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Long-term change in the burden of anisakid nematode parasites for marine mammal hosts

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Abstract

Long-term change in the burden of anisakid nematode parasites for marine mammal hosts

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Many marine mammal populations are failing to rebound despite legal protections; in this dissertation, I explore whether rising rates of parasitism may be placing an increasing energetic burden on already precarious host populations. Gastrointestinal parasites of the family Anisakidae are commonly found during necropsies of marine mammals. Anisakids have complex life cycles, requiring multiple intermediate hosts, including invertebrates, fishes, and cephalopods, to reach their definitive marine mammal hosts. These parasites have long been assumed to have only mild impacts on host health, but recent research suggests that the seriousness of these infections has been overlooked, with health effects ranging from energy loss and reduced immune system function to gastritis and ulceration in the gastrointestinal tract. Anisakid abundance in intermediate fish hosts is increasing globally. In some regions, increases in anisakids in fish have been linked to increasing marine mammal definitive host abundance. With legislation prohibiting their exploitation, many marine mammal populations have been able to recover to historic abundances, potentially increasing anisakid transmission in their ecosystems. However, other marine mammals have lagged in their recovery. As some marine

mammal species increase, they may be increasing the burden of parasitism on more vulnerable species, essentially engaging in "apparent competition." In this dissertation, I assess how anisakid abundances have changed in important prey species both globally and in the North Pacific, what factors may drive these changes, and estimate infection rates in a population of endangered killer whales. In Chapter 1, I used a meta-analytic dataset of anisakid abundance in fish and cephalopod species to assess how anisakid abundance has changed globally in important marine mammal prey species over the past few decades. However, this dataset does not include data from the marine-mammal-rich Northeast Pacific. In Chapter 2, I use museum specimens of ecologically important fish prey species collected in Puget Sound, Washington, USA, from 1920–2018 to determine how anisakid abundances have changed over time in relation to possible definitive host and environmental drivers. Museum collections lack adult salmon specimens, a common prey item for many marine mammals in this region. In Chapter 3, I use a novel data source, archived canned salmon, to assess changes in anisakid abundance in four species of Alaskan salmon from 1979–2021. Finally, in Chapter 4, I test for parasitism among southern resident killer whales, a highly endangered marine mammal population whose range includes Alaska and Puget Sound; I also assess whether infections are correlated with poor body condition. Taken together, these four chapters reveal recent increases in anisakids both globally and regionally in the Northeast Pacific. In Puget Sound, this increase coincides with and may be caused by increasing marine mammal abundances. Furthermore, southern resident killer whales face a high burden of anisakids, which may be putting additional energetic stress on this already vulnerable population. My findings reveal a changing landscape of infection risk, which could represent an increasing threat to vulnerable marine mammal populations in Puget Sound, Alaska, and beyond.

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PREAMBLE

Until 1972, many marine mammal populations were depleted due to unregulated anthropogenic harvest, and regulations have been enacted both nationally (Marine Mammal Protection Act, 1972) and internationally (International Whaling Commission's moratorium on commercial whaling, 1986) to protect remaining populations (Lotze et al. 2011, Roman et al. 2013, Magera et al. 2013). Some species have increased to carrying capacity (e.g., grey seals *Halichoerus grypus*, and California sea lions *Zalophus californianus*) and others are near the brink of extinction (e.g., southern resident killer whales *Orcinus orca*, North Atlantic right whales *Eubalaena glacialis*), largely as a function of the extent and timing of their prior exploitation (Lotze et al. 2011, Knowlton et al. 2012, Roman et al. 2013, Magera et al. 2013, NOAA 2016). The observed lack of recovery in some species is partially due the continuation of activities that were not strictly regulated by these pieces of legislation, including chemical contamination, ship strikes, bycatch, and entanglement (Laist et al. 2001, Cassoff et al. 2011, Trumble et al. 2013). Due to persistent stressors, there are currently 14 populations of marine mammals listed as endangered under the U.S. Endangered Species Act (ESA) and 20 listed as endangered or critically endangered by the International Union for Conservation of Nature (IUCN) Red List.

While many stressors on marine mammal populations are well understood, parasitism remains understudied. This is largely due to the difficulty of assessing a marine mammal for parasites. Parasitological examinations can only be conducted on difficult-to-obtain fecal samples from wild animals or necropsies of deceased animals, the latter being unrepresentative of a healthy, wild individual (Dailey and Stroud 1978, Aguilar and Borrell 1994, Ten Doeschate et al. 2017, Hermosilla et al. 2018). Because of the difficulty of studying parasite infections in wild, living animals, there is a deficit of knowledge on parasitism in marine mammals, including

the prevalence and intensity of infections. Through necropsies, we know that marine mammals host many parasites (Dailey et al. 1980, Dailey and Stroud 1978, Stroud and Roffe 1979). Parasites can cause mild to severe health problems for these hosts (Dailey 2001). For example, parasitic intestinal nematodes deprive their hosts of energy, and in some cases cause severe pathology (Dailey and Stroud 1978, Stroud and Roffe 1979, van Beurden et al. 2015).

One parasite family frequently found in the intestines of marine mammals are nematodes (i.e., roundworms) in the family Anisakidae, which include three prominent genera found in marine mammals: *Anisakis*, *Pseudoterranova,* and *Contracaecum*. Worms in each genus have complex life cycles that require both intermediate (i.e., required for larval development) and paratenic (i.e., not required for larval development) hosts to disperse the parasites to their definitive hosts via trophic transmission (McClelland 2005, Klimpel and Rückert 2005, Palm and Klimpel 2007, Klimpel and Palm 2011). The intermediate and paratenic hosts are invertebrates and fish, though the definitive hosts differ by parasite species.

A recent study reported a global increase in *Anisakis* spp. abundance in fish and squid intermediate hosts (Fiorenza et al. 2020). The authors conducted a meta-analysis of records of *Anisakis* and *Pseudoterranova* spp. in fish and squid host species published in peer-reviewed literature from 1967 to 2017. The data were spatially biased—many of the samples were collected from the Atlantic and Southern Oceans, and the Mediterranean Sea, while the Pacific was underrepresented. The authors found that there was a significant increase in *Anisakis* spp. over the past 53 years, but that trend did not hold true for *Pseudoterranova* spp. over a 37-year period. With this increased abundance of *Anisakis* in intermediate hosts, are marine mammals more prone to *Anisakis* infections today than they were in the past? If *Anisakis* spp. are increasing in abundance in their fish intermediate hosts, this implies that the risk of infection for

marine mammals that prey on those fishes is also increasing. In Chapter 1, I address these questions by assessing whether the global trend observed in Fiorenza et al. (2020) holds in the prey species of marine mammals.

However, few prior studies available for meta-analysis have assessed anisakid abundance in the North Pacific, so the fate of local marine mammals is largely unknown. Without this information, it is difficult to determine whether the observed global increase in anisakids poses a risk to marine mammals in this region. Previous work has shown that intermediate host anisakid infections are correlated with regional increases in marine mammal abundance (Buchmann and Kania 2012, Haarder et al. 2014, Mehrdana et al. 2014). Of the marine mammal species globally with enough data to detect a trend, 42% are increasing; overall, they have recovered to 61% of their historical abundance (Magera et al. 2013). Increases in the total number of marine mammal hosts in turn increases the potential for parasite transmission to both the marine mammal populations that are recovering and those that are not. In Chapter 2, I assess whether trends in anisakid abundance in fish are correlated with marine mammal abundance in Puget Sound over a 98-year period. This chapter makes use of museum specimens of common marine mammal prey species, but unfortunately does not incorporate common higher trophic level prey, like salmon.

Salmon (*Onchorhynchus* spp.) are culturally, economically, and ecologically important in the North Pacific (Carothers et al. 2021, Alaska Department of Fish and Game 2023). Salmon also make up a large part of the diet of many marine and terrestrial predators (Quakenbush et al. 2015, Morton 1990, Sigler et al. 2009, Helfield and Naiman 2006, Stanek et al. 2017). An increase in parasites in salmon could impact human consumers and wildlife, as well as the state’s economy, but estimating changes over time requires historical data on the parasite burden in salmon. Because salmon are so large, there are few adult salmon available in space-limited

museum collections. In Chapter 3, I address this deficit by determining how anisakid abundance has changed over time in four species of Alaskan salmon using canned salmon samples collected from 1979–2020.

Rising abundances of *Anisakis* spp. may pose a threat to many marine mammal species, but this is especially worrisome for populations that are already declining and facing cumulative stressors. Southern resident killer whales (*Orcinus orca*) are piscivorous killer whales that range from British Columbia to Northern California (Hanson 2015). Southern residents have been photo-identified in the Salish Sea since 1973, coinciding with the last years of the selected removal of killer whales for the aquarium trade, making them one of the most intensively studied cetacean populations in the world (Mann et al. 2000). The main threats to this population are a shortage of their preferred prey, Chinook salmon (*Oncorhynchus tshawytscha*) and chum salmon (*Oncorhynchus keta*, Ford and Ellis 2006); high concentrations of contaminants from major neighboring cities of Seattle, Vancouver, and Victoria (Krahn et al. 2007); and vessel noise from small boats and ships in the Salish Sea (Williams et al. 2014). Due in part to these stressors, the population was listed as endangered by the U.S. in 2005, yet the SRKW population continued to decline for two decades, and population currently comprises 74 whales (Lacy et al. 2017). Southern residents, and killer whales in general, have a low population growth rate, which leaves them particularly susceptible to stressors that impact survival and reproduction (Stark et al. 2004). While an individual might be able to withstand the impacts of one sublethal stressor, like a shortage of available prey, when acting in concert with others like contaminants and acoustic stress, the ability to withstand the stressors decreases (Wright 2012, Williams et al. 2016). Currently, modeling the cumulative effects of these known stressors of southern residents results in a projection that the population should be stable (Lacy et al. 2017). Why, then, is the

population declining? One stressor that has not been investigated is parasitism. Parasites reduce the energy available for immune response, resulting in a host that is more vulnerable to other infections, and increasing the impact of additional stressors (Beldomenico et al. 2008; Marcogliese and Pietrock 2011). This can lead to a negative feedback loop, in which killer whales undergo stress from multiple factors, leaving them unable to resist infection, which as a result, reduces their condition and leaves them more vulnerable to further stress (Beldomenico and Begon 2010). Additionally, host characteristics may influence susceptibility to parasite infections, meaning that parasitism may disproportionately affect some members of the population based on age or sex (Marcogliese and Pietrock 2011). If young whales are particularly susceptible to parasitism, then the southern resident population may not recover at the projected rate (Lacy et al. 2017).

