, Alaska pullock, Pacific cod, Pacific halibut, rock sole, starry flounder, sockeye salmon, threestripe rockfish, yellow striped flounder, dark flounder (Leidenberger et al. 2020), and Pacific salmon (Margolis 1958). Many of these are common prey items for the Pacific harbor seal (Orr et al. 2003). Once in the definitive host, *C. strumosum* is thought

to take about 2-3 weeks to reach sexual maturity (Ball 1930, Helle and Valtonen 1981), but the full length of their life cycle is not known. Even though acanthocephalans use their hooked proboscis to attach to the villi in the small intestines, they are not known for causing pathogenesis unless in extremely high numbers (Waindok et al. 2018). However, some species with much longer proboscises have been seen to penetrate deeply into the small intestine and may possibly cause intestinal perforation if in large numbers (Waindok et al. 2018). Acanthocephalans, like cestodes, lack a gastrointestinal tract of their own, and, therefore, passively absorb nutrients through their cuticle making the intestinal tract an ideal habitat (Hayunga 1991). Once they reach sexual maturity, they release their eggs and allow them to pass through the host in the fecal matter.

In this chapter, the parasites of the intestinal tract are discussed with a specific focus on the acanthocephalans as they were the dominant parasite found. The intensity of acanthocephalan infections was analyzed based on the host characteristics of age, sex, health status, and the external factors of seasonality and year of stranding (Aim 3). The distribution of the acanthocephalans along the intestinal tract was also evaluated.

Methods

Specimen Acquisition

Seals stranded dead off the coast of northern Oregon and southern Washington were collected and necropsied as described in Chapter Two.

Intestinal Processing and Parasite Collection

Intestines were removed and examined as a whole as described in Chapter Two and then processed for parasites by sections. The colon was identified by the presence of

the caecum, then separated and processed separately from the small intestine. Starting at the most posterior end (where the colon was separated) and working anteriorly (toward the stomach), the remaining small intestine was separated from the mesenteric tissue, and cut into 500cm sections (the last section was generally shorter than 500cm). Each section was flushed with lukewarm tap water, and the contents passed through a sieve to collect all grossly visible parasites. The sections were then cut open and laid flat to soak in water for 15-30 minutes to allow for the release of mucoid material. Each section was then physically examined for any remaining attached parasites, lesions, ulcers, and perforations. The soaking water was also passed through a sieve and all parasites and other contents of note (ie. plastic, bones, etc.) were collected and preserved. All notes, measurements, and parasite counts were recorded on intestinal datasheets (Appendix A).

Parasite Identification

Parasites were identified using general morphological characteristics (body shape, flatness, and the presence or absence of body segments) and the marine mammal identification key developed by Dailey and Gilmartin (1980) as described in Chapter Two. A subsample of acanthocephalans was confirmed to genus using Van Cleave (1923). Acanthocephalans from the genus *Corynosoma* differ from other genera by their general "club" shaped body with a more bulbous foretrunk, and body spines on the foretrunk with some combination of extended spines along the length of the trunk or just on the posterior end of the hindtrunk as can be seen in Figure 2.3 (Van Cleave 1953a, Van Cleave 1953b, Dailey and Gilmartin 1980). Morphological species identification was attempted only on specimens with fully extruded proboscises by viewing the proboscis

through a microscope as a temporary wet mount followed by staining with Semichon's stain (Dailey 1978) to produce permanent mounts. Prior to viewing or staining, frozen parasites were thawed at room temperature for 12-24 hours and allowed to relax in cold tap water for at least an hour. Parasites were then transitioned into 70% EtOH for long term storage. Specimens with fully extruded proboscises were punctured at the foretrunk and stained with Semichon stain until magenta in color and mounted in Canada balsam. The staining protocol I used was adapted from "*Helminth Slide Preparation with Semichon's Stain*" used by Dr. Reyda at the State University of New York College at Oneonta. See Appendix B for the full protocol used in this study. Once stained, the number of hook rows and hooks per row on the proboscis were counted (Figure 3.1).

Statistical Analyses

For intensity analyses, only seals with complete intestines were used (n=50), and to assess the distribution of acanthocephalans along the intestinal tract, only seals with intensity data for each intestinal section were used (n=41). All analyses were done using genus level acanthocephalan data in RStudio (version 1.4.17.17). Cestodes and nematodes were not included due to small sample sizes. One-way analysis of variance (ANOVA) was used with log-transformed acanthocephalan intensity data for each categorical variable (sex, age, health status, season, and year) and Kruskal-Wallis tests were run with raw acanthocephalan intensity data as supporting analyses. For statistically significant ANOVA results, a Tukey test was run as a post-hoc analysis to determine which variables were significant. For Kruskal-Wallis, a Dunn's test was used as the post-hoc analysis. Findings were considered significant when $p<0.05$. Linear regression

models were used for analyses of acanthocephalan intensity with body length, blubber layer, intestinal length, and polyparasitism. For analysis of acanthocephalan distribution throughout the intestinal tract, ANOVA and Kruskal-Wallis tests were performed and post-hoc analysis was conducted as described above.

Results

During the two-year collection period, 53 seals were examined for intestinal parasites. Three seals had incomplete intestines due to scavenging activities or a shark attack, and so were left out of the analyses. Of the remaining 50 seals, only two had no intestinal parasites, two pups, one compromised male from 2019 and one healthy female from 2018. Of the seals with intestinal parasites, 33 seals (16 female and 17 male) were collected in 2018, and 15 seals (9 female and 6 male) in 2019.

Parasite Diversity

Three main types of parasites were found in the intestinal tract; acanthocephalans, nematodes, and cestodes. The acanthocephalans were identified as belonging to the genus *Corynosoma* and were the most prevalent parasite found in the intestinal tract (48/50, 96%). Nematodes were identified as belonging to the family Anisakidae and were the second most prevalent (19/50, 38%). Cestodes were most likely from the family Diphyllobothriidae and were the least common intestinal parasite (4/50, 8%). Acanthocephalans occurred both by themselves or with nematodes and/or cestodes, but nematodes and cestodes were never found without acanthocephalans. This polyparasitism was seen in 42% (21/50) of all examined seals; 32% (11/34) in 2018 and 63% (10/16) in 2019. Total acanthocephala prevalence was 97% (33/34) in 2018 and 93% (15/16) in

2019. Seals with just acanthocephala had a prevalence of 65% (22/34) in 2018 and 31% (5/16) in 2019. Seals with both acanthocephala and nematodes were seen in 2018 (24%, 8/34) and in 2019 (56%, 9/16). Seals that had acanthocephala and cestodes were less common, 3% (1/34) in 2018 and 6% (1/16) in 2019. Just two seals were infected with all three parasites and both occurred in 2018 (Figure 3.2).

The intensity of acanthocephala infections ranged from 0–851 individuals per host (average 131.3), and a total of 6,590 acanthocephalans were collected during this study.

Acanthocephalan Intensity and Host Sex

Females had a mean of 156 acanthocephalans compared to 105 in males (Table 3.1). Both the ANOVA and Kruskal-Wallis found no statistical significance of acanthocephalan intensity between females and males (p-value's 0.344 and 0.299, respectively). The ANOVA model had a residual p-value of 0.03 indicating a decent fit. Both models were run using a 95% confidence interval with an alpha of 0.05 to establish significance.

Acanthocephalan Intensity and Host Age

Adult seals had the highest mean of acanthocephalan intensity at 195, followed by subadults at 126, yearlings at 88.8, and pups at 46.2 (Table 3.1 and Figure 3.4). Both ANOVA and Kruskal-Wallis analyses found that there was a statistically significant difference between one or more of the age class means (p-values 0.001 and 0.008, respectively). The ANOVA model had a residual p-value of 0.775 indicating a good fit. To determine which age classes had statistically significantly different means, a post-hoc analysis was performed. For the ANOVA, a Tukey's test determined that there was a

statistically significant difference in the intensity of acanthocephalans between pups and adults (p=0.001) and between pups and subadults (p=0.009). For the Kruskal-Wallis a Dunn's test only showed a significant difference between the pup and adult age classes (p=0.006). A summary of both post-hoc analyses is given in Table 3.2.

Acanthocephalan Intensity and Host Health Status

Compromised hosts had a mean of 142 acanthocephalans in their intestines, healthy hosts had a mean of 112, and the suspected ill hosts had a mean of 105. Both ANOVA and Kruskal-Wallis analyses indicated that there was no statistically significant difference between the three health categories (p-value 0.932 and 0.924). The residuals of the ANOVA had a p-value of 0.033 indicating that the model was a decent fit.

Acanthocephalan Intensity and Seasonality

Spring and summer both had means of 134 which were the highest means of acanthocephalan intensity. Fall had a mean of 130, and winter had the lowest mean of 115. Both ANOVA and Kruskal-Wallis analyses indicated that there were no statistically significant differences between the seasons (p-values 0.647 and 0.743) and acanthocephalan intensity (Table 3.1). The residuals of the ANOVA had a p-value of 0.146 indicating that this model was a good fit.

Acanthocephalan Intensity and Year

For year, 2018 had the higher mean of acanthocephalan intensity of 156 compared to 2019 with a mean of 86.4. There were two potential outliers in 2018 (Figure 3.5). Both ANOVA and Kruskal-Wallis analyses indicated there was no statistically significant

difference between the years (p-values 0.302 and 0.313) and acanthocephalan intensity (Table 3.1). The residuals of the ANOVA had a p-value of 0.023 indicating a decent fit.

Linear Regressions

A Pearson's correlation analysis showed that there was evidence of a positive linear relationship between acanthocephalan intensity and body length (0.53), intestinal length (0.19), average blubber layer (0.18), and polyparasitism (.19). Intestinal length was removed as it had a very strong colinear relationship with body length (0.73). Of the remaining three variables a linear regression showed that only body length was statistically significant ($p<0.001$, Figure 3.6). A linear regression of just acanthocephalan intensity and body length gave statistically significant results (p=9.29e-05, y=0.64852+(0.03022(Body length)), R^2=0.280, adjusted R^2=0.265, Figure 3.6), and an analysis of the residuals showed the model to be a good fit (p=0.611). However, the low R^2 value shows that while there is a positive correlation to acanthocephalan intensity and body length, it only attributes 26% of the variance seen.

The Pearson's coefficient of 0.79 showed a strong positive correlation of the intestinal length to body length (p=3.07e-11, y=352.256+(12.676(Body length)), R^2=0.621, and adjusted R^2=0.6123, Figure 3.7). The R^2 values suggest that in this model it accounts for 62% of the variance seen.

Acanthocephalan Intestinal Distribution

Seals with intestinal parasites, complete intestinal tracts, and intensities for each section were used for this analysis (n=39). Total length of intestinal tracts ranged from

1,059–2,748cm long (older animals had longer intestines, therefore more sections). The distribution of parasites found in each section of the intestines was not uniform (Figure 3.8). The second section (5-10m anterior to the colon) of the intestines had the highest mean acanthocephalan intensity of 44.8, followed by the third section with a mean of 40.6, the fourth with 34.9, the fifth with 13.7, the first with 12.1, and lastly the colon with a mean of 0.154 (Table 3.1). Both ANOVA and Kruskal-Wallis analyses showed there were statistically significant differences between the intestinal sections (p-values 2.00e-16 and 2.55e-16). The residuals of the ANOVA had a p-value of 0.158 indicating this was a good fit.

A Tukey test was used as a post-hoc analysis for the ANOVA to determine which sections were different. It showed that the acanthocephalan intensity in the colon was statistically significantly different from all other sections and that the second section was statistically significant from the fifth section (Table 3.4 and Figure 3.9). The post-hoc analysis for the Kruskal-Wallis, Dunn's test, showed that there was a statistically significant difference of acanthocephalan intensity between the colon and the first ($p=0.048$), second ($p<0$), third ($p<0$), and fourth ($p=0.015$) intestinal sections. The p-values for each section can be found in Table 3.4.

Discussion

The diversity of parasites found in the intestinal tract of stranded Pacific harbor seals from this study was similar to that found by Stroud and Dailey (1978) and Dailey and Fallace (1989). This study, like Dailey and Fallace (1989), found cestodes in the intestines not seen by Stroud and Dailey (1978). However, due to small sample sizes of

the nematodes and cestodes found in this study, the acanthocephalans were the focus of all intensity analyses. While the acanthocephalans were easily identified to the genus *Corynosoma* using morphological features and dichotomous keys (Van Cleave 1923, Dailey and Gilmartin 1980), getting species level identification proved challenging. Identifying *Corynosoma* to species using morphology alone is quite difficult as many species have been found to exhibit morphological plasticity (Aznar et al. 2016, Waindok et al. 2018). However, based on previous parasite research on Pacific harbor seals, just three species have been seen in the Pacific Northwest; *C. strumosum, C. semerme,* and *C. cameroni. Corynosoma strumosum* is typically 5-7mm long, may have body spines on its hindtrunk as well as genital spines (mostly in males) and the proboscis has 18 hook rows, with 10-11 hooks per row (Van Cleave 1953a, Van Cleave 1953b). But, this can vary as others have seen 17-19 hook rows with 9-12 hooks per row (Kuzmina et al. 2012, Lisitsyna et al. 2018). *Corynosoma semerme* is shorter, usually 3mm long, has body spines extending ventrally from their foretrunk all the way to their hindtrunk, and the proboscis has 22-24 hook rows with 12-13 hooks per row (Van Cleave 1953a, Van Cleave 1953b), but 21-26 hook rows with 12-14 hooks per row have been reported (Kuzmina et al. 2012). *Corynosoma cameroni* has a body length of 2.5-3.6mm, the hindtrunk is about the same length as the foretrunk with spines generally just on the foretrunk, and the proboscis has 16 hook rows with 9-11 hooks per row (Van Cleave 1953a, Van Cleave 1953b), but the body length has been reported to be longer (5.84-6.32mm) with 16 hook rows of 9-10 hooks per row by Kuzmina et al. (2012). Using these descriptions, I was able to positively confirm the presence of *C. strumosum* in some of

my harbor seal hosts. I did not find *C. semerme* in my samples as no specimen had body spines spanning the full length of the trunk, nor were any of the proper shape or size. The presence of *C. cameroni* is still possible if the longer body lengths reported by Kuzmina et al. (2012) hold true, however going by Van Cleave (1953a and b), I do not believe *C. cameroni* was found in this study. Other studies conducted along the West Coast had varying levels of acanthocephalan prevalence but showed that *C. strumosum* was the most prevalent species to be found in Pacific harbor seals (Delyamure et al. 1976, Stroud and Dailey 1978, Dailey and Fallace 1989), and is the only species identified in this study. To further provide species level identification, DNA analysis might be useful to complement detailed morphological measurements.