To conserve southern residents, a better understanding of the environmental prevalence and impact of intestinal parasites on marine mammal health is necessary. That includes determining if these parasites are a new problem or a persistent stressor in the environment, estimating the prevalence of intestinal parasites in southern residents, and determining whether parasite presence is correlated with poor health in living whales. Without baseline information about the history of anisakids in this region and their impact on southern residents today, parasitism as a sublethal stressor cannot be addressed in the management and conservation of southern residents. In Chapter 4, I address this data gap by investigating the prevalence of intestinal parasites in southern residents compared to other resident killer whales in the Northeast Pacific and determine the demographic correlates of parasite burden.

Taken together, these four chapters provide new evidence that the risk of anisakid nematode infection has changed over time in marine mammal prey species, both globally and in

the Northeast Pacific. My work reveals a link between marine mammal abundance and anisakid abundance in intermediate host fish species over the course of a century, a finding that has been observed elsewhere but not over a similar temporal range. It also generates the first long-term dataset of anisakid abundance in adult salmon, which are a major prey item for marine mammals in the Northeast Pacific. These new findings about changing anisakid prevalence provide context for my findings on killer whale parasite assemblages, and show how the parasite infections observed today may relate to parasite infections in previous decades. Finally, my work provides the first assessment of parasite infections in living southern resident killer whales, uncovers what factors are likely to increase parasite prevalence, and offers support for further parasitic monitoring in this highly endangered population.

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Chapter 1: Meta-analysis suggests that, for marine mammals, the risk of parasitism by anisakids has changed over a half-century

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ABSTRACT

Gastrointestinal parasites are a potentially consequential stressor for marine mammal species due to their health impact. Although they are infrequently identified as a cause of mortality, gastrointestinal parasites could drive cryptic declines in individual fitness – including declines in nutritional status – by acting synergistically with other stressors. Nematodes in the family Anisakidae are ubiquitous throughout the world's oceans and are transmitted to marine mammal hosts through the consumption of fish and cephalopod prey. We sought to assess whether marine mammals face a rising risk of gastrointestinal infection due to an increase in anisakid burden of their prey species, using data compiled from a recent meta-analysis of anisakid abundance in fish and invertebrates from 1967 to 2015. We reviewed the diets of 113 marine mammal species to identify their known prey species. We extracted data on anisakid burden for each of the prey species and tested whether anisakid abundance in these intermediate hosts had increased over time. Our findings suggest that *Anisakis* spp. burden in marine mammal prey species has increased over the past half-century, while *Pseudoterranova* spp. burden showed no significant

change. Our findings suggest that the risk of *Anisakis* spp. infection is increasing for marine mammal hosts.

INTRODUCTION

Marine mammals have faced substantial changes in anthropogenic stressors over the past 50 years. In that time, several pieces of legislation have reduced the burden of commercial harvest and placed protections on many marine mammal populations, including the U.S. Marine Mammal Protection Act of 1972 (MMPA), Europe's Habitats Directive of 1992, the U.K.'s Wildlife and Countryside Act of 1981, Australia's Environment Protection and Biodiversity Act of 1999, and the International Whaling Commission's global moratorium on commercial whaling in 1986. At a global scale and among the 47 marine mammal species with at least 10 years or 3 generations of data, 61% have exhibited an increase (Magera et al. 2013). Within the US and among the marine mammal species with sufficient data, there are reports of population recoveries over the past 40 years (Gulland and Hall 2007; Roman et al. 2013). Since the enactment of the MMPA, 7–21% of marine mammal stocks have increased in US waters (Roman et al. 2013). Despite these observed increases, 2% of marine mammal stocks in the US have not recovered, and an additional 3% have exhibited declines; this is in addition to the 71% of stocks that lack sufficient data to detect a trend (Roman et al. 2013). Outside the US, many marine mammal species lack substantive protection (Roman et al. 2013). While direct mortality via harvest has declined, other anthropogenic stressors like fishing interactions, vessel strikes, and indirect effects of climate change, as well as less detectable sublethal stressors (i.e., stressors that do not cause direct mortality), threaten the recovery of many species (Roman et al. 2013).

Parasitic disease may be one factor contributing to reported marine mammal declines or recovery failures. Gastrointestinal nematodes are commonly reported during necropsies of

cetaceans and pinnipeds (Dailey 2001), including species in the family Anisakidae, which are among the most prevalent parasites in marine mammals (Dailey 2001; Iñiguez et al. 2011). The anisakids include the genera *Anisakis* Dujardin, 1845, commonly known as whaleworm, *Pseudoterranova* Mozgovoi, 1951, commonly known as sealworm, and *Contracaecum* Railliet and Henry, 1912.

Anisakis and *Pseudoterranova* have complex life cycles (Figure 1.1). *Anisakis* spp. nematodes infect cetaceans as their definitive host, and *Pseudoterranova* spp. infect pinnipeds, though larval forms of *Pseudoterranova* have been found in cetaceans and larval *Anisakis* have been found in pinnipeds (Dailey and Brownwell 1972; Dailey 2001). The life cycles involve four to five larval phases (summarized in Klimpel and Palm 2011; Anderson 1992; McClelland 2002; Mattitucci et al. 2018). Unembryonated eggs are expelled from the marine mammal host via feces. They embryonate in seawater, maturing within the egg (Køie 2001). The larvae molt once or twice and emerge as an ensheathed, free-swimming larva (McClelland 2002, Køie et al. 1995). Anisakid larvae continue their life cycle when ingested by the first intermediate host, a crustacean (McClelland 1982; Køie et al. 1995). Fish and cephalopods that prey on this crustacean host can then act as paratenic hosts for *Anisakis* spp., which are not obligately required for the parasite's development but which efficiently pass the parasite up the food web to their definitive marine mammal host (e.g., Klimpel and Palm 2011). For *Pseudoterranova* spp., fish serve as an obligately required second intermediate host (McClelland 1995; McClelland and Martell 2001). When the larva is ingested, it either remains in the visceral cavity or organs, or migrates to the muscle tissue, depending on the species (e.g., Mattiucci et al. 2018; Cipriani et al. 2016; Karl and Levsen 2011; Levsen and Lunestad 2010; Levsen et al. 2017; Roepstorff et al. 1993). Marine mammals become infected by ingesting a paratenic or intermediate host harboring

one or more larva, at which point the pre-adult larva reaches sexual maturity (Klimpel and Palm 2011; McClelland 2002).

Once inside their marine mammal definitive host, anisakids can cause both direct and indirect fitness costs. After being consumed with an intermediate host, the nematodes reproduce in the host stomach, inhabiting the stomach compartments either in the gastric lumen or attached to the gastric or stomach wall mucosa (Dailey 2001; Geraci and St. Aubin 1987; Iñiguez et al. 2011). It is unknown what adult nematodes and subadult larvae feed on while in the gastrointestinal tract, but whether they consume the food of the host or the host itself, anisakids are an energy sink, sequestering nutrients away from their hosts (Combes 2001). The proportion of host energy taken by these parasites is currently unknown. At the site of anisakid infection, the host immune system forms granulomas, often in the stomach lining, causing gastritis, ulceration, and secondary bacterial infections (Cattan, Babero, and Torres 1976; Martineau et al. 2002; Dailey 2001). Individual worms cluster in groups of 50–100 with their anterior ends embedded in an ulcer, which can reach up to 6 cm in diameter (Geraci and St. Aubin 1987.; Motta et al. 2008; Audicana et al. 2003). The ulcers may be acute and hemorrhagic, or chronic, and can be associated with edema (Raga et al. 2009; Alves Motta et al. 2008; Haebler and Moeller 2021). In stranded cetaceans off the coast of Brazil, six of eight animals with anisakid infections exhibited chronic lymphoplasmocytic gastritis (Motta et al. 2008). In severe infections, the perforations of the stomach wall can cause peritonitis, ultimately leading to hemorrhage, anemia, and rarely, death (Dailey 1985; Dailey and Stroud 1978; Smith 1989; Stroud and Roffe 1979; Margolis et al. 1997; Ballachey et al. 2002; van Beurden et al. 2015). Whether or not these infections result in disease or mortality, they reduce the hosts' fitness, which can have individual and population-level consequences (Shanebeck et al. 2022).

With some marine mammals increasing while others languish or decline, threats that can cross species barriers become especially salient for conservation. While some marine mammals are able to recover, as they increase in density, they facilitate parasite transmission (Anderson and May 1978; Arneberg et al. 1998), increasing the availability of parasite propagules in the environment and the potential for infection in declining marine mammal species. Marine mammals have high energetic demands (McNab 2012; Berta et al. 2020), and populations that are declining due to sub-lethal stressors are likely to face even higher energy demands (McNab 1989; McNab 2012; King et al. 2015, Berta et al. 2020). This can result in individuals having less energy available to devote to immune response, and therefore being more susceptible to infection (Beldomenico et al. 2008; Shanebeck et al. 2022; e.g., Lair et al. 2016; Colegrove et al. 2016; Seguel et al. 2018). In turn, an increased susceptibility can lead to greater pathology from parasite infections or higher fitness costs, and ultimately population level consequences (King et al. 2015; May et al. 2019; Shanebeck et al. 2022), thereby increasing the impact of existing energetic stressors (Beldomenico et al. 2008). Healthy marine mammal populations with few stressors, on the other hand, may increase in abundance and tolerate parasite infections, further serving as breeding grounds for parasites and possibly increasing the population of infectious helminths.

Recently, Fiorenza et al. (2020) demonstrated that there has been a global increase in the abundance of *Anisakis* sp. in fish and cephalopod intermediate hosts. The authors conducted a meta-analysis of records of *Anisakis* and *Pseudoterranova* spp. in fish and squid host species published in peer-reviewed literature from 1967 to 2017. They found that there was a 283-fold increase in *Anisakis* spp. between 1978 and 2015, and no change in the abundance of *Pseudoterranova* spp. Does this finding mean that marine mammals are at greater risk of

Anisakis infections today than they were in the past? Fiorenza et al. (2020) could not answer this question, because they included in their meta-analysis all species of fish and invertebrates that were sampled and screened for anisakid infections, not all of which are diet components for marine mammal hosts. While Fiorenza et al. (2020) found a global increase in *Anisakis* spp., they could not determine whether this represented an increase in risk to marine mammal definitive hosts.

We sought to determine whether marine mammals face an increased risk of anisakid infection by assessing how anisakid prevalence has changed in prey species of marine mammals over four decades (1967–2007). This time period is especially important, as this 35-year window immediately followed the passage of the MMPA and the Endangered Species Act (1973) in the US and encompasses the beginning of the international moratorium on commercial whaling. We made use of Fiorenza et al. (2020)'s existing meta-analytic database by extracting those records that pertained to (1) marine mammal prey species and (2) geographically overlapped with the range of their marine mammal predator.

We used anisakid burden within prey species as a proxy for marine mammal risk rather than obtaining infection data directly from marine mammals for a number of reasons. While there is evidence from direct sampling of hosts that viral and bacterial diseases of marine mammals have increased from 1966 to 2007, metazoan parasites, which were well studied in the 1960s, have not received much research attention in recent years, and therefore the temporal trend in helminth burden is currently unknown (Gulland and Hall 2007). Direct parasitological examinations can only be conducted on difficult-to-obtain fecal samples from wild animals or necropsy samples from deceased individuals, the latter being skewed toward overrepresentation of unhealthy individuals (Aguilar and Borrell 1994; Dailey and Stroud 1978; Hermosilla et al.

2018; Ten Doeschate et al. 2017). Gastrointestinal helminths rarely cause mortality, but may contribute to poor marine mammal health in vulnerable species threatened by multiple stressors. By investigating how anisakid burden has changed in marine mammal prey over time, we were able to quantify temporal trends in infection risk for marine mammals. We sought to answer the following questions: (1) have *Anisakis* spp. and *Pseudoterranova* spp. abundance increased over time in the prey species marine mammals commonly eat, and (2) does the trajectory of change in anisakid abundance differ between two major marine mammal groups (i.e., cetaceans versus pinnipeds).

METHODS

Data collection

Literature search and data extraction

We used a publicly available and published dataset (Fiorenza et al. 2020) to obtain estimates of *Anisakis* spp. and *Pseudoterranova* spp. abundance from 1978 to 2015. To compile their meta-analytic dataset, Fiorenza et al. (2020) conducted a search in Web of Science in October 2017 using the search terms anisak* or "herring worm" or "herringworm" or Pseudoterranova or whaleworm or "whale worm" or phocanema or "whale-worm" (Figure 2). The search resulted in 2,284 papers, which were then screened for suitability using their titles (2,284 papers), abstracts (1,336 papers), and full text (576 papers). In this process, the authors removed any papers that focused on non-fish/non-cephalopod hosts or non-target parasites, experimental manipulation studies, and reviews. The final pool of papers (123) reported host and parasite species identity, location and year of collection, size of host, how parasitological examinations were conducted, and prevalence and intensity or abundance of infection with an error estimate. From these papers,

the authors extracted 755 data points, or unique estimates of parasite abundance for a host species in a particular location at a particular time. Of these data points, 69.7% represented *Anisakis* spp. and 30.3% *Pseudoterranova* spp. (Fiorenza et al. 2020).

Identifying records pertinent to marine mammal health

To extract the records from the Fiorenza et al. (2020) dataset that were germane to marine mammal health, we sought the subset of records from marine mammal prey species in geographic areas where their marine mammal predators occur. We first compiled all marine mammals included in the IUCN database (IUCN 2018), regardless of Red List status, including all marine species in the genera *Arctocephalus*, *Callorhinus*, *Enhydra*, *Eumetopias*, *Lontra*, *Neophoca*, *Otaria*, *Phocarctos* and *Zalophus* and Families Balaenidae, Balaenopteridae, Delphinidae, Dugongidae, Eschrichtiidae, Iniidae, Monodontidae, Neobalaenidae, Odobenidae, Phocidae, Phocoenidae, Physeteridae, Pontoporiidae, Trichechidae, Ursidae, and Ziphiidae. We used IUCN range maps for each species, which were the most detailed and up-to-date maps available at the time of our analysis (IUCN 2020). Many marine mammal species are subdivided into distinct populations, but because IUCN range data are available only at the species level, our analysis was conducted at the species level. We only considered extant ranges – that is, areas where the species is known or thought to occur in the present day (IUCN 2020). This includes areas with current or recent (past 30 years) records of suitable habitat. As habitat records for each species were developed using sighting data from about 75% of the meta-analysis data period, we found these habitat ranges suitable for our filtering process. We then determined which marine mammal species ranges overlapped with the data points from the meta-analysis through visual identification and filtered out marine mammal species whose ranges did not overlap with any data points.

We compiled diet data for each of the remaining marine mammal species. Many of these data were derived from species summaries through the IUCN Red List, which contains detailed diet information from literature reviews compiled by experts on each species (IUCN 2020). For marine mammal species where diet data were not included in IUCN species summaries, we compiled diet data by reviewing published literature for each species, searching Google Scholar for publications including the species and region of interest that surveyed the prey species eaten through any method (e.g., stable isotopes, necropsy, fecal samples). In some cases, prey data were only reported at the family level. In these instances, we reviewed each prey family in FishBase (Froese and Pauly 2019) and determined which species of those families had ranges that overlapped geographically with the marine mammal's range. Through this effort, we generated a list of potential prey species for each marine mammal. Then, we filtered the Fiorenza et al. (2020) records to include only those records that fell within both the geographic and the diet range of at least one marine mammal species. We then performed a final filter to ensure that the remaining prey species were collected in the geographic range of the marine mammal that preys upon it. This was completed by visually comparing the specific location where the intermediate host from Fiorenza et al. (2020) was caught to ensure that the definitive host could have theoretically consumed that prey item. We obtained 396 overlapping data points, including 278 *Anisakis* spp. data points and 118 *Pseudoterranova* spp. data points, which were then used in the analysis.

Analysis

Data Standardization

We standardized the data prior to analysis using the methods described in Fiorenza et al. (2020). Briefly, the dataset included information on host species; collection location and year; portion of

the host examined and examination method; parasite genus, prevalence, intensity, abundance; and error associated with intensity and abundance. Some, especially older, articles only identified parasites to the genus level (e.g., *Anisakis* sp.). In order to maximize the proportion of available data that we could use, maximize the temporal scope of our analysis, and increase the sample size and power for our analysis, we grouped parasites by genus. Because fish length was reported in different ways across studies, we standardized values using a standard linear conversion equation $Length_{standard} = a + b*Length_{reported}$ using a and b values, or length–weight parameters, for each species from FishBase (Froese and Pauly 2019). The mean parameter values were calculated and used if there were multiple values reported (Froese and Pauly 2019). Parasite abundance, if not reported, was calculated from parasite intensity and prevalence by multiplying intensity and prevalence and propagating the error through in quadrature (the square root of the sum of squares; Fiorenza et al. 2020). Locations were grouped based on FAO fishing region (FAO 2008) using ESRI ArcGIS (ESRI 2011). As standard deviation was required for the meta-analytic framework, if a study reported other forms of error (standard error, confidence intervals, or range), we calculated standard deviation using formulae (Fiorenza et al. 2020). If the range of parasite abundance was provided, we estimated standard deviation by using the properties of the negative binomial distribution (Shaw et al. 1998). We optimized the negative binomial distribution for the dispersion parameter, assuming that the maximum value was the 95th quantile of the negative binomial distribution, and the mean value as the mean of the negative binomial distribution (Shaw et al. 1998). An optimization algorithm was used to estimate the best fit dispersion parameter, which was then used to calculate the error of the mean. If other forms of error were reported they were converted back to standard deviation. For example, standard error was multiplied by the square root of sample size and, for confidence intervals, we took the

difference of the upper bound and the mean and then divided it by the appropriate z-score, then multiplied by the square root of the sample size, as in Fiorenza et al. (2020).

Data Analysis

We generated columns for definitive host species, definitive host suborder/infraorder (Pinnipedia or Cetacea), parasite genus (*Anisakis* spp. or *Pseudoterranova* spp.), and paper ID (i.e., the paper from which the datum arose) to group the data for analysis. There was one instance in which multiple marine mammal definitive hosts had the same intermediate host species in their diet. In that instance, we chose to include the intermediate host row for the definitive host with the smaller sample size, as duplicating the row would complicate interpretation of the analysis. Anisakid abundance and standard deviation were fourth-root transformed to fit normality assumptions (Ekstam et al. 2011; Mandonnet et al. 2001; Stevens and Connolly 2005). We corrected for standard errors that were equal to zero (e.g., when only one sample was taken) by adding 1 to every variance to prevent over-weighting, as meta-regression uses the inverse of variance to weight observations (Fiorenza et al. 2020). We subset the data into two datasets based on parasite species occurrence, one for *Anisakis* spp. and one for *Pseudoterranova* spp., to run separate models on each genus.

We ran four generalized linear mixed-effects models on the data to determine whether **(1)** there has been a change in the abundance of (1a) *Anisakis* spp. or (1b) *Pseudoterranova* spp. in prey species of marine mammals over time, and **(2)** there has been a change in the abundance of (2a) *Anisakis* spp. or (2b) *Pseudoterranova* spp. in prey species of each marine mammal host sub/infraorder (Table 1). Each model was run using *rma.mv()* in the metafor package (Viechtbauer 2010) in R (Version 4.0.0, R Core Team 2020).