The prevalence of acanthocephalans seen here was much higher (96%) than the 56% previously found for Pacific harbor seals by Stroud and Dailey (1978). Dailey and Fallace (1989) suggested that the prevalence of *C. strumosum* decreases as you move south down the coast as seals in Canada have been reported to have 100% prevalence (Margolis 1956) and they found 93% prevalence in their study in Washington, while Stroud and Dailey (1978) reported 34% prevalence of *C. strumosum* in Oregon, and just 11% prevalence in California (Dailey and Hill 1970). If the main species of acanthocephala found in this study is indeed *C. strumosum* this could mean either the report by Stroud and Dailey (1978) underreported acanthocephala prevalence due to their sample size (18 harbor seals), that the parasite has become more widely present in our area or that perhaps one or more of its intermediate hosts had a population increase leading to the increased prevalence rates seen in this study.

This study has added to our understanding of intestinal parasite infections of *Corynosoma sp.* for Pacific harbor seals by comparing intensity with host characteristics (sex, age, and health status), seasonality, and year along the Oregon coast. There were significant differences of acanthocephalan intensities depending on the age of the host (Figure 3.4 and Tables 3.1 and 3.2) The pups had the lowest intensities significantly different from the subadult and adult intensity levels. This was supported by the linear regression using body length as an indicator of host age (Figure 3.6). This would suggest that it takes a certain amount of time for pups to be exposed to and acquire acanthocephalans. The fact that parasite intensities increase over time is a well-supported idea with respect to general parasite exposure; i.e. the longer an individual lives, the more likely they are to become infected with parasites (Nickol et al. 2002, Kaimoto et al. 2018, Lisitsyna et al. 2018, Waindok et al. 2018). This is the only study conducted on stranded Pacific harbor seals along the coast of Oregon that has evaluated acanthocephalan intensities among age groups, but this trend has been seen in California in stranded California sea lions (Lisitsyna et al. 2018) and in stranded harbor seals from the North and Baltic Seas (Waindok et al. 2018). However, this age difference was not seen in collected (free-ranging, not stranded) Pacific harbor seals from Washington (Dailey and Fallace 1989), raising the question of acanthocephalan intensity differences between free-ranging and stranded seals.

While no significant differences in acanthocephalan intensities were seen between females and males, consistent with Dailey and Fallace (1989), the means appeared different (female 156 and male 105) with females having higher intensities of

acanthocephalans. The differences seen in our study may have been due to two potential female outliers that had the highest acanthocephalan intensities (550 and 851). These outliers may have increased overall variance of intensities leading to the differences in the means without impacting statistical significance. Herreman et al. (2011) reported seeing acanthocephalans only in female harbor seals in Washington. This combined with our apparent differences between sexes suggests that more research is needed in this area.

Our study also conducted a broad comparison between healthy and compromised hosts and their acanthocephalan intensities. While there were no statistically significant findings between the health categories, it is worth noting that the four highest individual intensities of acanthocephalans were all in compromised adult seals from 2018. Three were females from the summer, and the fourth was a male from the fall. While they were all compromised, they were classified compromised for different reasons so no conclusions could be drawn, but they all stranded within a month of each other which might indicate another area to investigate further. One reason we did not see a statistically significant difference between acanthocephalan intensities and host health could be that the majority of compromised hosts had intensities of less than 300, and just four individuals had higher intensities causing the means and variance between the compromised and healthy hosts to be more similar than anticipated (Figure 3.3).

While acanthocephalan intestinal intensity ranged from 0-851 parasites per seal, there were two outliers that required further investigation. Both outliers were compromised adult females that stranded in the summer of 2018 within two weeks of each other. They both had parasites in their stomach and intestines. The host with 851

parasites (PSU 18-08-11B Pv) had yellow blubber, was quite thin (blubber layer of 0.9cm) and had signs of trauma supporting a possible fisheries interaction. It stranded in Warrenton, OR. The other host had 550 acanthocephalans (PSU 18-08-01C Pv) was recorded as being "clearly ill" but with significant hemorrhage indicating human interaction. A possible bacterial infection was noted on the intestinal examination, but the seal had a very thick blubber layer (2.3cm). It stranded in Long Beach, WA. Considering this information, not much can be discerned as to why they had the highest intensities of acanthocephalans, but perhaps they shared similar dietary preferences that exposed them to similar acanthocephalan infection rates. Further research into the stomach contents of these animals could help determine why their intensities were so much higher than the rest of the examined seals. Interestingly, Dailey and Fallace (1989) found that presence of *C. strumosum* increased with the number of total parasites found in the host ($p < 0.001$), which could be a possibility with these hosts as they both had stomach parasites which could be different species. Perhaps these hosts had a higher diversity of parasites species than some of the other hosts causing them to have higher intensities of *Corynosoma sp.*

Seasonality did not seem to play a role in the intensity of acanthocephalan infections, also consistent with previous research (Helle and Valtonen 1981, Dailey and Fallace 1989). This suggests that the intermediate hosts for the acanthocephalans are present year-round in our area. On the other hand, the two years of collection had different means of acanthocephalan intensity (2018, mean=152 and 2019, mean=86.4) and 2018 had the four highest intensities (339, 436, 550, and 851) which were potential outliers. This may have caused a failure to reject the null hypothesis due to increasing the

overall variance. However, the data used for the ANOVA was log transformed and had a near normal distribution with no outstanding outliers and a Kruskal-Wallis, which is a non-parametric analysis for data that does not fit all ANOVA assumptions, also showed no significant differences between the two years. Therefore, it could be that there truly is no difference between our two years, or that our sample size is just too small to draw any other conclusions at this time.

The linear regression model analyzing acanthocephalan intensity with body length, blubber thickness, and polyparasitism showed that those variables all play a role in intensity levels, but do not explain the entire picture. Body length was the strongest predictor of acanthocephalan intensity, which supports our findings that intensities increase as hosts get older. While these variables may play a role in acanthocephalan intensity, they did not fully explain the differences we saw, leading us to believe that the are other variables at play that we did not anticipate. The strong colinear relationship found between body length and intestinal length (Figure 3.7) supports existing research that intestinal length is a function of body size and that pinnipeds in particular have much longer intestines than terrestrial carnivores (McGrosky et al. 2016).

Interestingly, this study found that acanthocephalans had higher intensities in certain areas of the intestines. Specifically, the highest intensities were found between 5-10m anterior to the colon (Figures 3.8 and 3.9, and Tables 3.3 and 3.4). The lowest intensities were in the colon and the sections closest to the stomach (4 and 5). The sections closest to the stomach have the highest rates of peristalsis, so this may be the reason why these sections had lower intensities (Hayunga 1991). This is the first study to

analyze the distribution of these parasites along the intestinal tract in Pacific harbor seals in Oregon and Washington, though previous authors have gathered data in one stranded Pacific harbor seal pup in California (Ball 1930), Kuril harbor seals in Japan (Kaimoto et al. 2018), and gray seals in the Baltic Sea (Nickol et al. 2002). Ball (1930) reported the vast majority of the parasites were found within the first 630cm of the intestines. Kiamoto et al (2018), as in this study, found that the majority were found in the posterior end of the intestines. Nickol et al. (2002) found that *C. strumosum* could be found throughout the small intestine. But all offered further support that the vast majority of the parasites found in the small intestines in this current study were most likely *C. strumosum* especially as it has also been documented that *C. semerme* is mainly found in the most posterior section of the small intestine or entirely in the large intestine and rectum in seals (Valtonen 1983, Valtonen and Helle 1988, Nickol et al. 2002).

In conclusion, the findings of this study suggest that *Corynosoma sp.* are year-round inhabitants of prey of the Pacific harbor seals on the Northern Oregon and Southern Washington coasts. They are found in both male or female hosts, there was no clear difference in prevalence in healthy versus compromised hosts, and they accumulate within their host over time. They are specific to the small intestine, rarely being found in the colon in large numbers. They seem to favor the microenvironment of the small intestine roughly 5-10m anterior to the colon, but will colonize any part of the small intestine. Lastly, species level identification of the acanthocephalans using morphology alone proved to be extremely challenging and should be followed up with DNA analysis to confirm species identification.

Chapter Four: Summary and Broader Impacts

Summary

During the years 2018-2019, 53 stranded Pacific harbor seals were collected by the Northern Oregon/Southern Washington Marine Mammal Stranding Program (NOSWSP) and examined for grossly visible parasites in their heart, lungs, stomach, and intestines. This level of parasite analysis had not conducted for Pacific harbor seals along the Oregon coast since Stroud and Dailey (1978). The prevalence of parasites in each organ were analyzed for correlations to host sex, age, health status, season, and year, which to our knowledge has never been documented in Oregon (Chapter Two). The intestinal tract was closely examined for parasites and the intensity of the acanthocephalan parasites was analyzed for correlations with host sex, age, health status, season, and year (Chapter Three). Lastly, the distribution of acanthocephalans along the intestinal tract was also evaluated (Chapter Three). The work presented here was conducted to establish a new baseline for the parasites that infect stranded Pacific harbor seals off the Northern Oregon and Southern Washington coast and to compare parasite diversity and prevalence with similar reports along the Pacific Northwest coast outside of Oregon.

Parasites were found in 94% (50/53) of the Pacific harbor seals stranded off the Northern Oregon and Southern Washington coast during 2018-2019. Parasite prevalence varied for each organ, but were found in the heart, lungs, stomach, and intestines of the seals. While parasites were present in each of the examined organs, the stomach and intestines consistently had the highest rates of prevalence regardless of host sex, age,

health status, season, and year. Using chi-square analyses, parasite prevalence by organ was analyzed against host sex, age, health status, season, and year. The only statistically significant findings were for parasites in the lungs with regard to host age class and season. Yearlings had a significantly higher prevalence rate of parasites in the lungs when compared to the other age classes. The prevalence of parasites in the lungs had a significant relationship with seasonality, increasing over the course of the year and peaking during the winter. The increased prevalence of parasites in the lungs of young pinnipeds has been reported previously by other work along the West coast (Stroud and Roffe 1979, Dailey and Fallace 1989, Elson-Riggins et al. 2001). The seasonality of parasites in the lungs has also been reported in pinnipeds from California by Gulland et al. (1997).

Based on previous studies, the parasites in the heart are believed to be *Acanthocheilonema spirocauda* (Stroud and Dailey 1978, Eley 1981, Dailey and Fallace 1989, Leidenberger et al. 2007) or *Dirofilaria immitis*, the canine heartworm, from a histological report and previous finding of this parasite in harbor seals in Portugal (Alho et al. 2017). The parasites in the lungs may be *Otostrongylus circumlitus* according to a histological report and previous studies (Dailey and Fallace 1989, Gerber et al. 1993, Elson-Riggins et al. 2001, Colegrove et al. 2005); *Parafilaroides sps.* (Herreman et al. 2011, Rhyan et al. 2018); or *Parafilaroides gullandae* (Dailey 2006). The stomach parasites are strongly suspected to be from the family Anisakidae based on morphological characteristics and previous reports (Stroud and Dailey 1978, Stroud and Roffe 1979, Dailey and Fallace 1989, Gerber et al. 1993, Herreman et al. 2011). The intestinal

nematodes are believed to be anisakids like those found in the stomach, but there is the possibility that there may have been a few larval lungworms present as well. The identified cestode from the intestine is believed to be from the family Diphyllobothriidae, which has been documented in Pacific harbor seals along the West coast previously (Daily and Fallace 1989, Gerber et al. 1993, Herreman et al. 2011). Lastly, the acanthocephalans found in the intestines were all identified to the genus *Corynosoma,* with a few specimens being identified to species level as *C. strumosum* based on morphology. These parasites have routinely been found in harbor seals with *C. strumosum* regularly being the dominant species (Margolis 1956, Delyamure et al. 1976, Dailey and Stroud 1978, Dailey and Fallace 1989, Herreman et al. 2011).