Models 1a and 1b tested whether the abundance of *Anisakis* spp. and *Pseudoterranova* spp. changed over time. The models included parasite abundance of *Anisakis* or *Pseudoterranova* spp. as the response variable. Year and host length were included as fixed effects. As we expected that host species, fishing region, the portion of fish analyzed for parasites, and method of detection could all influence the count of anisakids differently, we included random effects of portion of fish sampled nested within intermediate host species, FAO region, method of detection, and paper ID. This resulted in the following model:

Equation 1.1

$$(Parasite_abundance_{ijkl})^{1/4} \sim Host_length_{ijkl} + Year_{ijkl} + (1 \mid Host_species_{l} / Portion_of_fish_{ijkl}) + (1 \mid FAO_region_{j}) + (1 \mid Method_of_detection_{ijkl}) + (1 \mid Paper_ID_{i})$$

where the response variable$_{ijkl}$ represents a measurement of parasite abundance from the ith study in the jth location at the kth time in the lth intermediate host species. Models 2a and 2b tested whether the change over time in *Anisakis* spp. or *Pseudoterranova* spp. differed between those prey items consumed by cetaceans versus those consumed by pinnipeds. Model 2 was identical to Model 1, except that it also included an interaction term of $Year_{ijkl} * Definitive_host_suborder_{l}$. To assess the interaction of time and definitive host order (Model 2), we ran identical models, changing the host order in the reference position and recording the coefficient for each as represented by the intercept in its respective model.

For Models 1 and 2, all marine mammal definitive hosts were used, regardless of the temporal range or sample size of their prey data. While *Pseudoterranova* spp. and *Anisakis* spp. have different definitive hosts, we included both *Pseudoterranova* spp. and *Anisakis* spp. data

points for the analysis of all species of marine mammal definitive host regardless of preferred host, as there is evidence that larval forms of parasites can infect non-preferred hosts (*Anisakis* spp. in pinnipeds, *Pseudoterranova* spp. in cetaceans; Dailey 2001).

As in Fiorenza et al. (2020), we were interested in determining whether certain fish species or regions contributed disproportionately to the observed patterns. We performed two sub-analyses of Models 1a and 1b: one to investigate whether any one intermediate host species was responsible for driving patterns, and another to investigate whether any one FAO region was responsible for driving patterns. We sequentially excluded single host species or FAO regions and then reran the model on the reduced dataset. We extracted an estimate of the change over time for each iteration to assess whether a single host or region was responsible for the observed change over time. When significant influence of a particular species or region was detected, we ran the model (excluding the random effect of either FAO or intermediate host species) on the subset of data for the influential FAO region / intermediate host species, to determine if a significant trend would be observed within that subset of the data.

RESULTS

We compiled and reviewed diet data for 113 marine mammal species listed in the IUCN database (Figure 1.2). After compiling range data for each species, we compared ranges to the data points extracted in the meta-analysis and found that 30 marine mammal species overlapped spatially with the records collected in the Fiorenza et al. (2020) dataset (Appendix Ch. 1: Table S1.1). The number of fish or invertebrates analyzed totaled 46,359, representing 66 wild prey species collected in 64 studies from 1978 to 2015 (Appendix Ch. 1: Figure S1.1).

In Model 1, we detected a significant increase (estimate = 0.0234, *SE* = 0.0067, *Z* = 3.5055, *p* = 0.0005) in abundance of *Anisakis* spp. over time and no significant change in abundance of *Pseudoterranova* spp. (Figure 1.3, Table 1.2). In Model 2, there was a significant increase in the abundance of *Anisakis* spp. (estimate for effect of year = 0.0218, *SE* = 0.0113, Z = 1.9249, *p* = 0.0542) detected in the prey species consumed by both cetaceans and pinnipeds (estimate for effect of year * definitive host suborder = 0.0086, *SE* = 0.0142, *Z* = 0.6084, *p* = 0.5429). We detected no difference in the rate of change in *Pseudoterranova* spp. abundance in prey species of cetaceans versus pinnipeds (Figure 1.4, Table 1.3).

When testing whether particular intermediate host species drove the trend observed in Model 1, we found that black scabbardfish (*Aphanopus carbo*) was the most influential intermediate host driving the temporal increase in *Anisakis* spp., though once this species was removed and the model rerun, the trend remained significant for *Anisakis* spp. (estimate = 0.0169, *SE* = 0.0071, *Z* = 2.3648, *p* = 0.0180). The prey species that was most influential for *Pseudoterranova* spp. was Greenland halibut (*Reinhardtius hippoglossoides*), and when this intermediate host species was removed, the overall trend for *Pseudoterranova* spp. was significantly negative (estimate = -0.0281, *SE* = 0.0104, *Z* = -2.6955, *p* = 0.0070). When the model was run on *R. hippoglossoides* exclusively, the temporal trend was nonsignificant (estimate = 0.1046, *SE* = 0.2826, *Z* = 0.3701, *p* = 0.7113). When testing which FAO region contributed most to the observed pattern in Model 1, the model failed to converge when we removed region 57 (the Eastern Indian Ocean), so we were unable to evaluate whether this region drove the observed pattern. We were able to proceed with sequential removal of the remaining FAO regions and we found that FAO region 37 (the Mediterranean and Black Seas) had the greatest effect on *Anisakis* spp., although when it was removed, the trend remained

significantly positive (estimate = 0.0292, *SE* = 0.0077, *Z* = 3.8104, *p* = 0.0001; Appendix 1: Figures S2–S4). The FAO region 21, the Northwest Atlantic, had the biggest effect on *Pseudoterranova* spp., and when removed, the trend became significantly negative (estimate = -0.0279, *SE* = 0.0105, *Z* = -2.6495, *p* = 0.0081). When run independently, the trend for *Pseudoterranova* spp. in the Northwest Atlantic was non-significantly positive (estimate = 0.0692, *SE* = 0.0429, *Z* = 1.6145, *p* = 0.1064).

DISCUSSION

We detected a significant increase in the abundance of *Anisakis* spp. in the prey species of marine mammals over a 36-year period (Figure 1.3), but no change in *Pseudoterranova* spp. Prey species eaten by cetaceans and pinnipeds showed an increase in *Anisakis* spp. This study is retrospective and correlational, constraining our ability to identify causal drivers of the patterns observed. With the caveat that our data cannot discriminate among competing hypotheses to explain these patterns, we offer some potential explanations below.

The observed increase in *Anisakis* spp. over time is consistent with findings for the broader dataset in Fiorenza et al. (2020), and may correspond to a number of drivers. One possible driver is the recovery of some marine mammal species following protections. Our filtered meta-analysis dataset spanned from 1978 to 2015. This period immediately follows the passage of the Marine Mammal Protection Act in the US in 1972, and encompasses the enactment of the International Whaling Commission's global moratorium on commercial whaling in 1986. While the MMPA protected only marine mammals in U.S. waters, the moratorium was an international protection on great whale species. Prior to the moratorium, many whale species were heavily overexploited, some nearly to the point of extirpation (Clapham, Young, and Brownell 1999; Thomas, Reeves, and Brownell 2016). Following the

moratorium, some cetacean species, especially small coastal species, have increased and even recovered to historical levels (Magera et al. 2013; Roman et al. 2013). This recovery in some cetaceans, the definitive hosts of *Anisakis* spp., could lead to increased prevalence of *Anisakis* spp. in the prey species of the many marine mammals surveyed in this meta-analysis. We posit that increasing *Anisakis* spp. burdens driven by marine mammal populations that are increasing in abundance could spill over into data-deficient and declining marine mammal species.

As these parasites have complex life cycles with multiple hosts, it is possible that the observed increase in *Anisakis* spp. is attributable to changes in intermediate hosts as well. Increased nutrient inputs drive plankton blooms (Beman, Arrigo, and Matson 2005), thus increasing the number of copepod intermediate hosts (Siokou-Frangou and Papathanassiou, 1991; Uriarte and Villate 2004) available for marine mammal parasites to infect, as suggested in Fiorenza et al. (2020). If copepods or other low-trophic-level crustaceans were previously the bottleneck in marine mammal parasite life cycles (Lafferty 2012), this increased nutrient input could release that constraint, allowing anisakids more opportunities to survive to transmit to paratenic and definitive hosts. Similarly, fish paratenic hosts could drive the increase in several ways. Changing fishing pressures away from preferred paratenic hosts could result in a greater number of infected fish available for marine mammals to eat (Dobson and May 1987; McCallum, Gerber, and Jani 2005). Such a change would increase paratenic host density, and could then increase infection if these hosts were previously a limiting factor (Dobson and May 1987; Wood, Lafferty, and Micheli 2010). Furthermore, if fish have moved into ranges that they previously did not inhabit due to climate change or species introductions, they could potentially increase overlap with novel marine mammal hosts, thus increasing the number of exploitable hosts in the ecosystem for marine mammal parasites (Brooks and Hoberg 2007; Marcogliese

2001). As anisakids include many species that collectively infect a wide range of intermediate and paratenic hosts, this could increase their prevalence (Klimpel and Palm 2011).

Climate change could drive the observed changes. Increased temperatures associated with climate change are predicted to increase growth and decrease generation time in fishes (Magnuson et al. 1997), which could speed up the development of their parasites as well (Marcogliese, 2001; Fiorenza et al. 2020). With increasing temperatures, earlier onset of spring, and longer growing seasons, parasites with complex life cycles could increase the number of generations produced annually (Magnuson et al. 1997, Marcogliese 2001; Measures 1996). The resulting increase in the fecundity of some parasite species could be responsible for the increase in *Anisakis* spp. prevalence observed in this study. Increasing temperature can also reduce immunocompetence or behavioral resistance of fish hosts, which could lead to increases in abundance in anisakids (Burgess, Polasky, and Tilman 2013; Harvell et al. 2002; Claar and Wood 2020). These predicted trends could be species-specific, however-- if certain parasite species or their requisite hosts are adapted to colder climates, climate change could have the opposite effect, which may in part explain the observed differences in trends in *Anisakis* and *Pseudoterranova* spp. Additionally, climate change could result in one species outcompeting another. In general, *Anisakis* spp. use pelagic hosts (Klimpel and Palm 2011), which could mean that, in areas that face increases in pelagic productivity (Lannuzel et al. 2020; Meier et al. 2014), *Anisakis* spp. have more hosts available to exploit. Regions that have experienced a loss of multi-year ice, like the Arctic, have the potential to increase primary productivity in pelagic environments (Lannuzel et al. 2020; Meier et al. 2014). Decreases in multi-year ice have led to a greater prevalence of younger ice, which is more permeable to light and drives phytoplankton blooms (Meier et al. 2014). These blooms may in turn increase the number of pelagic

intermediate hosts in some regions (Lannuzel et al. 2020). This could serve to increase the hosts available for *Anisakis* spp., while the more benthic *Pseudoterranova* spp. (Klimpel and Palm 2011) would not experience the same increase.