Parasites were present in the intestines of 96% (51/53) of the stranded Pacific harbor seals examined, and included nematodes from the family Anisakidae, cestodes from the family Diphyllobothriidae, and acanthocephalans from the genus *Corynosoma.* Of these parasites, the acanthocephalans, specifically *Corynosoma sp.* and *C. strumosum,* were by far the most prevalent and were the focus of Chapter Three in this study. Intensity ranged from 0-851 parasites per host, and a total of 6,590 acanthocephalans were collected in this study. Using one-way ANOVA analyses, the intensity of *Corynosoma sp.* infections were compared with host sex, age, health status, season, and year. The only statistical significance found was with host age class and level of parasite intensity. Statistically, pups had significantly lower intensities of *Corynosoma sp.* than the subadults and adults. This difference in host age and parasite intensity has been seen before in California sea lions along the California coast (Lisitsyna et al. 2018), and in

harbor seals from the North and Baltic Seas (Waindok et al. 2018). As there was no difference in intensities seen between host sex, health status, season, or year, it indicates that the intermediate hosts for *Corynosoma* are well adapted to our environment, present year-round, and are more common. They were seen in higher intensities in older animals. While *Corynosoma sp.* was found throughout the intestinal tract, distribution was not uniform and appeared to favor some areas of the intestine over others. Specifically, they were seen in significantly lower intensities in the colon compared to any other section, and in significantly higher intensities in the second intestinal section (5m anterior to the colon), especially when compared with the fifth section which was closest to the stomach. It has been documented that *C. strumosum* can be found along the full length of the intestinal tract in grey seals from the Baltic Sea (Nickol et al. 2002) but has also been seen in the more posterior sections by Kaimoto et al. (2018) in harbor seals in Japan. The distribution of these parasites along the intestinal tract is interesting and warrants further investigation as these findings suggest that some areas in the intestines are favored by the parasites but little research has been conducted to determine why.

Development of a Parasite Course-based Undergraduate Research Experience (CURE)

As a result of this work, a parasitology course-based undergraduate research experience (CURE) was developed in collaboration with Dr. Kathryn Weglarz at Westfield State University in Westfield, Massachusetts. Students in this parasitology CURE are conducting research on the acanthocephalan specimens collected in this study and will share their findings to the broader Westfield community through presentations at

during the Center for Undergraduate Research and Creative Activity (CURCA). As part of this CURE, 17 students have participated in mounting, staining, identification, and genetic sequencing of these parasites. The results of the genetic sequencing have identified *Corynosoma strumosum*, confirming the morphological findings from our study. They have also developed their own research proposals and are currently conducting experiments that involve environmental impacts on parasite intensities (i.e. ocean pH, temperature, and productivity), heavy metal analysis, parasite hormone secretions, and reproductive status of parasites.

CUREs bring novel research experiences into traditional labs allowing undergraduates to participate in scientific research to which they might otherwise not have access. These courses have been shown to increase student retention (Nagda et al. 1998, Rodenbusch et al. 2016) and sense of belonging in biology courses (Hunter et al. 2009). CUREs also increase the accessibility of research experiences by eliminating many of the barriers that prevent students from participating in traditional undergraduate research experiences, such as financial limitations, time commitment, and awareness of the opportunities for undergraduate research (Bangera and Brownell 2017).

Future Research

Parasites, specifically acanthocephalans, have been shown to be extremely well-suited bioaccumulators of heavy metals (Sures 1999 and Siddall, Sures et al. 1999, Sures 2004, Nachev and Sures 2016). An extension to the parasite CURE at Westfield, future work could compare the levels of heavy metals, such as lead, that are found in *Corynosoma* to the levels found in the host tissues of Pacific harbor seals. A second

extension of study from this CURE could be a comprehensive morphological description of *C. strumosum* with their genetic sequence to further contribute to the growing work of parasite identification (Waindok et al. 2018). Furthermore, genetic sequencing to determine the genetic variation and diversity of this parasite population could also be used to contribute to the idea that parasite genetic diversity may be an indicator of ecosystem stability (Marcogliese 2005, Hudson et al. 2006, Nachev and Sures 2016). The acanthocephalans found in this study are ideal candidates for this kind of work because they were present year-round, in all age classes, and in both sexes of seals. If the acanthocephalans or anisakid nematodes found in this study disappeared from the Pacific harbor seals from this area, it would indicate that their prey populations are being disrupted, and thus indicate major disturbance in the ecosystem. This study shows that these parasites are easily accessible and ideal organisms to use for robust studies that examining ecosystem changes over long periods of time.

The seals used in this study also had their stomach contents used for dietary analysis. The comparison of diet analysis with parasite diversity and intensity could be combined to further elucidate the life cycle of *Corynosoma sp.* in our area. As it is known that *Corynosoma sp.* may use as many as 20-30 different species of fish as their intermediate hosts (Moles 2007, Kuzmina et al. 2012, Lisitsyna et al. 2018), the dietary analysis may help narrow down the main sources of infection for the Pacific harbor seals in our area. This could contribute to the understanding of why the prevalence of *Corynosoma sp.* is higher in our area than seen the previous work of Stroud and Dailey (1978) and explain why some of the seals in this study had such high levels of intensity compared to others.

The majority of the stranded marine mammals that NOSWSP responds to are collected as museum specimens and stored in the Museum of Vertebrate Biology at Portland State University. All the parasites in this study were collected opportunistically while operating under the normal practices of the NOSWSP and required minimal supplies and skill to collect. The collection of these parasites allowed the involvement of 10 undergraduate researchers to participate in hands on research at Portland State University and in addition led to the development of a parasite CURE that included undergraduates at Westfield State University. As natural history museums continue to evolve and expand their research capabilities with advances like DNA sequencing and digitizing specimens through imaging, this study supports the call for the collection of parasites from mammal specimens to become standard practice (Galbreath et al. 2019). The parasites collected during this study, and those from other specimens being collected on behalf of museum research, are potential subjects of a wide variety of future research topics that deserve to be explored.

Study Limitations

It should be acknowledged that this study was conducted on dead stranded Pacific harbor seals and therefore is a biased population sample that may not fully reflect what could be found in free-ranging seals. It also bears repeating that this study focused solely on grossly visible parasites and did not evaluate the hosts for microscopic parasites so may have missed smaller parasites that passed through the sieves. Lastly, the fixation technique in this work was not optimal for the full relaxation and extrusion of the proboscis of the acanthocephalans impeding the ability to identify them to the species

level. Future collection of acanthocephalans should follow improved fixation techniques to ensure optimal relaxation and full extrusion of the proboscis in order to better identify the parasites to species.

Tables

Table 1.1: Pacific Harbor Seal Strandings and Necropsies

	2018	2019
Necropsied (intestines processed)	36	17
Necropsied (intestines missing)	14	4
Not Necropsied (unrecoverable or too decomposed)	44	19
Total Reported Stranded Seals	**94**	**40**

A total of 94 harbor seals stranded in 2018 compared to 40 in 2019. This was due to an ongoing mass mortality event that began in 2017 and continued into 2018. In 2018, 72% of the necropsied seals had their intestines processed and in 2019, 81% of the seals necropsied also had their intestines processed. In 2018 38% of the total number of stranded seals had their intestines processed, and 43% were processed in 2019. Seals that were unreachable, or too decomposed were not necropsied.

Table 2.1. Parasite Diversity and Prevalence by Anatomical Location

Parasite	Location	Prevalence
Nematoda		
Onchocercidae	Heart	11/53 (21%)
Strongylida (order)	Lung	15/53 (28%)
Anisakidea	Stomach	38/53 (72%)
	Intestines	20/53 (38%)
Cestoda		
Diphyllobothrium	Intestines	4/53 (8%)
Acanthocephala		
Corynosoma	Intestines	50/53 (94%)

Percent prevalence of parasites found by organ and suspected identification. Parasites were found in each of the four examined organs. The stomach and intestines had the highest prevalence of parasites. Nematodes were found in each organ, but cestodes and acanthocephalans were only found in the intestines.

Table 2.2. Prevalence of Parasites by Host Sex

	Female	Male	X^2
Heart	3/28 (11%)	8/25 (32%)	2.459^{ns}
Lung	6/28 (21%)	9/25 (36%)	0.757^{ns}
Stomach	19/28 (68%)	19/25 (76%)	0.124^{ns}
Intestines	26/28 (93%)	24/25 (96%)	$2.638E\text{-}31^{ns}$

Percent prevalence of parasites by organ and host sex. No significant findings between parasite location of host sex was found. Yates corrected chi-square value (X^2), ^{ns} = not significant.

Table 2.3. Prevalence of Parasites by Host Age

	Pup	Yearling	Subadult	Adult	X^2
Heart	3/11 (27%)	3/8 (38%)	2/11 (18%)	3/23 (13%)	2.524^{ns}
Lung	4/11 (36%)	7/8 (88%)	2/11 (18%)	2/23 (9%)	19.081***
Stomach	7/11 (64%)	6/8 (75%)	8/11 (73%)	17/23 (74%)	0.457^{ns}
Intestines	9/11 (82%)	8/8 (100%)	10/11 (91%)	23/23 (100%)	5.332^{ns}

Percent prevalence of parasites by organ and host age. Lungworm prevalence was found to have a highly significant relationship with host age class. Pearson chi-square value (X^2), ^{ns} = not significant,

Table 2.4. Prevalence of Parasites by Host Health

	Compromised	Healthy	Suspected Ill	X^2
Heart	10/32 (31%)	1/19 (8%)	0/2 (0%)	5.439^{ns}
Lung	10/32 (31%)	5/19 (26%)	0/2 (0%)	0.973^{ns}
Stomach	25/32 (78%)	11/19 (58%)	2/2 (100%)	3.225^{ns}
Intestines	31/32 (97%)	17/19 (89%)	2/2 (100%)	1.348^{ns}

Percent prevalence of parasites by organ and host health status. While it appears that parasites were more common in the compromised hosts, no statistical significance was found. Yates corrected chi-square value (X^2), ^{ns} = not significant.

Table 2.5. Prevalence of Parasites by Season

	Spring	Summer	Fall	Winter	X^2
Heart	0/4 (0%)	6/28 (21%)	3/18 (17%)	2/3 (67%)	5.083^{ns}
Lung	0/4 (0%)	5/28 (18%)	7/18 (39%)	3/3 (100%)	11.679**
Stomach	2/4 (50%)	20/28 (71%)	14/18 (78%)	2/3 (67%)	1.294^{ns}
Intestines	4/4 (100%)	26/28 (93%)	17/18 (94%)	3/3 (100%)	0.536^{ns}

Percent prevalence of parasites by organ and season. Parasites in the lungs have a very significant relationship to seasons and appear to become increasingly more prevalent as the year progresses, however the small sample size for winter may not represent actual prevalence. Pearson chi-square value (X^2), ^{ns} = not significant, ** = $P < 0.01$.

Table 2.6. Prevalence of Parasites by Year

	2018	2019	X^2
Heart	9/36 (25%)	2/17 (12%)	0.557^{ns}
Lung	7/36 (19%)	8/17 (47%)	2.179^{ns}
Stomach	27/36 (75%)	11/17 (65%)	0.202^{ns}
Intestine	35/36 (97%)	15/17 (88%)	0.469^{ns}

Percent prevalence of parasites by organ and year. While parasites were seen in each organ both years, it appears that parasites in the lungs were more prevalent in 2019, however there is no statistical significance to support this. Also, it is clear that the stomach and intestines had the highest rates of parasites of all organs for both years. Yates corrected chi-square value (X^2), ^{ns} = not significant.

Table 3.1. Acanthocephalan Intensity Summary Table

		Sample Size	Range	Mean	Standard Deviation	ANOVA p-value	Kruskal-Wallis p-value
Sex	Female	26	0 - 851	156	189	0.344	0.299
	Male	24	0 - 436	105	115		
Age	Pup	11	0 - 266	46.2	78.4	**0.001**	**0.008**
	Yearling	8	10 - 259	88.8	103		
	Subadult	10	19 - 247	126	84		
	Adult	21	5 - 851	195	206		
Health Status	Healthy	17	0-266	112	95.1	0.932	0.924
	Compromised	32	0-851	142	186		
	Suspected Ill	1	105	105	NA		
Season	Spring	4	72-213	134	58.6	0.647	0.743
	Summer	27	0-851	134	191		
	Fall	16	8-436	130	127		
	Winter	3	21-244	115	116		
Year	2018	34	0-851	152	180	0.302	0.313
	2019	16	0-259	86.4	86.9		

Summary statistics of all variables analyzed with acanthocephalan intensity. Of all variables analyzed, only host age had significant results (bold). Post-hoc analysis of these results can be found in Table 3.2.

Table 3.2. Post-hoc Analysis for Host Age and Acanthocephalan Intensity

	Tukey p-value	Dunn's p-value
Pup - Yearling	0.210	0.809
Pup - Subadult	**0.009**	0.097
Pup - Adult	**0.001**	**0.006**
Yearling - Subadult	0.685	0.875
Yearling - Adult	0.377	0.473
Subadult - Adult	0.981	0.998

Post-hoc analysis of ANOVA and Kruskal-Wallis analyses. The Tukey test from the ANOVA showed that there were statistically significant differences between acanthocephalan intensities between the pups and subadults and the pups and adults (bold). The Dunn's test from the Kruskal-Wallis shows only a significant difference between the pups and adults (bold).

Table 3.3. Intestinal Distribution of Acanthocephalans Summary Table

	Mean	Standard Deviation	ANOVA	Kruskal-Wallis
Colon	0.154	0.376	**2.00E-16**	**2.55E-16**
First	12.1	11.7		
Second	44.8	57		
Third	40.6	39.3		
Fourth	34.9	69.8		
Fifth	13.7	20.3		

Statistical summary of intestinal distribution of acanthocephalans. Both the ANOVA and Kruskal-Wallis showed significant results (bold). Post-hoc analyses are found in Table 3.4.