The global trend in *Pseudoterranova* spp. was non-significant, but fragile; it changed depending on the species and regions included in the model. When we tested for intermediate host species and FAO regions that heavily influenced the observed trend, we found that removing data for Greenland halibut (*Reinhardtius hippoglossoides*) and the Northwest Atlantic resulted in significant declines in *Pseudoterranova* spp. abundance through time. When examined independently, the temporal trend for Greenland halibut showed no significant trend. These stocks were fished heavily until the 1990s, with catches in the Northeast Atlantic exceeding the advised limit and catches in the Northwest Atlantic proceeding relatively uncontrolled (Bowering and Nedreas 2000). Since then, recruitment has increased in both locations (Treble and Nogueira 2020; MFRI 2021), which would have allowed *R. hippoglossoides* and their parasites to increase or remain stable, which may have masked the declining trend observed across the other host species. When the data from the Northwest Atlantic were examined independently, there was a non-significant increase in *Pseudoterranova* spp. This suggests that the Northwest Atlantic may have experienced a slight increase in *Pseudoterranova* spp., which, when combined with global data, masks a significant decline observed across other regions. The Northwest Atlantic has experienced an increase in harbor seals and grey seals on the Atlantic coast of the U.S. since the enactment of the MMPA (Roman et al. 2013), and in other systems increasing abundances of grey seals have correspondingly increased the local prevalence of anisakids in intermediate hosts (Buchmann and Kania 2012; Hiby et al. 2007; Horbowy, Podolska, and Nadolna-Ałtyn 2016; Mehrdana et al. 2014; Galatius and Olsen 2014). It is possible the federal protections put in place

have increased the density of definitive hosts of *Pseudoterranova* spp. in the Northwest Atlantic. A similar trend is not detectable on the west coast of the U.S., where pinnipeds have also been increasing, but this region of the Pacific was not well-represented in our dataset.

Of the two anisakid genera, we expected *Pseudoterranova* spp. to fare better than *Anisakis* spp., given the strong recoveries of pinniped compared to cetacean populations (Magera et al. 2013). Globally, half of pinniped populations assessed by Magera et al. (2013) were found to be significantly increasing. However, the lack of protections in place for pinniped species on an international level and a myriad of additional threats may drive an opposite trend in other, less monitored pinniped populations, resulting in the appearance *Pseudoterranova* spp. remaining unchanged. Pinnipeds are often caught in conflicts with fisheries and have been subject to culls (Bowen and Lidgard 2011; Olsen, Galatius, and Härkönen 2018; Roman et al. 2013), and have also faced large infectious disease mortality events (Duignan et al. 2014; Runstadler and Puryear, 2020). Pinnipeds are also subject to harvest in some countries, a threat that many cetaceans do not face as pelagic species (Kovacs et al. 2011). Many of the Arctic seals, which overlap in range with the meta-analysis dataset, face habitat loss and other threats rooted in climate change, which may cause declines in pinniped populations (Kovacs et al. 2011). Declines in these species are likely to go unnoticed. For example, a decline in any ice-obligate pinniped species would be impossible to detect using current survey efforts (Taylor et al. 2007). The lack of protections for pinnipeds on a global scale and the cumulative threats from climate change and other anthropogenic stressors may leave them more vulnerable to undetected declines, reducing the number of pinniped definitive hosts. This, in tandem with regional increases in pinnipeds in the Northwest Atlantic and elsewhere, may have leveled out the prevalence of *Pseudoterranova* spp. in the marine environment, leading to the non-significant trend detected in our analysis.

The prey species that both cetaceans and pinnipeds are eating have experienced an increase in *Anisakis* spp. prevalence over the past 40 years. This increase suggests marine mammals are facing mounting risks of *Anisakis* infections, especially cetaceans (the appropriate definitive hosts) but also pinnipeds and other dead-end hosts that can nonetheless become infected (Dailey 2001). The fragile trend in *Pseudoterranova* spp. suggests pinnipeds may face less of a burden of gastrointestinal nematodes than in the past 40 years depending on the region (i.e., outside of the Northwest Atlantic). Importantly, the study period encompasses an era immediately following the protection of marine mammals from extensive hunting. The changes we detected in this study may be very different from a baseline prior to the removal of marine mammals from much of the world's oceans—it is possible that anisakids were much more abundant before whaling when their definitive hosts were much more abundant. At this moment, this remains an untested hypothesis. Whatever the past burden of anisakids, persistent sublethal stressors on many contemporary marine mammal populations may make anisakid infections more dangerous than they would have been in the past. Marine mammals should be monitored for digestive tract helminths when conducting health assessments using fecal samples, or at necropsy. Future research using population-based models to assess the impacts of multiple stressors on endangered cetacean species could incorporate the energetic impact of gastrointestinal parasites on their hosts via increased prevalence in key prey species.

We used the information available for 113 marine mammal species, but the data used to inform these analyses are limited by low study effort for many marine mammal species. Thorough diet analyses for marine mammals over their entire range are difficult to conduct for some species and nearly impossible for others; diets require adequate sample sizes from a representative population in order to be determined, and such samples can be difficult to obtain,

especially in pelagic cetaceans that eat and defecate at depth and rarely strand (Pauly 1998; Trites and Spitz 2017). As a result, for many marine mammal species, only the family of prey items are known, and we were therefore compelled to include all intermediate host species that fall within the reported family, some of which will not actually be prey of the marine mammal species in question. Additionally, it is known that geographical ranges of marine mammals can be biased by the survey method (Tyne et al. 2016; Williams et al. 2014). While density maps would have given a more accurate account of where marine mammals concentrate within their ranges, because these were not available for all species considered in this analysis, we instead chose to use the most recent range maps compiled by species experts for IUCN (IUCN, 2020).

For our analysis we compiled data reaching back to the 1960s and 1970s, and over this period techniques in parasite detection and identification improved significantly (Wood and Vanhove 2022). To achieve the statistical power needed to detect a trend in these two genera, and to include older data on parasite abundance collected at the genus level with older techniques, we performed our analysis by genus and accounted for any potential differences in detection methods in our models. However, it is important to note that we grouped together parasite species with diverse ecologies, specific life cycles, and varying pathology. As such, we cannot detect changes in individual species, and the general trends observed in our analysis may mask differences in trends among species that are not ecologically equivalent.

While our aim was to be inclusive when determining which intermediate host species were likely to fall within marine mammal diets, there were factors we did not take into account in preparing the dataset for analysis. We did not consider shifts in marine mammal range over the 36-year period, either short-term (migration) or long-term (changes in range as species recover or decline). While it would have been possible to incorporate these long-term shifts for some well-

studied marine mammal species, many more species are data-deficient. We therefore used the most up-to-date marine mammal ranges (IUCN, 2020). Instead of incorporating seasonality in migration patterns, we erred on the side of inclusivity—if a prey species was sampled in the marine mammal's range, it was included regardless of the time of year sampled. This may be an issue in calving grounds where large cetaceans are known to fast. Similarly, we did not consider seasonal or temporal shifts in diet. Some species have had documented shifts in their diet as fish stocks change (e.g., humpback whales in the Southern Gulf of Maine). However, as many understudied marine mammals do not include this level of detail in their diet data, we included any diet species or families regardless of the period documented.

CONCLUSION

We found an increase in *Anisakis* spp. and no change in *Pseudoterranova* spp. prevalence in the fish that marine mammals consume using a long-term, global meta-analysis dataset spanning nearly four decades. This period encompasses the 1986 moratorium on commercial whaling, and immediately follows the 1972 enactment of the US Marine Mammal Protection act. The observed increase in *Anisakis* spp. may reflect a global trend of increasing cetacean abundance, powered by international conservation efforts, while the lack of change in *Pseudoterranova* spp. suggests variable changes in pinniped host recovery, driven by changes in definitive host abundance or the availability of requisite intermediate hosts. The observed increase in *Anisakis* spp. should be considered when assessing the threats to marine mammals globally. Parasitism may not be the primary source of mortality in marine mammals, but it is an additional stressor in the growing list of threats that marine mammals face. The additional health and energetic burden of *Anisakis* spp. nematodes, a threat that is currently unrecognized, will be important to include

when considering the impact of multiple stressors in marine mammal conservation going forward.

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CH. 1 TABLES

Table 1.1: The research questions and the generalized linear mixed effect models run to detect a change in anisakid prevalence over time for 1) all marine mammal prey species, and 2) prey species grouped by marine mammal order. Each model was run separately for a subset of data with Anisakis sp. and Pseudoterranova sp. Prey intermediate host standard length (Std length) was included as a fixed effect, and the portion of the host assessed nested within the intermediate host species, FAO geographical region, method of counting (candling, etc.), and a unique identifier for each paper were included as random effects in each model. Model 1 included year as a fixed effect, while Model 2 included an interaction of definitive host order (pinniped or cetacean) and year.

Has anisakid prevalence changed over time in...	**Model number**	**Model**
… marine mammal prey species generally?	1	[Parasite abundance]$^{1/4}$ ~ Std length + Year + (1| Intermediate host/Portion of Body) + (1|FAO)+ (1|Method of Counting) + (1|Paper ID)
…pinniped versus cetacean prey species?	2	[Parasite abundance]$^{1/4}$ ~ Std length + Definitive Host Order*Year + (1| Intermediate host/Portion of Body) + (1|FAO)+ (1|Method of Counting) + (1|Paper ID)

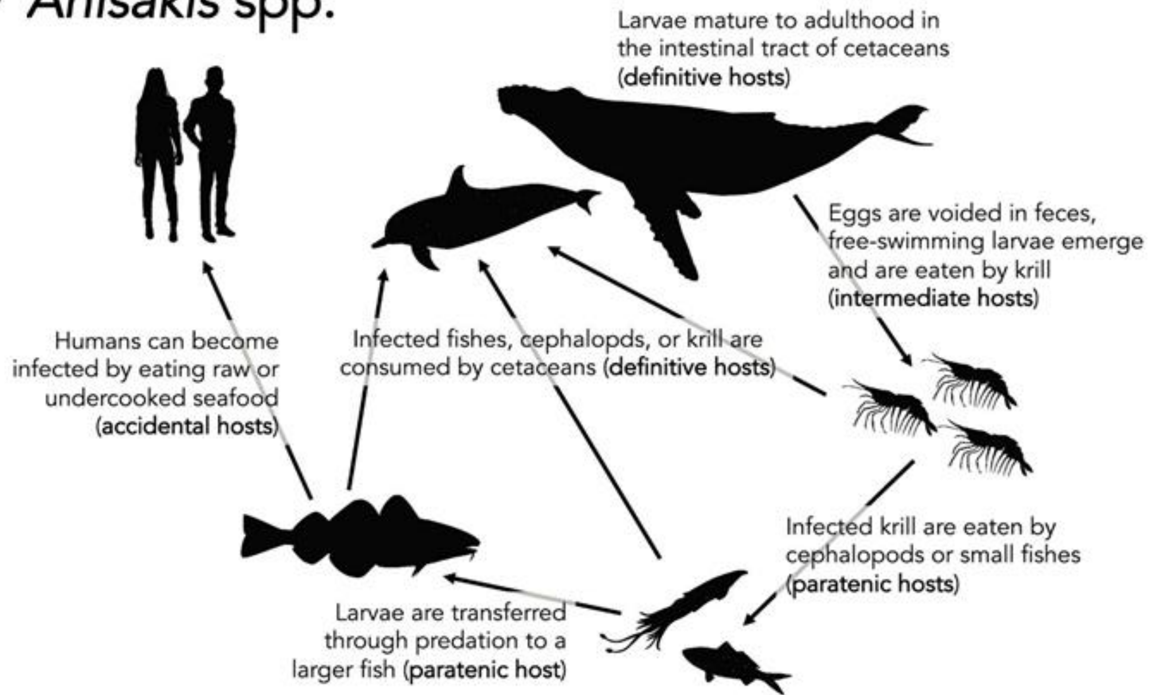
Table 1.2: Results of Model 1 – assessing the effect of year on *Anisakis* spp. (1a) and *Pseudoterranova* spp. (1b) independently.

Model		**estimate**	**se**	**zval**	**pval**
Model 1a - *Anisakis* spp.	intercept	-46.7131	13.3812	-3.491	0.0005
	Standard Length	0.0246	0.0031	7.9789	<0.0001
	Year	0.0234	0.0067	3.5055	0.0005
Model 1b - *Pseudoterranova* spp.	intercept	23.3783	21.6688	1.0789	0.2806
	Standard Length	0.004	0.0059	0.6882	0.4913
	Year	-0.0115	0.0109	-1.0573	0.2904

Table 1.3: Coefficients for the effect of time on each host taxonomic order (Model 2) for both *Anisakis* spp. (2a) and *Pseudoterranova* spp. (2b) Estimates represent the effect of order*year when the Cetacea is in the reference position.

Model		estimate	se	zval	pval
Model 2a - *Anisakis* spp.	intercept	-43.5278	22.714	-1.9163	0.0553
	Standard Length	0.0245	0.0031	7.9844	<.0001
	Pinnipedia	-17.0561	28.2814	-0.6031	0.5465
	Year	0.0218	0.0113	1.9249	0.0542
	Pinnipedia * Year	0.0086	0.0142	0.6084	0.5429
Model 2b - *Pseudoterranova* spp.	intercept	-10.6582	42.8568	-0.2487	0.8036
	Standard Length	0.0035	0.0057	0.6178	0.5367
	Pinnipedia	27.3422	49.4813	0.5526	0.5806
	Year	0.0054	0.0214	0.2503	0.8023
	Pinnipedia * Year	-0.0134	0.0248	-0.5402	0.5891

CH. 2 FIGURES



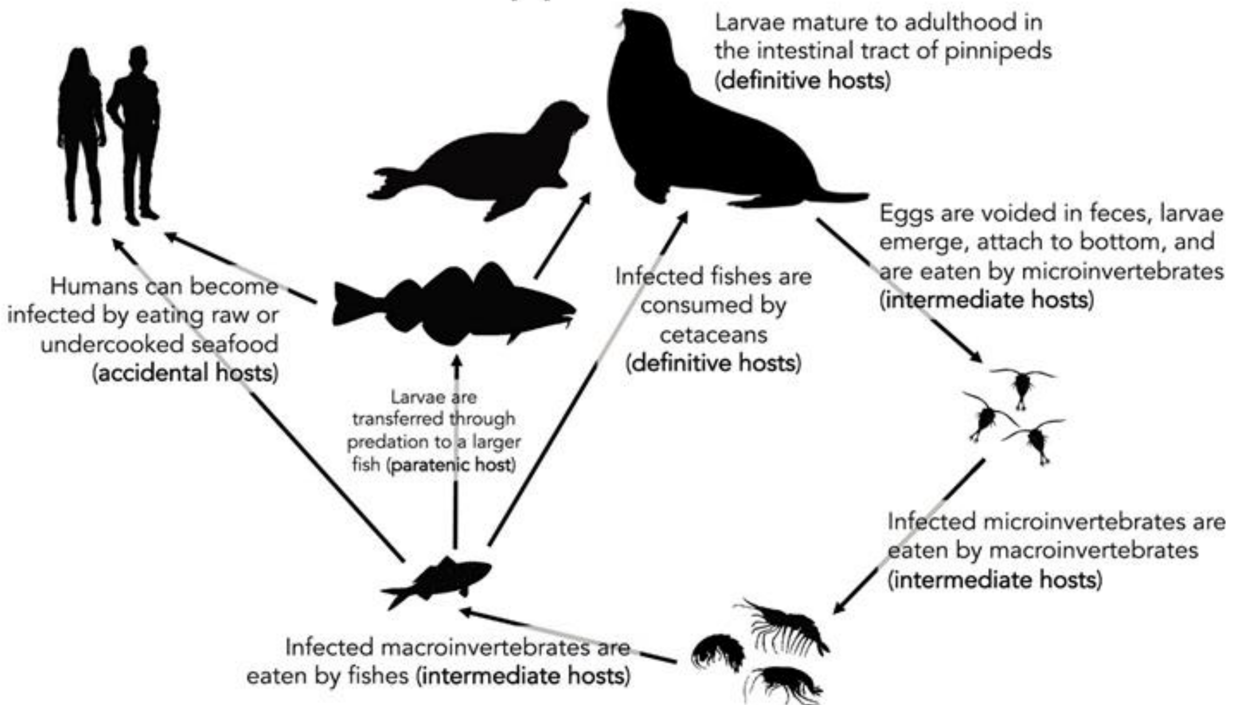


Figure 1.1. General life cycle of *Anisakis* spp. (a) and *Pseudoterranova* spp. (b) nematodes. The *Anisakis* spp. life cycles involve four larval phases (L1-L4) which take place mainly in the pelagic environment (Klimpel and Palm, 2011). Reproduced with permission from Fiorenza et al. 2020.

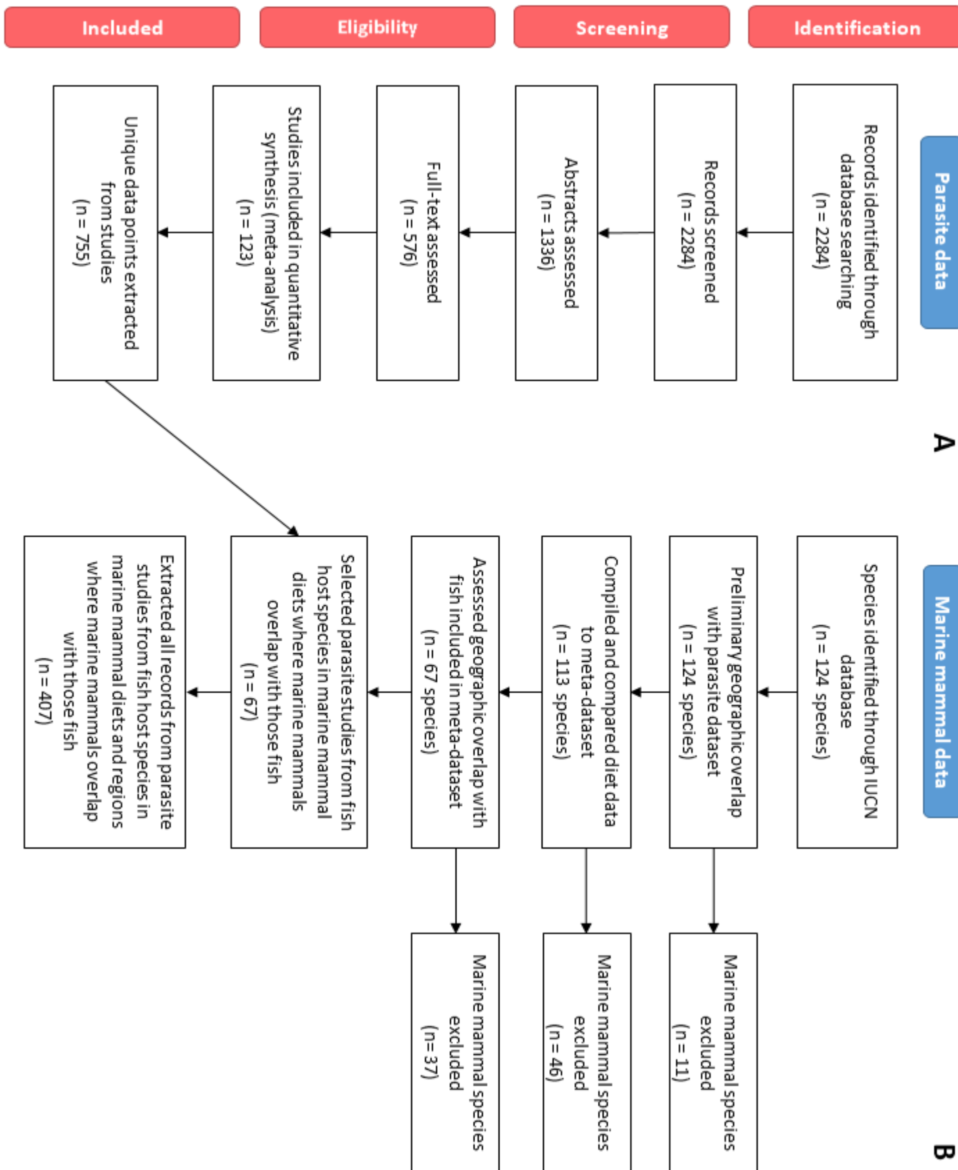


Figure 1.2. A PRISMA flow diagram of the process of determining inclusion of parasite publications for the meta-analysis (A) and marine mammal diet species with which to filter the meta-dataset (B). The process of screening papers for the meta-analysis (A) was conducted by Fiorenza et al. (2020).