Table 3.4. Post-hoc Analysis for Host Age and Acanthocephalan Intensity

	Tukey's p-value	Dunn's p-value
Colon - First	**0.000**	**0.048**
Colon - Second	**0.000**	**0.000**
Colon - Third	**0.000**	**0.000**
Colon - Fourth	**0.000**	**0.015**
Colon - Fifth	**0.000**	0.079
First - Second	0.078	0.674
First - Third	0.499	0.660
First - Fourth	1.000	1.000
First - Fifth	0.740	1.000
Second - Third	0.930	1.000
Second - Fourth	0.088	0.908
Second - Fifth	**0.015**	0.536
Third - Fourth	0.467	0.900
Third - Fifth	0.098	0.522
Fourth - Fifth	0.871	1.000

Post-hoc analyses of ANOVA and Kruskal-Wallis for intestinal sections. The Tukey test (for the ANOVA), showed that the colon has statistically significantly different acanthocephalan intensities than any other section (bold). It also found that the second and fifth section have statistically significantly different levels of acanthocephalan intensity (p-value 0.015). The Dunn's test (from Kruskal-Wallis) showed that the colon was statistically significantly different from all sections but the fifth (bold).

Figures

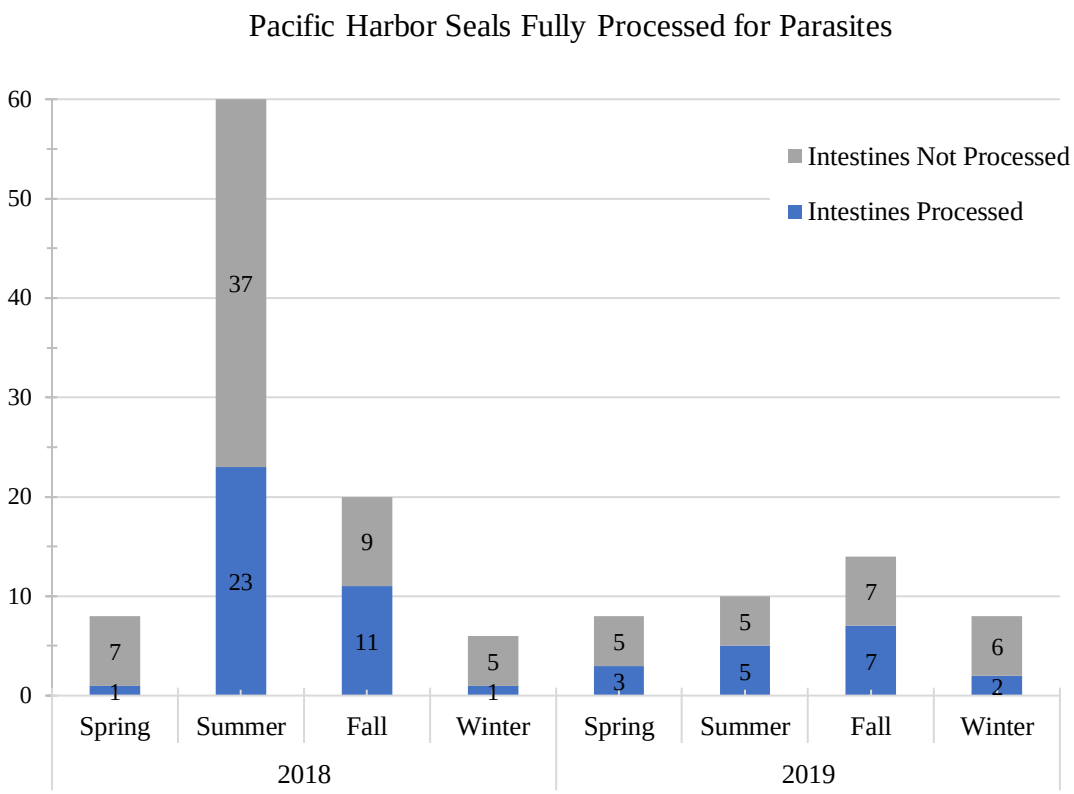

Figure 1.1: Seasonal distribution of necropsied Pacific harbor seals during the two-year collection. Note the spike of strandings in the summer of 2018 compared to a more normal stranding rate as seen in 2019.

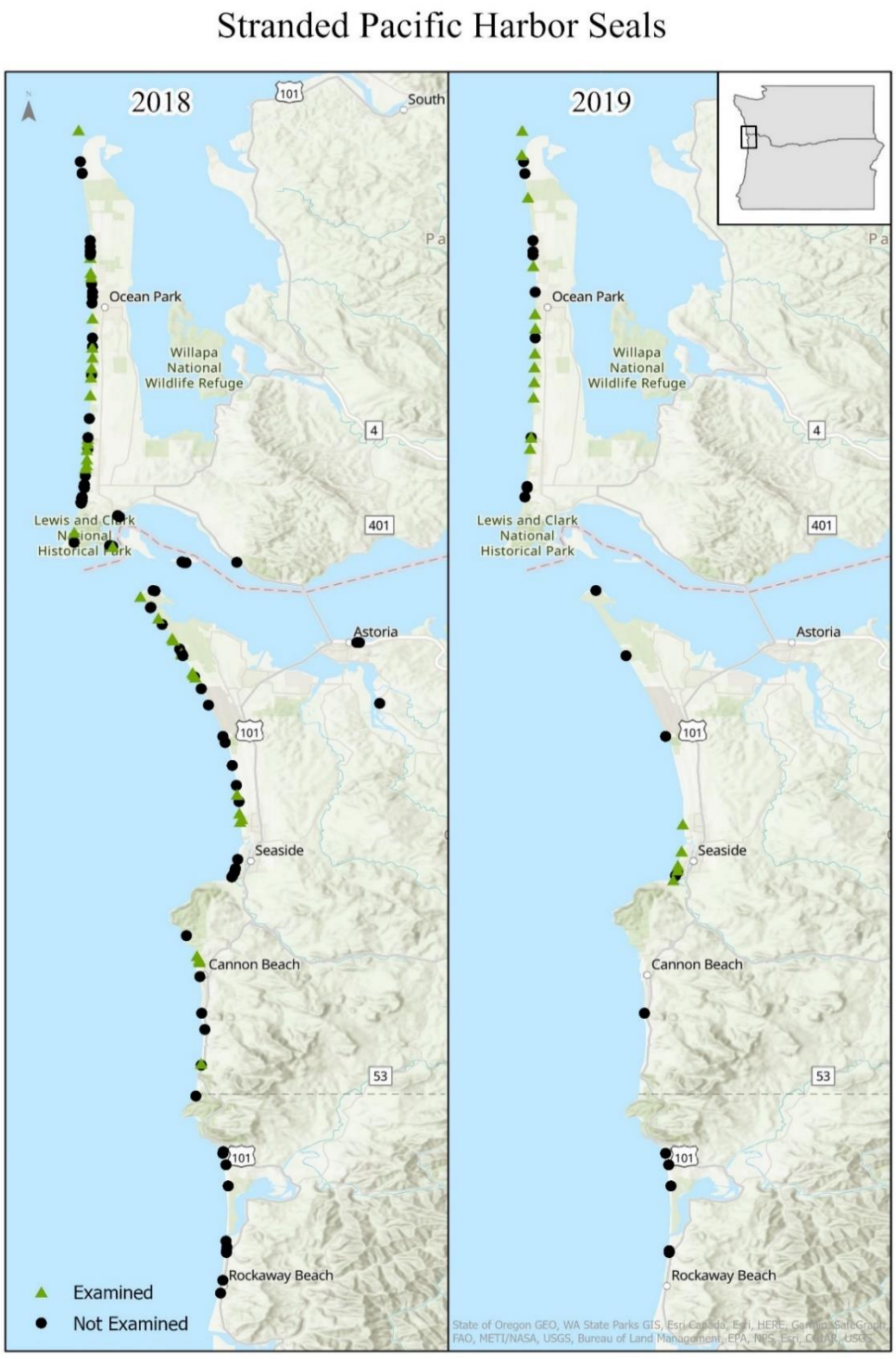

Figure 1.2: Spatial distribution of all stranded Pacific harbor seals from 2018 (left) and 2019 (right). The fully necropsied animals are represented by green triangles. The black circles are stranded seals that were not used in this study because they were either unreachable, too decomposed, or did not have intestines.

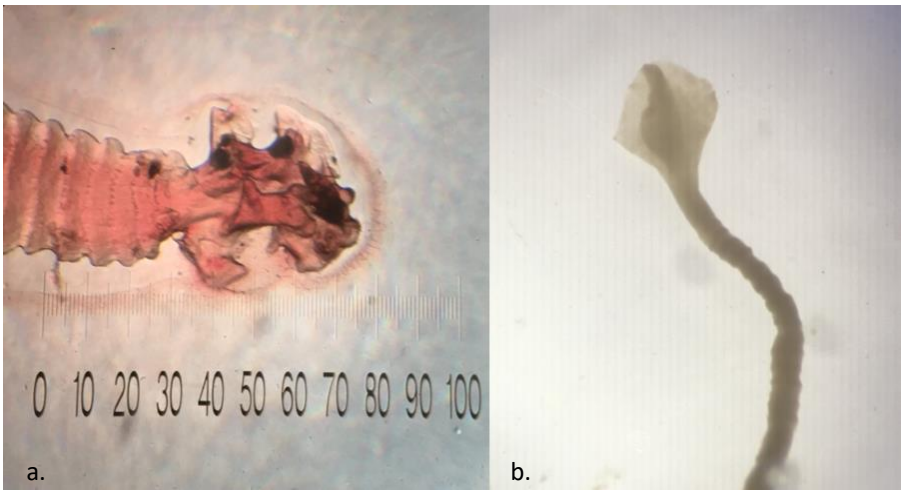

Figure 2.1: Cestode specimens from the intestines. Two cestode specimens recovered from the intestines of two harbor seals. On the left (**a**) a 10x magnified scolex of unidentified cestode, (**b**) scolex and body section of cestode from the family Diphyllobothriidae under dissection microscope. Both specimens have flat segmented bodies.

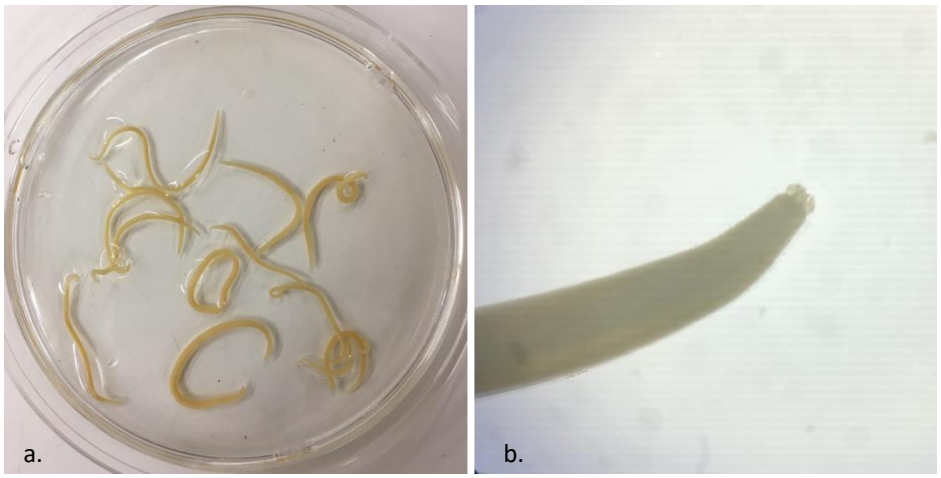

Figure 2.2: Nematode specimens from the stomach. On the left (**a**) the specimens are round bodied, of varying lengths, and do not have body segments. On the right (**b**) a magnified view of the anterior region with the mouth. These specimens are believed to be from the Anisakidae family.

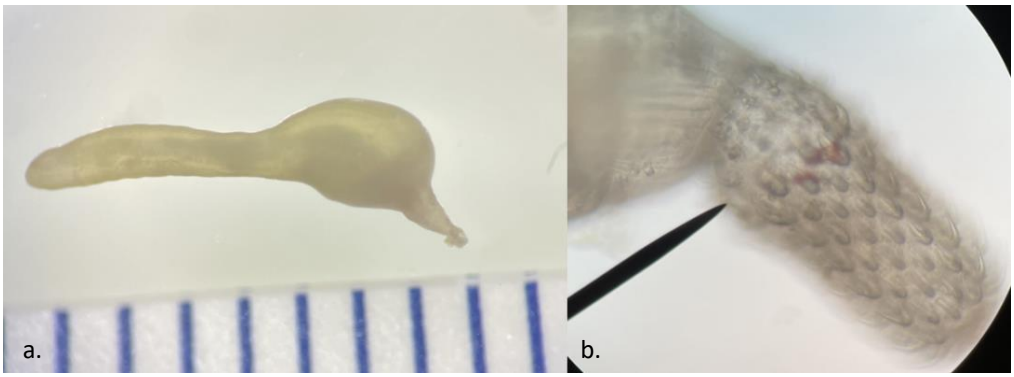

Figure 2.3: Acanthocephalan from the intestine. (**a**) The body is robust, the hind and foretrunk are easily distinguishable under the magnification of a dissection microscope, under fine focus spines are observed on both the posterior and anterior ends. (**b**) Under 20x the fully extruded proboscis is clearly covered in spines. These specimens belong to the genus *Corynosoma*.

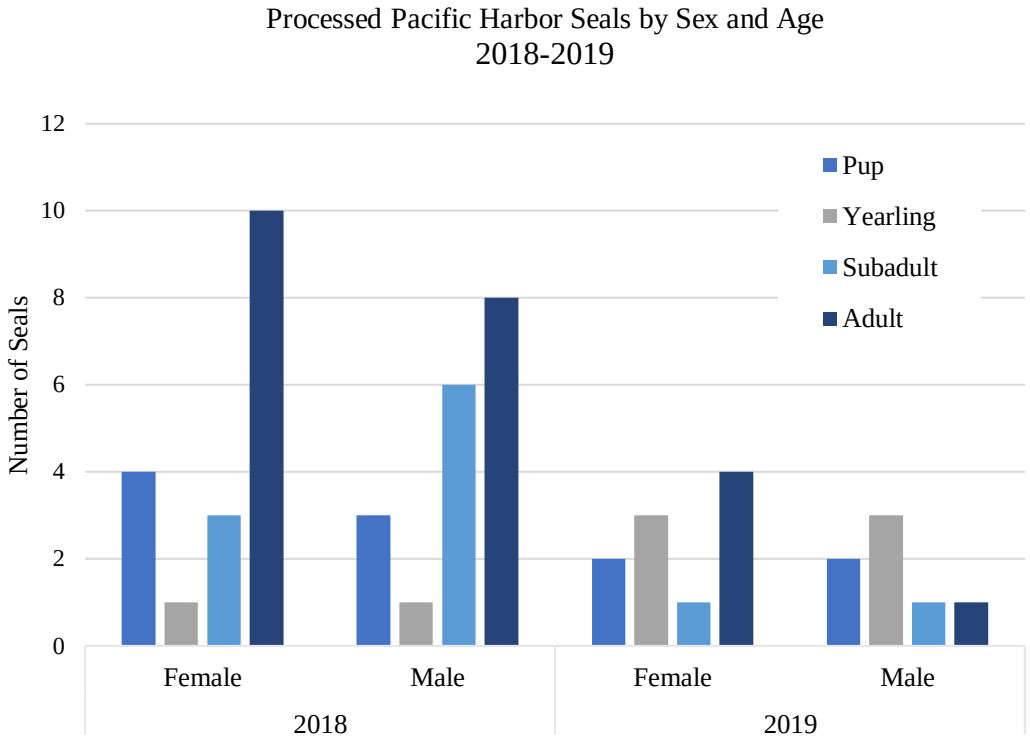

Figure 2.4: Stranded seals by sex and age class. In 2018, 7 pups were processed (4 female, 3 male), 2 yearlings (1 female, 1 male), 9 subadults (3 female, 6 male), and 18 adults (10 female, 8 male). In 2019, 4 pups were processed (2 female, 2 male), 6 yearlings (3 female, 3 male), 2 subadults (1 female, 1 male), and 5 adults (4 female, 1 male). In total, 18 females and 18 males were processed in 2018 and 10 females and 7 males in 2019.

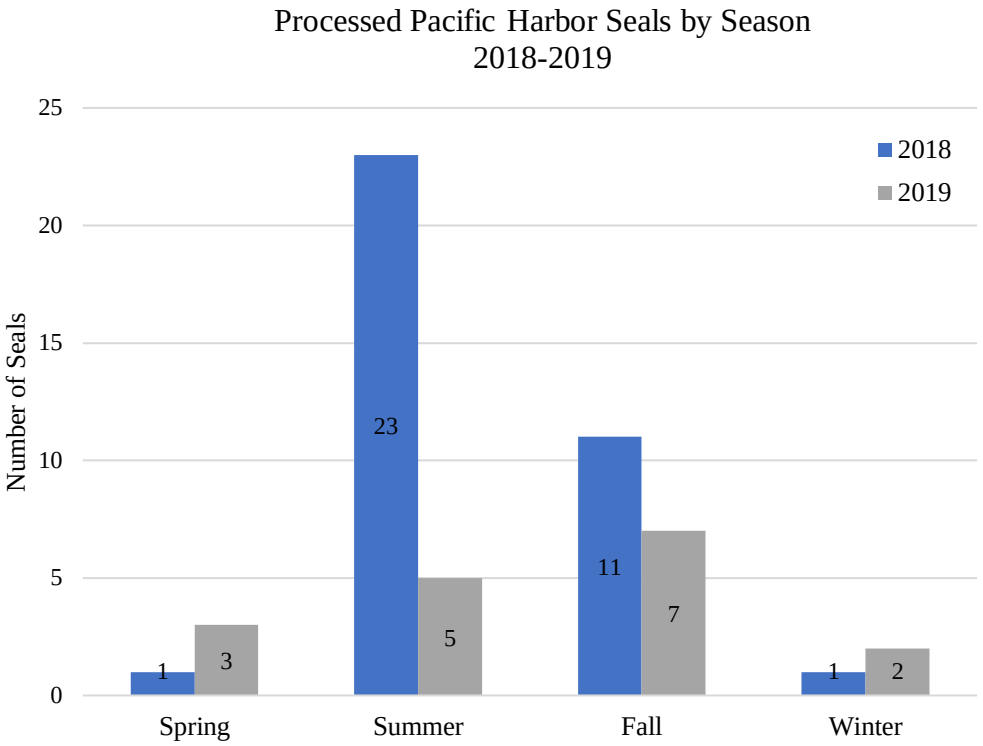

Figure 2.5: Processed harbor seals by season. For both years, the spring and winter had the lowest numbers of processed seals, but the differences between the years is not large. The summer of 2018 had almost five times the number of seals processed compared to 2019. The fall of 2018 had 4 additional seals processed compared to 2019.

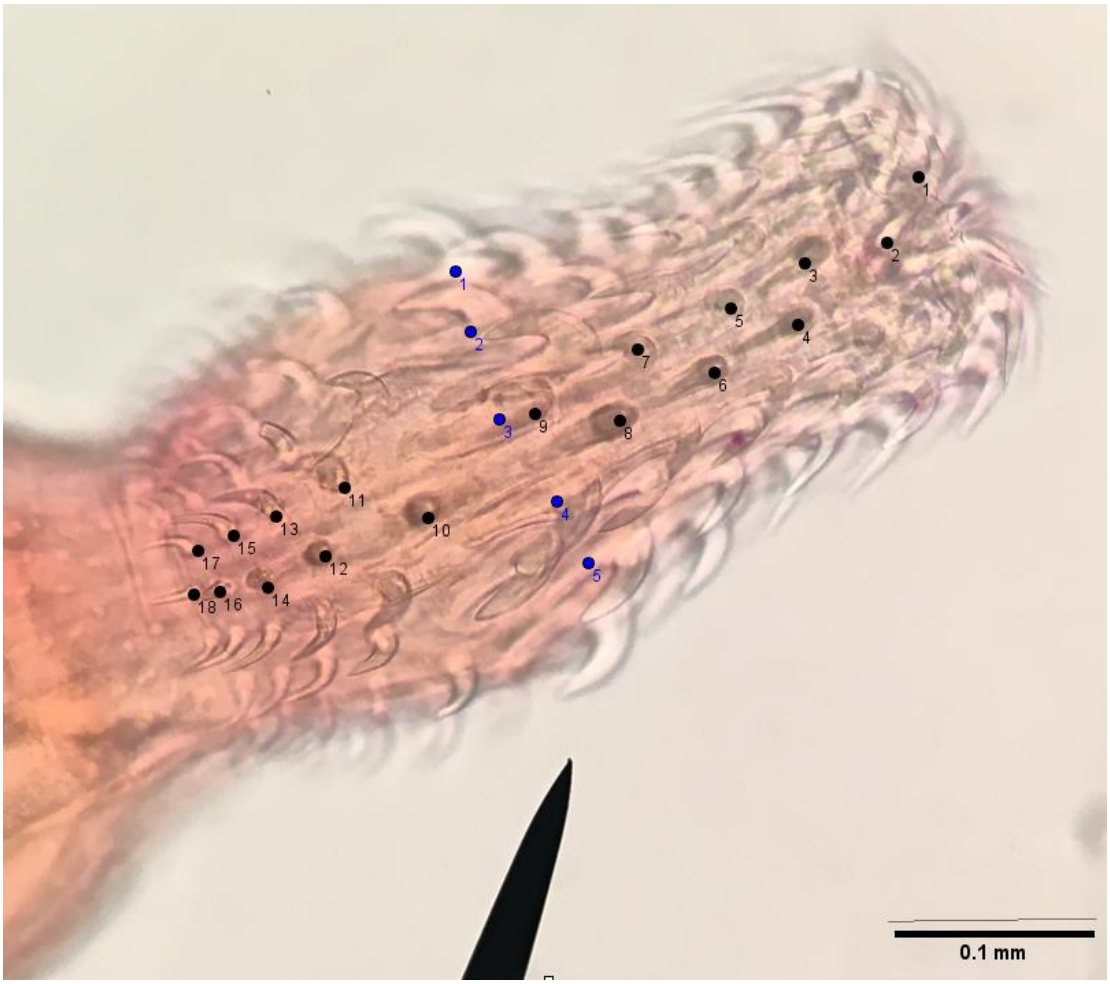

Figure 3.1: Proboscis of stained *Corynosoma* specimen magnified 20x. The black dots count the number of hook rows, the blue dots count the number of hooks in that row. The number of hooks per row are multiplied by 2 to give an approximate number around the entire proboscis. From the number of hook rows and hooks per row, this specimen is tentatively identified as *C. strumosum*.

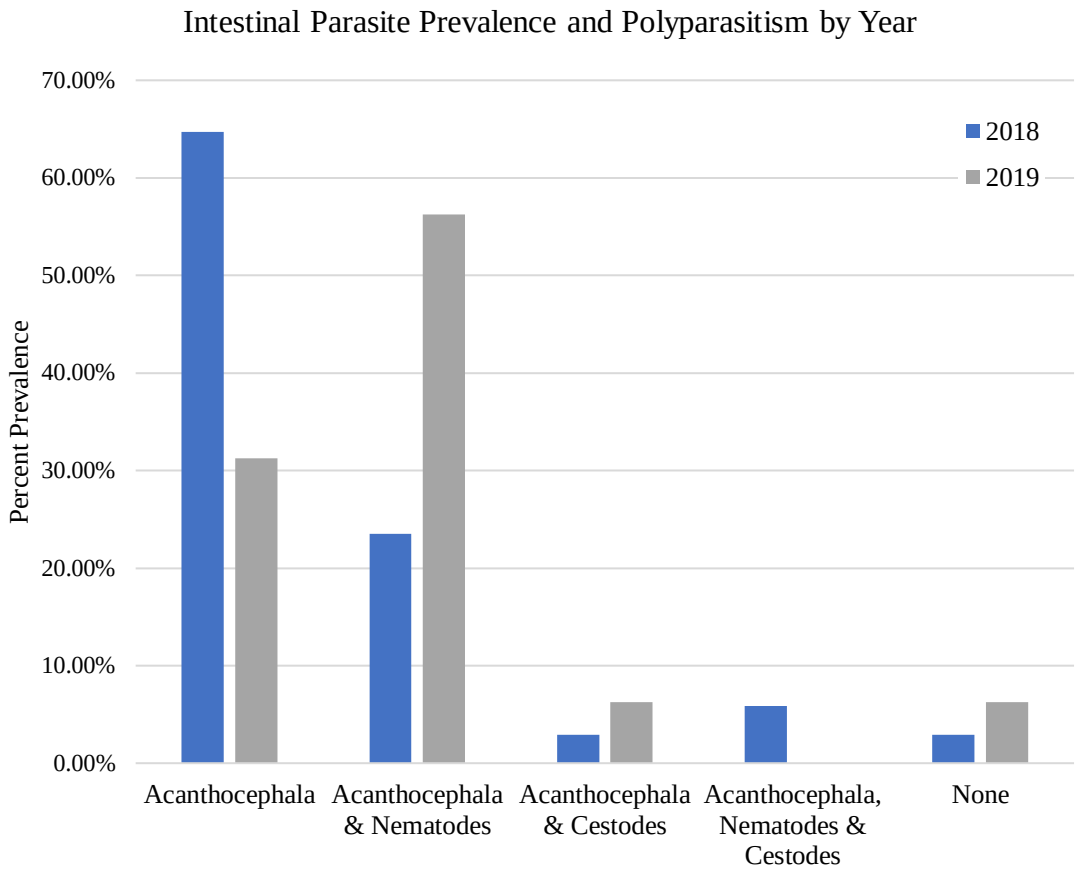

Figure 3.2: Intestinal Parasite Diversity and Polyparasitism. Acanthocephalans were the most common parasites found in the intestines. Nematodes and cestodes, while present, were never seen without acanthocephalans.