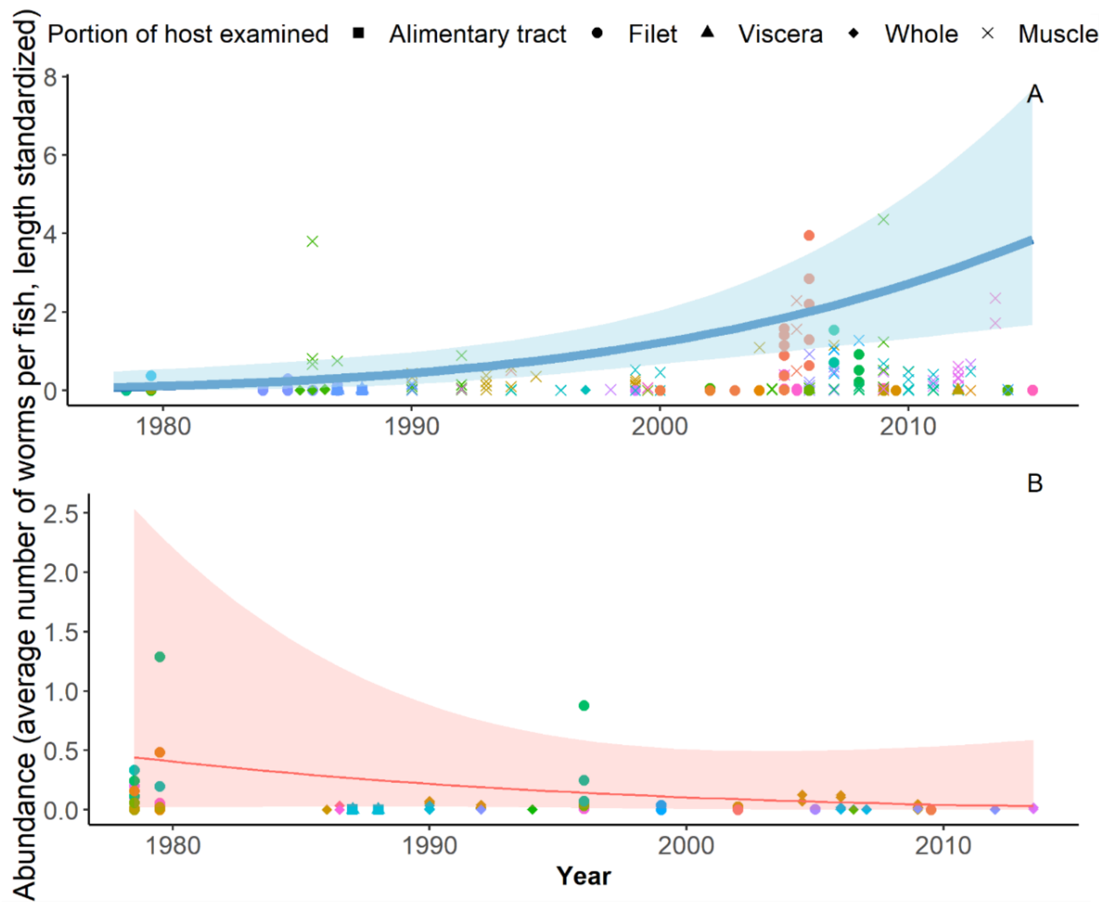
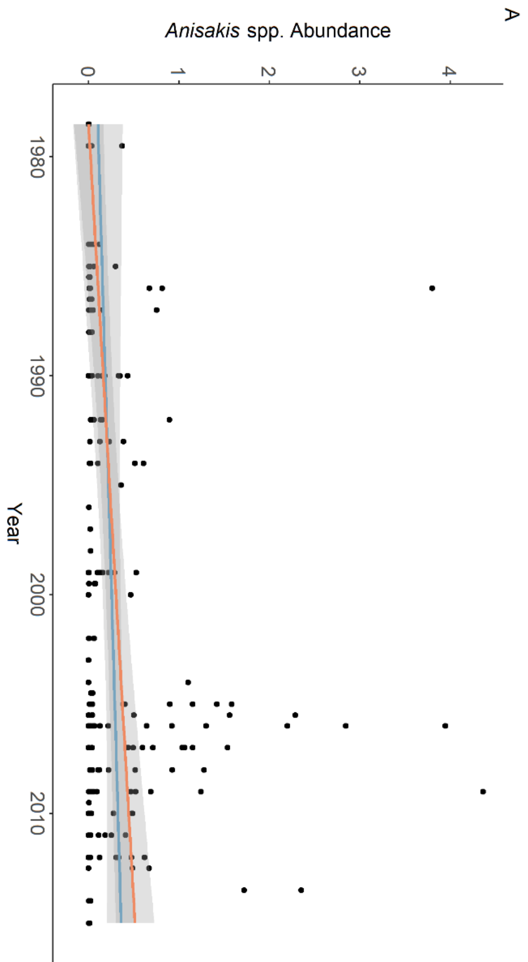
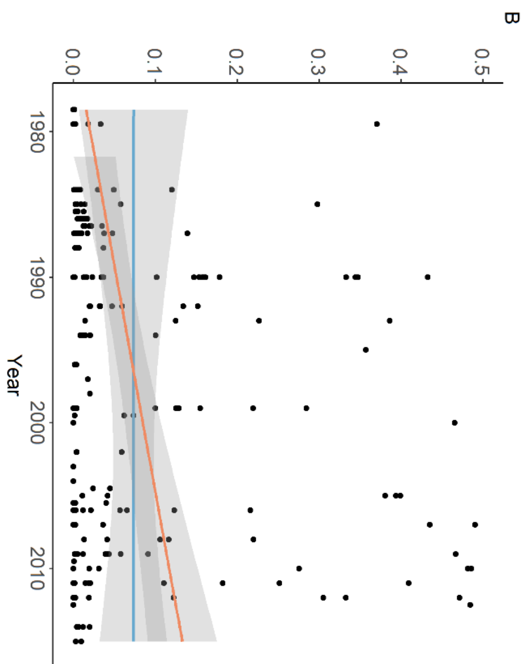
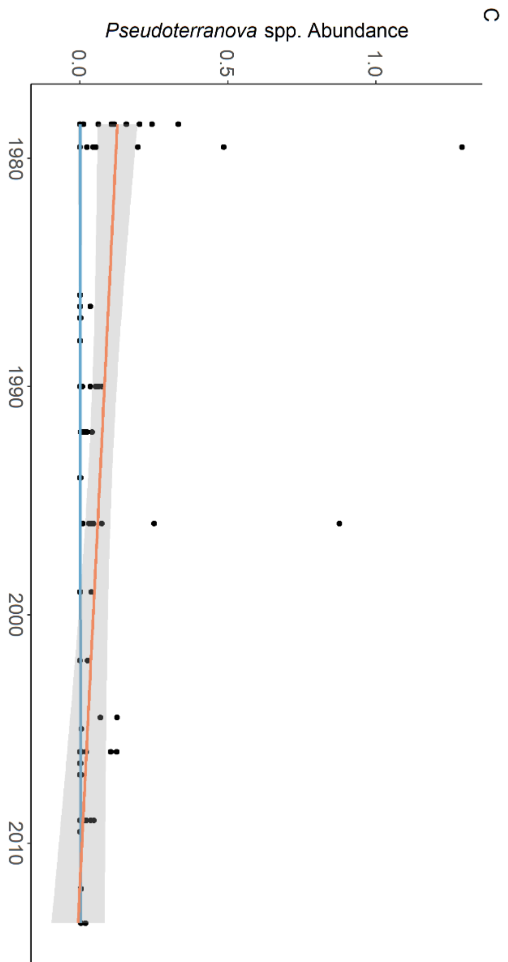
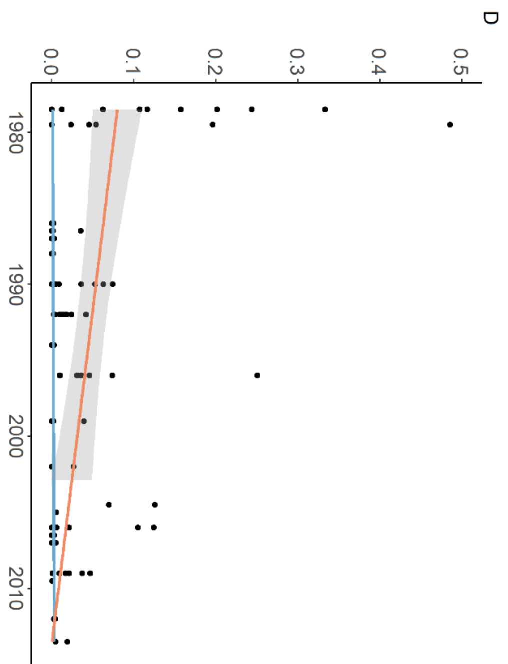


Figure 1.3: The trend in *Anisakis* spp. (A) and *Pseudoterranova* spp. (B) abundance, corrected for host length, colored by the intermediate (fish) host species, as predicted from Model 1. Predictions were derived in the predict.rma function in the metafor package in R, and are based on the average standard length of the hosts in the dataset.