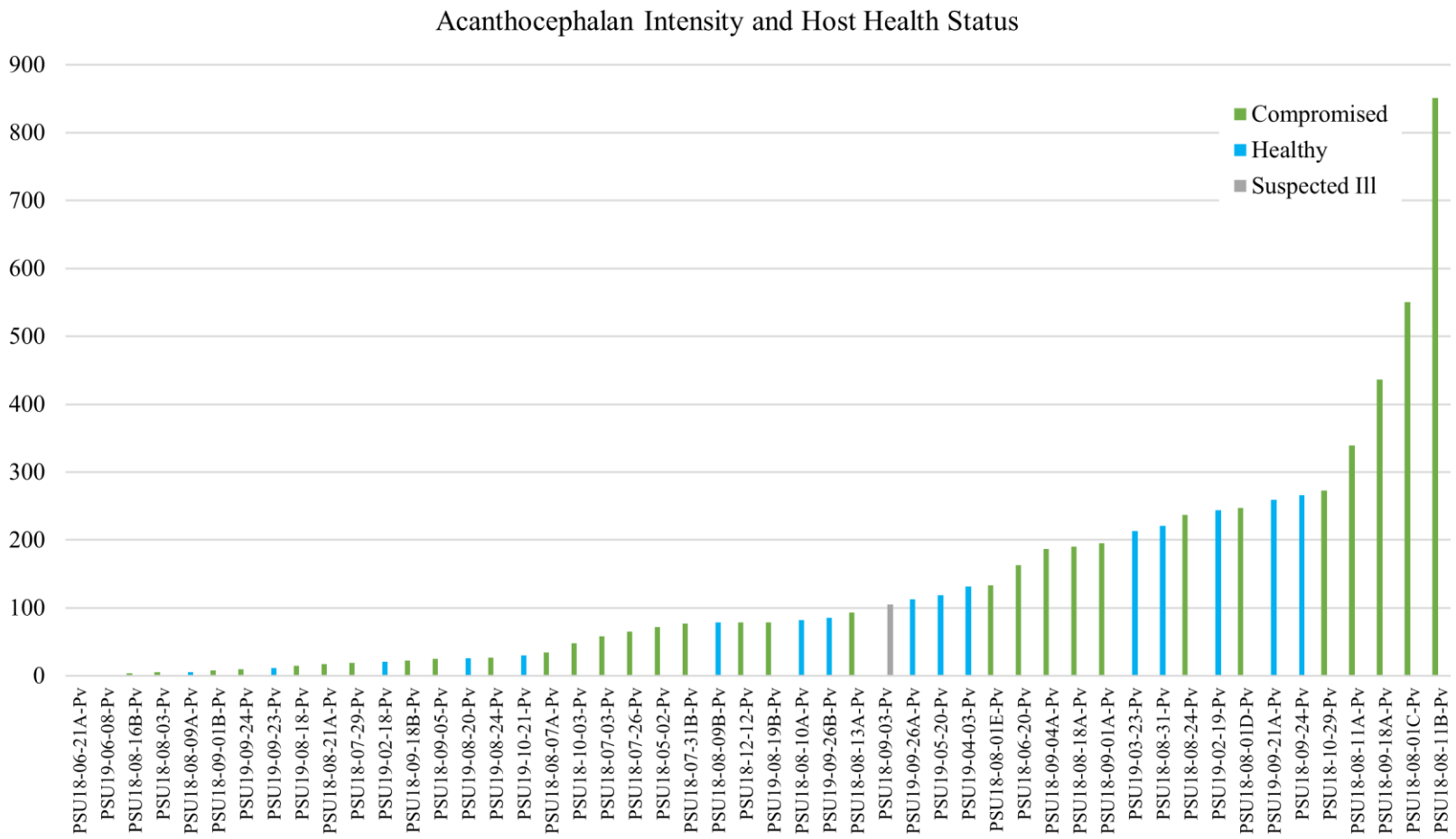

Figure 3.3: All recorded acanthocephalan intensities for each host. The average intensity was 131.3, and the range for healthy hosts was 0-266, but compromised hosts had a range from 0-851. However, there are only 5 compromised hosts with intensities that are above the range of healthy hosts which may have caused the lack of significance between the two health categories Two of these were considered outliers.

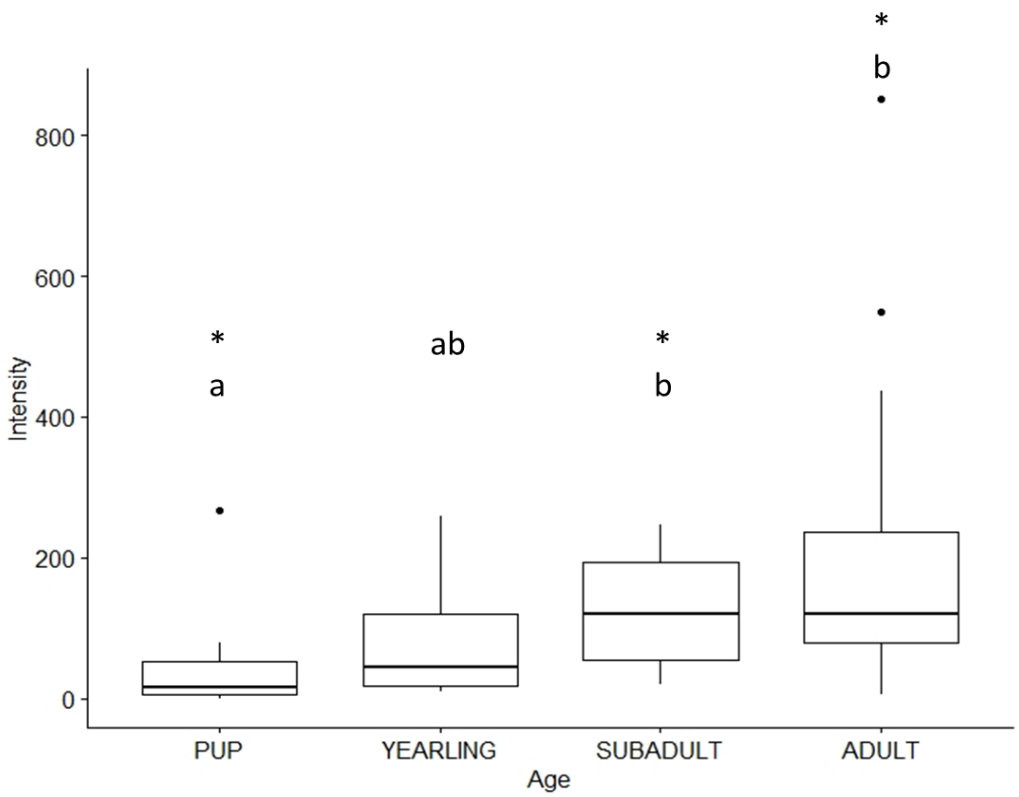

Figure 3.4: ANOVA boxplot of acanthocephalan intensity and host age class. Letters with * designate significance. The pups had significantly lower acanthocephalan intensities than the subadults and adults ($p < 0.001$).

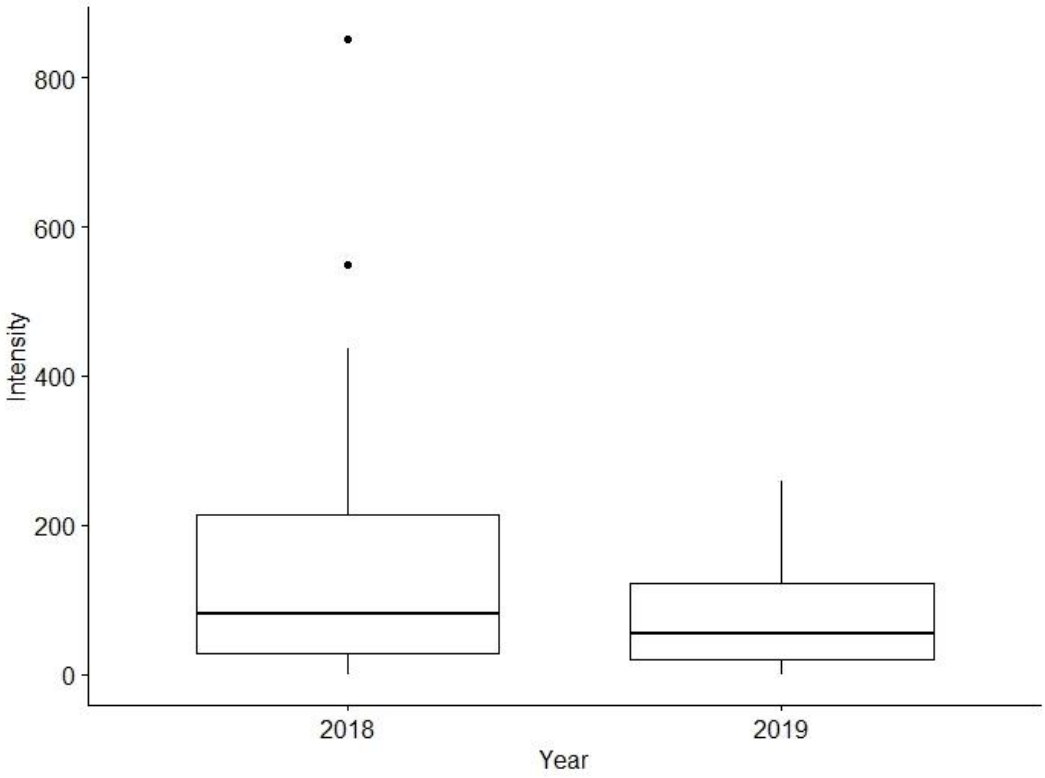

Figure 3.5: Boxplot of acanthocephalan intensity and year. The center line in the boxes represent the medians. This plot shows that although the means for the years were different (152 and 86.4), the medians were very similar. The two outliers seen above the 2018 box may have driven the difference between the means, increasing the variance between the two years.

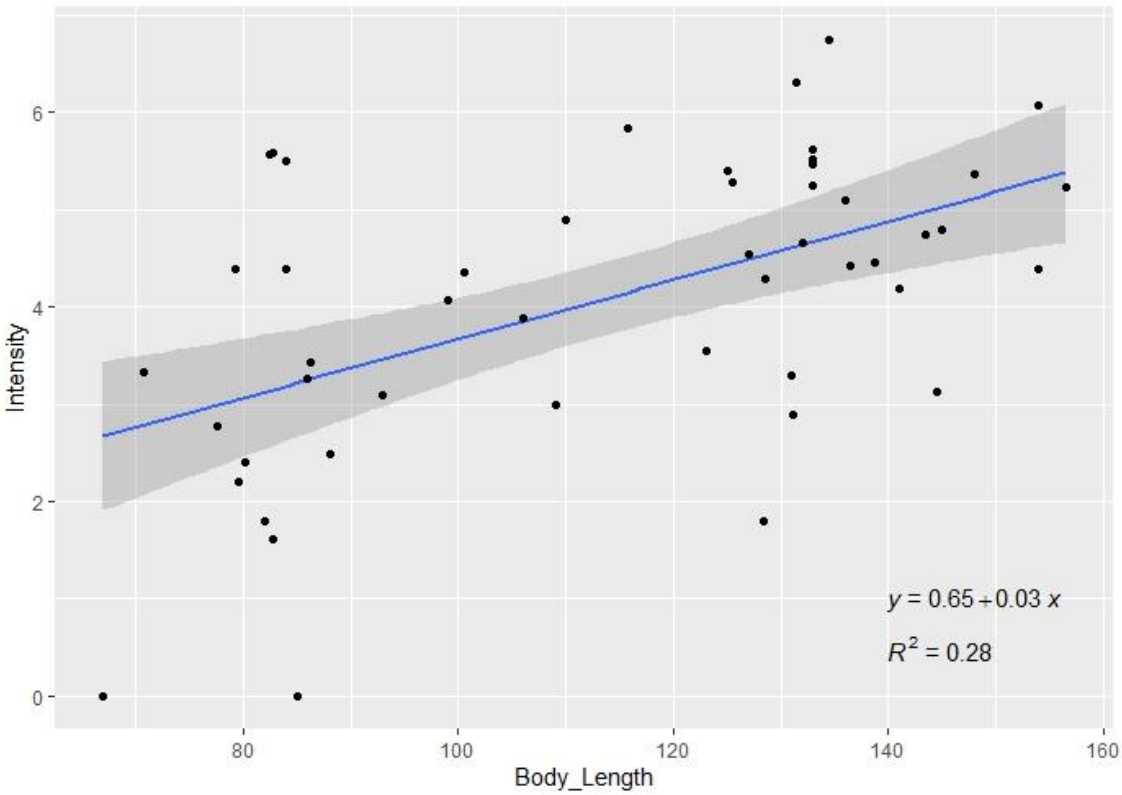

Figure 3.6: Linear regression of body length and acanthocephalan intensity. This relationship indicates that acanthocephalan intensity increases as the host gets larger (increases in age). Which is supported by the ANOVA results of acanthocephalan intensity and age class.

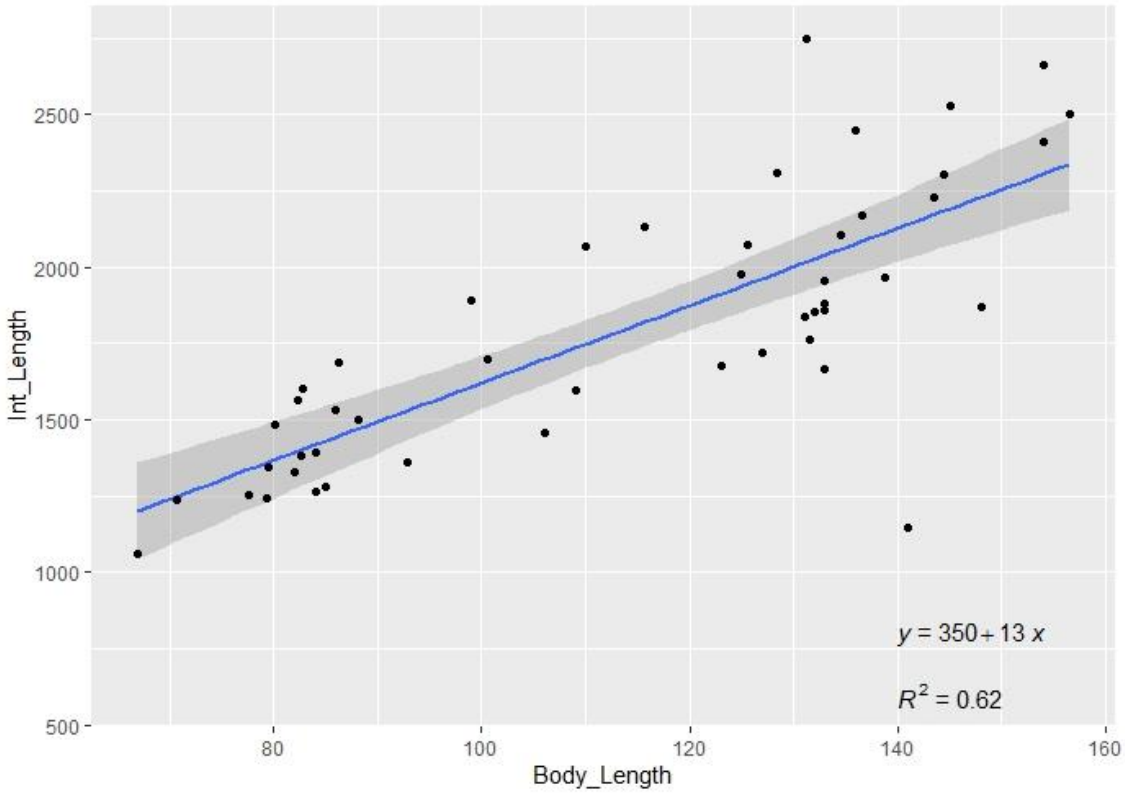

Figure 3.7: Linear regression of body length and intestinal length. This indicates a strong positive correlation between body length and intestinal length, supporting the idea that intestinal length is a factor of body length.

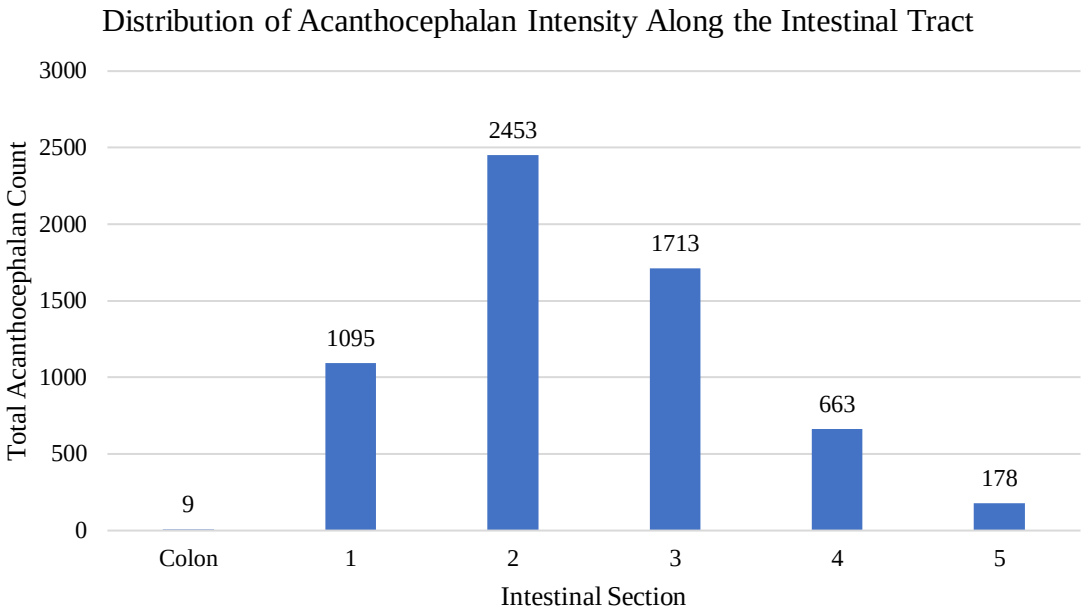

Figure 3.8: Distribution of total parasite intensity for each intestinal section. Section 1 is the most posterior covering the first 5 meters from the colon, and the following sections moving progressively anterior until the stomach was reached. Section 2 (starting 5m anterior to the colon), had the highest total intensity out of all the sections.

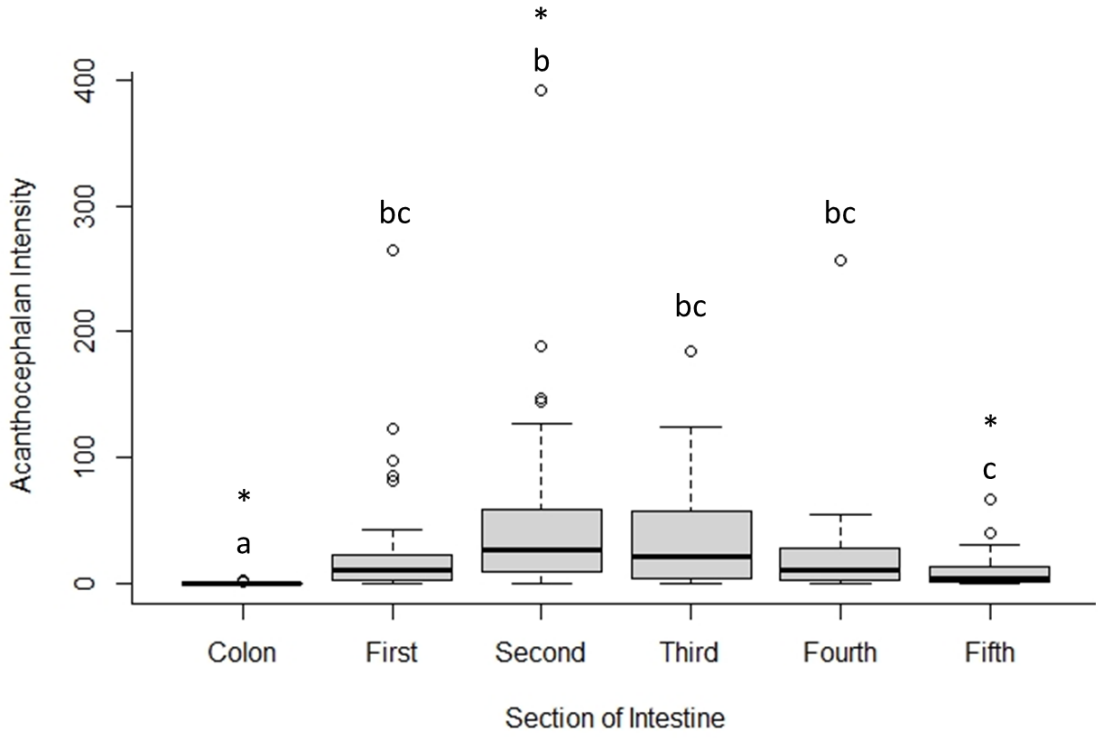

Figure 3.9: ANOVA of the distribution of acanthocephalan intensity throughout the intestinal tract. Letters with * indicate significance. The colon had significantly lower intensities of acanthocephalans than any other section ($p = 0$). The second section was statistically different from the fifth section, having higher acanthocephalan intensities ($p < 0.05$).

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Appendix A. Pinniped Intestinal Tract Analysis Form

Pinniped Intestinal Tract Analysis Form

Field ID: _____ Colon Length: _____

Date Processed: _____ Small Intestine Length: _____

Processor: _____ Total Length (Colon and Small):_____

Feces Collected (Circle one): Yes / No Amount: _____

Photographs Taken (Circle one): Yes / No

Total Parasites Collected: _____

General Notes:
(Ex. Overall coloration, texture, twisting, necrosis, nodules, ulcers, bones…)

Colon Analysis:
(Ex. Fecal material, parasites, ulcers, coloration…)

Ant. Diameter: _____

Mid. Diameter: _____

Post. Diameter: .

Small Intestine Analysis:
500cm sections working from the colon towards the stomach (duodenum). Note any abnormalities, parasites, and coloration of each section here. Flush in the natural direction of the system (stomach to colon). Your last section may not be exactly 500cm, so record its length in the proper section notes. Record diameters of each section (anterior end, posterior end, and middle).

Section #1 (first section after the colon):

Ant. Diameter: _____

Mid. Diameter: _____

Post. Diameter: .

Parasites: _____

Section #2:

Ant. Diameter: _____

Mid. Diameter: _____

Post. Diameter: .

Parasites: _____

Section #3:

Ant. Diameter: _____

Mid. Diameter: _____

Post. Diameter: .

Parasites: _____

Section #4:

Ant. Diameter: _____

Mid. Diameter: _____

Post. Diameter: .

Parasites: _____

Section #5:

Ant. Diameter: _____

Mid. Diameter: _____

Post. Diameter: .

Parasites: _____

Appendix B. Acanthocephala Staining Protocol

Acanthocephala Staining Protocol

This protocol was adapted from "*Helminth Slide Preparation with Semichon's Stain*" from Dr. Reyda at State University of New York College at Oneonta. A list of materials and solutions are provided below, be sure to wear appropriate PPE (googles, gloves, and lab coat) and have adequate ventilation when dealing specifically with Methyl Salicylate (it has a strong odor). From start to finish, this protocol takes about two and half hours. This includes some set up time, specimen preparation, staining procedure, and mounting time. The time for the specimens to cure will take a number of weeks depending on the concentration or type of mounting media. Very slightly dilute Canada balsam will take at least 5 weeks to fully cure. So be sure to take care with storing freshly made slides.

Materials needed:

- Glass microscope slides and cover slips
- Insect pins (#2?)
- Transfer pipettes
- Small glass petri dishes
- Sharp probe/fine tip forceps
- Kim wipes
- Small paint brush
- Cotton swabs
- Waste jars
- Timer
- Dissection and compound microscope
- Slide labels or etching pen

Solutions Needed:

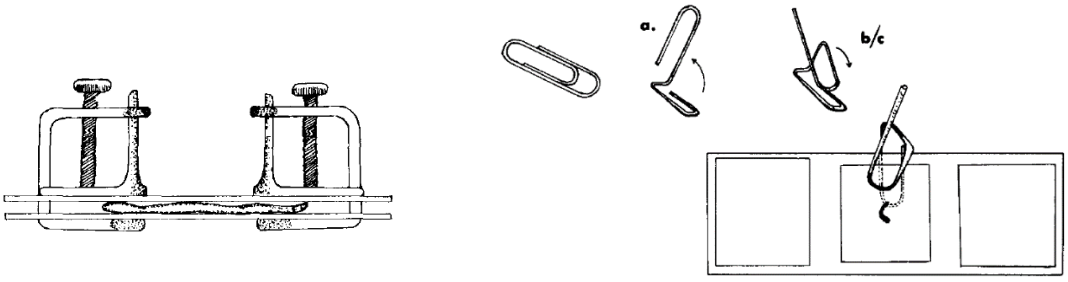
- Semichon's Acetocarmine Stain (filtered but not diluted)
- 70% Ethanol
- 70% Acidic Ethanol (HCl)
- 70% Basic Ethanol (Ammonium Hydroxide)
- 95% Ethanol
- 100% Ethanol
- Methyl Salicylate
- Canada Balsam

All parasites are stored in 70% EtOH. Throughout this protocol while you are adding in solutions, just add enough to fully cover the parasite. Do not let the parasite dry out. In your lab notebook, record the specimen ID you are working with, and take notes during the staining procedure (times left in solutions, male/female, copulatory cap present, etc.).

1. Using the transfer pipet, place a few parasites in one of the small glass petri dishes and add 70% ethanol.
2. Check parasites for fully extruded proboscis' using a dissection scope (REFER TO IMAGE).
3. If necessary, carefully remove attached tissues from the proboscis as needed using the sharp probe and fine tip forceps. Pay close attention to how the spines on the proboscis curve and pull attached tissues going with the direction of the curve to avoid damaging the spines.

4. Select the best specimen that has a fully extruded proboscis and transfer to a new small glass petri dish using the paint brush or transfer pipet. DO NOT USE FORCEPS! Pinching the specimen with forceps will damage it.
5. Puncture the parasite near the mid body (just below the bulge of the "head") with insect pins approximately four times and flatten by gently rolling the pin along the body of the parasite and then cover with a glass cover slip, let sit for 5ish minutes. Upon puncturing, there may be a slight 'popping' feeling and a milky substance may be released. These are eggs, no need to panic. You are puncturing the parasite to allow the stain to be fully absorbed and are also attempting to flatten it to make it easier to mount on a slide.
6. Remove the cover slip with forceps, pipette out the ethanol, and replace with Semichon stain for 15 minutes (until a nice dark purple color).
7. Pipette out the Semichon stain, add in clean 70% EtOH and wash (pipette up and down x 5) remove ethanol and replace with clean ethanol in between washes. Continue this for 3 minutes.
8. Pipette out the ethanol, add in acidic ethanol to destain for ~5 min or until it is a lighter pink/magenta color.
9. Pipette out the acidic ethanol, add in basic ethanol to stop the destaining process and let sit for 10 minutes.
10. Pipette out the basic ethanol, wash with fresh 70% ethanol for three minutes as you did in step 4 (**you can stop here if you need a break, can leave in this ethanol for 24 hours if needed**).
11. Pipette out the 70% ethanol and begin dehydration by adding in 95% ethanol and let sit for 10 minutes.
12. Pipette out the 95% ethanol, add in 100% ethanol for 10 minutes, and repeat with fresh 100% ethanol for another 10 minutes.
13. **Under proper ventilation**, pipette out the ethanol and add in methyl salicylate and hold parasite down with glass cover slip, let sit for 10 minutes.
    a. While the parasite is sitting in this stage, label your microscope slide and add a drop of the very slightly diluted Canada balsam.
14. Using the paint brush or a transfer pipette, carefully place parasite onto the drop of Canada balsam on your slide. Make sure it is resting on its side with its proboscis unobstructed and lying flat.
    a. You can adjust the positioning as best you using the sharp probe or insect pin while observing under a dissection scope.
15. Add another drop of Canada balsam on top of your parasite and carefully add your cover slip.
16. You can view the slide immediately to check the positioning of the parasite under the dissection scope or under a compound scope if you are extremely careful to not disturb the cover slip.
17. Because these parasites are not completely flat, you may need to use a weight or a molded paper clip to keep the cove slide compressed and level as your slide cures.

18. Leave in a cool and relatively dust free area to rest and dry (can take a few weeks to fully cure).



Techniques for flattening specimens mounted on slides. Images from "The Collection and Preservation of Animal Parasites" by Mary Hanson Pritchard and Gunther O.W. Kruse.

Appendix C. Parasite Taxonomy List

Species	**Taxonomy Credit**
Heartworm	
Acanthocheilonema spirocauda	Leidy, 1856
Dirofilaria immitis	Leidy, 1856
Echinophthirius horridus	Von Olfers, 1816
Lungworm	
Otostrongylus cicrumlitus	Railliet, 1899
Parafilaroides sps.	Dougherty 1946
Parafilaroides gullandae	Dailey, 2006
Parafilaroides decorus	Dougherty & Herman, 1947
Stomach Nematodes	
Anisakis sp.	Dujardin, 1845
Anisakis simplex	Rudolphi, 1809
Contracaecum sp.	Railliet & Henry, 1912
Contracaecum osculatum	(Rudolphi, 1802) Baylis, 1920
Pseudoterranova decipiens	(Krabbe, 1878) Gibson, 1983
Cestodes	
Anophryocephalus sp.	Baylis, 1922
Diphyllobothrium sp.	Cobbold, 1858
Diphyllobothrium alascense	Rausch & Williamson, 1958
Diplogonoporus sp. (Diphyllobothrium sp.)	Lönnberg, 1892
Adenocephalus pacificum (Diphyllobothrium pacificum)	Nybelin, 1931
Acanthocephalans	
Corynosoma sp.	Lühe, 1904
Corynosoma strumosum	(Rudolphi, 1802) Lühe, 1904
Corynosoma semerme	(Forssell, 1904) Lühe, 1911
Corynosoma cameroni	Van Cleave, 1953

Appendix D. Stranded Pacific Harbor Seal Data

Field ID	County	State	Latitude	Longitude	Sex	Age Class	Health Status	Year	Season	Parasites by Organ	Intestinal Parasites	Acanthocephala Intensity
PSU18-05-02-Pv	Clatsop	OR	46.03112	-123.9285	Female	Subadult	Compromised	2018	Spring	Stomach, Intestines	Acanthocephala & Nematodes	72
PSU18-06-20-Pv	Clatsop	OR	45.81092	-123.9644	Female	Adult	Compromised	2018	Summer	Stomach, Intestines	Acanthocephala & Nematodes	163
PSU18-06-21A-Pv	Clatsop	OR	46.02866	-123.9301	Female	Pup	Healthy	2018	Summer	None	No	0
PSU18-07-03-Pv	Pacific	WA	46.48165	-124.0605	Male	Yearling	Compromised	2018	Summer	Heart, Stomach, Intestines	Acanthocephala	58
PSU18-07-26-Pv	Pacific	WA	46.37323	-124.0646	Male	Adult	Compromised	2018	Summer	Intestines	Acanthocephala, Nematodes & Cestodes	65
PSU18-07-29-Pv	Clatsop	OR	45.9024	-123.966	Male	Subadult	Compromised	2018	Summer	Heart, Lungs, Stomach, Intestines	Acanthocephala & Nematodes	19
PSU18-07-31B-Pv	Clatsop	OR	46.17934	-123.9814	Female	Yearling	Compromised	2018	Summer	Heart, Lungs, Stomach, Intestines	Acanthocephala	77
PSU18-08-01A-Pv	Pacific	WA	46.36925	-124.0649	Female	Adult	Suspected Ill	2018	Summer	Stomach, Intestines	Acanthocephala	1
PSU18-08-01C-Pv	Pacific	WA	46.36326	-124.0655	Female	Adult	Compromised	2018	Summer	Stomach, Intestines	Acanthocephala	550
PSU18-08-01D-Pv	Pacific	WA	46.34696	-124.0665	Male	Subadult	Compromised	2018	Summer	Intestines	Acanthocephala	247
PSU18-08-01E-Pv	Clatsop	OR	46.0531	-123.9329	Male	Subadult	Compromised	2018	Summer	Stomach, Intestines	Acanthocephala	133
PSU18-08-03-Pv	Clatsop	OR	45.9079	-123.9681	Female	Adult	Compromised	2018	Summer	Stomach, Intestines	Acanthocephala	5
PSU18-08-07A-Pv	Pacific	WA	46.65104	-124.0727	Male	Adult	Compromised	2018	Summer	Heart, Stomach, Intestines	Acanthocephala	34
PSU18-08-09A-Pv	Clatsop	OR	45.90427	-123.9661	Female	Pup	Healthy	2018	Summer	Intestines	Acanthocephala	5
PSU18-08-09B-Pv	Clatsop	OR	46.192	-123.9889	Female	Adult	Healthy	2018	Summer	Stomach, Intestines	Acanthocephala	79
PSU18-08-10A-Pv	Pacific	WA	46.45537	-124.0605	Female	Adult	Healthy	2018	Summer	Stomach, Intestines	Acanthocephala	82
PSU18-08-11A-Pv	Clatsop	OR	46.19368	-123.9901	Female	Adult	Compromised	2018	Summer	Stomach, Intestines	Acanthocephala	339
PSU18-08-11B-Pv	Clatsop	OR	46.15854	-123.9696	Female	Adult	Compromised	2018	Summer	Stomach, Intestines	Acanthocephala	851
PSU18-08-13A-Pv	Pacific	WA	46.27622	-124.0425	Female	Adult	Compromised	2018	Summer	Intestines	Acanthocephala	93
PSU18-08-16B-Pv	Clatsop	OR	46.03614	-123.9308	Male	Pup	Compromised	2018	Summer	Stomach, Intestines	Acanthocephala	4
PSU18-08-18A-Pv	Pacific	WA	46.53627	-124.0623	Female	Subadult	Compromised	2018	Summer	Intestines	Acanthocephala	190
PSU18-08-21A-Pv	Pacific	WA	46.52264	-124.062	Male	Adult	Compromised	2018	Summer	Heart, Lungs, Stomach, Intestines	Acanthocephala	17
PSU18-08-24-Pv	Clatsop	OR	46.1801	-123.9819	Male	Adult	Compromised	2018	Summer	Stomach, Intestines	Acanthocephala	237
PSU18-08-31-Pv	Pacific	WA	46.41257	-124.0623	Female	Subadult	Healthy	2018	Summer	Intestines	Acanthocephala	221
PSU18-09-01A-Pv	Pacific	WA	46.42852	-124.0618	Male	Subadult	Compromised	2018	Fall	Heart, Lungs, Stomach, Intestines	Acanthocephala	195
PSU18-09-01B-Pv	Pacific	WA	46.51882	-124.0616	Male	Pup	Compromised	2018	Fall	Heart, Lungs, Stomach, Intestines	Acanthocephala & Nematodes	8

Field ID	County	State	Latitude	Longitude	Sex	Age Class	Health Status	Year	Season	Parasites by Organ	Intestinal Parasites	Acanthocephala Intensity
PSU18-09-03-Pv	Clatsop	OR	46.16073	-123.9711	Male	Subadult	Suspected Ill	2018	Fall	Stomach, Intestines	Acanthocephala & Cestodes	105
PSU18-09-04A-Pv	Clatsop	OR	46.16275	-123.9721	Male	Adult	Compromised	2018	Fall	Stomach, Intestines	Acanthocephala	187
PSU18-09-05-Pv	Pacific	WA	46.35482	-124.0653	Female	Pup	Compromised	2018	Fall	Intestines	Acanthocephala & Nematodes	25
PSU18-09-14-Pv	Pacific	WA	46.43704	-124.0613	Male	Adult	Healthy	2018	Fall	Stomach, Intestines	Acanthocephala	51
PSU18-09-18A-Pv	Pacific	WA	46.44644	-124.0605	Male	Adult	Compromised	2018	Fall	Heart, Lungs, Stomach, Intestines	Acanthocephala	436
PSU18-09-18B-Pv	Clatsop	OR	46.23114	-124.0181	Male	Adult	Compromised	2018	Fall	Intestines	Acanthocephala	22
PSU18-09-24-Pv	Clatsop	OR	46.16281	-123.972	Male	Pup	Healthy	2018	Fall	Stomach, Intestines	Acanthocephala, Nematodes & Cestodes	266
PSU18-10-03-Pv	Pacific	WA	46.34955	-124.0656	Male	Subadult	Compromised	2018	Fall	Stomach, Intestines	Acanthocephala	48
PSU18-10-29-Pv	Pacific	WA	46.28913	-124.0765	Female	Adult	Compromised	2018	Fall	Stomach, Intestines	Acanthocephala & Nematodes	273
PSU18-12-12-Pv	Clatsop	OR	46.21171	-124.0023	Female	Pup	Compromised	2018	Winter	Heart, Lungs, Stomach, Intestines	Acanthocephala & Nematodes	79
PSU19-02-18-Pv	Pacific	WA	46.65066	-124.072	Female	Yearling	Healthy	2019	Winter	Heart, Lungs, Stomach, Intestines	Acanthocephala & Nematodes	21
PSU19-02-19-Pv	Clatsop	OR	45.97607	-123.9387	Male	Yearling	Healthy	2019	Winter	Lungs, Intestines	Acanthocephala & Nematodes	244
PSU19-03-23-Pv	Pacific	WA	46.59047	-124.0668	Female	Adult	Healthy	2019	Spring	Stomach, Intestines	Acanthocephala & Nematodes	213
PSU19-04-03-Pv	Pacific	WA	46.47265	-124.0604	Female	Adult	Healthy	2019	Spring	Intestines	Acanthocephala	131
PSU19-05-20-Pv	Pacific	WA	46.37447	-124.0641	Female	Adult	Healthy	2019	Spring	Intestines	Acanthocephala	119
PSU19-06-08-Pv	Clatsop	OR	45.98923	-123.9346	Male	Pup	Compromised	2019	Summer	None	No	0
PSU19-08-18-Pv	Pacific	WA	46.45018	-124.0608	Male	Pup	Compromised	2019	Summer	Heart, Lungs, Stomach, Intestines	Acanthocephala & Nematodes	15
PSU19-08-19B-Pv	Clatsop	OR	46.00209	-123.9313	Female	Pup	Compromised	2019	Summer	Stomach, Intestines	Acanthocephala	79
PSU19-08-20-Pv	Pacific	WA	46.43775	-124.0611	Male	Subadult	Healthy	2019	Summer	Stomach, Intestines	Acanthocephala & Cestodes	26
PSU19-08-24-Pv	Clatsop	OR	46.02634	-123.9303	Female	Pup	Compromised	2019	Summer	Lungs, Stomach, Intestines	Acanthocephala & Nematodes	27
PSU19-09-21A-Pv	Pacific	WA	46.52877	-124.0622	Female	Yearling	Healthy	2019	Fall	Lungs, Intestines	Acanthocephala	259

Field ID	County	State	Latitude	Longitude	Sex	Age Class	Health Status	Year	Season	Parasites by Organ	Intestinal Parasites	Acanthocephala Intensity
PSU19-09-23-Pv	Pacific	WA	46.41045	-124.0619	Female	Yearling	Healthy	2019	Fall	Lungs, Stomach, Intestines	Acanthocephala & Nematodes	11
PSU19-09-24-Pv	Pacific	WA	46.36441	-124.065	Male	Yearling	Compromised	2019	Fall	Lungs, Stomach, Intestines	Acanthocephala & Nematodes	10
PSU19-09-26A-Pv	Pacific	WA	46.48569	-124.0606	Male	Adult	Healthy	2019	Fall	Intestines	Acanthocephala & Nematodes	113
PSU19-09-26B-Pv	Pacific	WA	46.62889	-124.0724	Female	Adult	Healthy	2019	Fall	Stomach, Intestines	Acanthocephala & Nematodes	85
PSU19-09-30-Pv	Clatsop	OR	45.98525	-123.9348	Female	Subadult	Healthy	2019	Fall	Stomach	No	0
PSU19-10-21-Pv	Pacific	WA	46.42431	-124.0613	Male	Yearling	Healthy	2019	Fall	Lungs, Stomach, Intestines	Acanthocephala	30