Definitive Host Order — Cetacea — Pinnipedia

Figure 1.4: Trends in *Anisakis* spp. (C and D) and *Pseudoterranova* spp. (A and B) abundance over time in cetaceans and pinnipeds using raw data. In plots B and D, the y-axis is truncated to make the temporal relationship more apparent. Lines represent smoothed conditional means of parasite abundance to time by order, calculated in the ggplot2 package in R using the glm method in the geom_smooth() function. Shaded areas represent confidence intervals around the generalized linear model regression line, generated by the geom_smooth() function, which constructed the normal confidence interval on the link scale and back-transformed it to the response scale, parasite abundance.

APPENDIX CH 1

Table S1.1: The marine mammal species and their diet species included in the full analysis for Models 1 and 2. Species listed overlap in range and family of reported diet species, asterisks indicate confirmed diet species.

Definitive Host	**Common name**	**Confirmed Intermediate Hosts (Used in AllDat3**	**Level of certainty**	**Location**	**Citation**
Arctocephalus gazella	Antarctic fur seal	*Electrona antarctica, Nototheniops nybelini, Trematomus newnesi, Trematomus lepidorhinus, Gymnoscopelus nicholsi, Trematomus bernacchii, Trematomus eulepidotus, Trematomus hansoni, Gobionotothen gibberifrons, Notothenia rossii, Notothenia coriiceps, Dissostichus mawsoni, Lepidonotothen squamifrons*	Overlaps in range, family myctophidae and notothenidae	Heard Island, Macquarie Island, Iles Crozet, Prince Edwards Islands	Reviewed by Hofmeyr, G.J.G. 2016.
Arctocephalus pusillus	Afro-Australian fur seal	*Thyrsites atun, Rexea solandri*	Overlaps in range, known as Anchovy, Barracouta, and Cuttlefish	Marine mammal's range	Reviewed by Hofmeyr, G.J.G. 2015.
Arctocephalus townsendi	Guadalupe fur seal	*Citharichthys sordidus*, Sebastes miniatus*	First three certain, last two overlap in range from families	Farallon Island, and Guadalupe island	Reviewed by Aurioles-Gamboa, D.

			Scopelogadus and Sebastes		2015.
Balaenoptera acutorostrata	Common minke whale	*Scomber scombrus**	Overlaps in range, family Ammodytidae, herring, and sandeels, last species is certain	Noth Atlantic-Icelandic shelf, North Sea, Northern Norway	Reviewed by Cooke, J.G. 2018.
Balaenoptera borealis	Sei whale	*Mallotus villosus, Scomber japonicus*	Overlaps in range	Western North Pacific and Japan	Reviewed by Cooke, J.G. 2018.
Balaenoptera edeni	Byde's whale	*Trachurus picturatus*	Overlaps in range, Anchovies and myctophids	South Africa	Reviewed by Cooke, J.G. & Brownell Jr., R.L. 2018.
Callorhinus ursinus	Northern fur seal	*Oncorhynchus gorbuscha, Oncorhynchus nerka, Oncorhynchus keta*	Overlaps in range, of family Gadidae and Salmonids, *Clupea pallasii pallasii* is certain	California to Washington, Pribilof Islands	Reviewed by Gelatt, T., Ream, R. & Johnson, D. 2015; Zeppelin and Orr 2010
Cystophora cristata	Hooded seal	*Reinhardtius hippoglossoides, Sebastes fasciatus, Sebastes mentella, Gadus morhua*	Overlaps in range	Marine mammal's range	Reviewed by Kovacs, K.M. 2016.
Delphinus delphis	Short-beaked common dolphin	*Engraulis encrasicolus, Sprattus sprattus*	Overlaps in range, also known as Anchovy and sprat	Black Sea	Reviewed by Hammond, P.S. et al. 2008.

Globicephala melas	Long-finned pilot whale	*Sepietta oweniana, Alloteuthis media, Aphanopus carbo, Centrophorus squamosus, Scyliorhinus canicula, Mora moro*	Overlaps in range, known as squid, mackerel, dogfish, herring, turbot, cod, and hake	Marine mammal's range	Reviewed by Minton, G., Reeves, R. & Braulik, G. 2018
Halichoerus grypus	Grey seal	*Gadus morhua, Reinhardtius hippoglossoides*	Overlaps in range, known as cod, sandeels, redfish, ling, and turbot	Canada, North Sea, Iceland, Faroes, UK, Outer hebrides	Reviewed by Bowen, D. 2016.
Hyperoodon ampullatus	Northern bottlenose whale	*Helicolenus dactylopterus*	Overlaps in range, known as herring and redfish	Marine mammal's range	Reviewed by Taylor et al. 2008
Lagenorhynchus acutus	Atlantic white-sided dolphin	*Argentina silus, Gadus morhua*	Overlaps in range, known as squid, sandlance, mackerel, cod, smelt, herring, and hake	Marine mammal's range	Reviewed by Braulik, G. 2019.
Lagenorhynchus albirostris	White-beaked dolphin	*Salmo trutta, Micromesistius poutassou*	Overlaps in range, known as whiting, squid, and hake, family Gadidae	North Sea along the coast of the Netherlands, Scotland	Reviewed by Kiszka, J. & Braulik, G. 2018.
Lagenorhynchus australis	Peale's dolphin	*Doryteuthis gahi*	Overlaps in range	Southern Atlantic	Reviewed by Heinrich, S. & Dellabianca, N. 2019.

Lagenorhynchus obscurus	Dusky dolphin	*Merluccius hubbsi, Stromateus brasiliensis, Rexea solandri*	Overlaps in range, known as lanternfish, anchovy, squid, hatchet fish, hake, sardine, trachurus sp, lampanyctus sp., and horse mackerel	Southern Africa, New Zealand, Northern and Central Patagonia	Reviewed by Alafaro-Shiguieto, J. et al. 2019
Megaptera novaeangliae	Humpback whale	*Scomber scombrus, Clupea harengus*	Overlaps in range	Alaska, North Atlantic, Newfoundland and Labrador	Reviewed by Cooke, J.G. 2018.
Mirounga leonina	Southern elephant seal	*Lindbergichthys nudifrons, Nototheniops larseni, Trematomus scotti, Trematomus lepidorhinus, Trematomus eulepidotus*	Overlaps in range, notothenid fish, myctophids, and squid	Marine mammal's range	Reviewed by Hofmeyr, G.J.G. 2015
Monachus monachus	Mediterranean monk seal	*Sparus aurata, Spicara smaris, Boops boops, Diplodus annularis, Sarpa salpa**	Overlaps in range, fish of the family sparidae	Greece, Cabo Blanco, Desertas Islands	Reviewed by Karamanlidis, A. & Dendrinos, P. 2015.
Monodon monoceros	Narwhal	*Arctogadus glacialis, Reinhardtius hippoglossoides*	Overlaps in range	Marine mammal's range	Reviewed by Lowry, L., Laidre, K. & Reeves, R. 2017.
Orcinus orca	Killer whale	*Oncorhynchus mykiss**	Certain	Marine mammal's range	Reviewed by Reeves, R., Pitman, R.L. & Ford, J.K.B. 2017.

Otaria byronia	South American sea lion	*Raneya brasiliensis**	Certain	Marine mammal's range	Reviewed by Cárdenas-Alayza, S., Crespo, E. & Oliveira, L. 2016; Koen Alonso et al. 2000
Pagophilus groenlandicus	Harp seal	*Sebastes mentella*	Overlaps in range, Arctic cod, polar cod, herring, and redfish.	Greenland, Newfoundland, Labrador, Gulf of St. Lawrence, Barents Sea	Reviewed by Kovacs, K.M. 2015.
Phocoena phocoena	Harbor porpoise	*Clupea harengus*	Overlaps in range, Arctic cod, herring, smelt, capelin, salmon, flatfish, rockfish	Marine mammal's range	Reviewed by Braulik, et al. 2020.
Phocoena spinipinnis	Burmeister's Porpoise	*Merluccius hubbsi**	Certain	Marine mammal's range	Reviewed by Félix et al. 2018; García-Godos et al. 2007; E. Crespo, unpub. data
Physeter macrocephalus	Sperm whale	*Merlangius merlangus, Lophius piscatorius, Lophius budegassa, Electrona carlsbergi*	Overlaps in range, of Myctophidae family	Southern Australia, Northeast Atlantic, North Sea	Reviewed by Taylor et al. 2019; Martin and Clarke 1986; Evans and Hindell 2004; Santos et al. 2006; Santos et al 2002; Santos et al 1999; Pierce et al. 2018

Pseudorca crassidens	False killer whale	*Macruronus magellanicus*	Overlaps in range	Tierra del Fuego	Reviewed by Baird, R.W. 2018; Koen Alonso et al 2006
Pusa hispida	Ringed seal	*Arctogadus glacialis*, Sebastes mentella, Mallotus villosus*	Overlaps in range	Marine mammal's range	Reviewed by Lowry, L. 2016.
Sotalia guianensis	Guiana dolphin	*Plagioscion squamosissimus, Cynoscion guatucupa*	Overlaps in range	Southeast Brazil	Reviewed by Secchi et al. 2018; Madeira Di Beneditto and Siciliano 2007; Flores et al 2018; Lopes et al. 2012
Stenella coeruleoalba	Striped dolphin	*Benthosema glaciale, Diaphus holti, Myctophum punctatum, Alloteuthis media, Hygophum benoiti, Ceratoscopelus maderensis, Lampanyctus crocodilus, Illex coindetii*	Overlaps in range, squid, cod, and lanternfish	Marine mammal's range	Reviewed by Braulik, G. 2019.

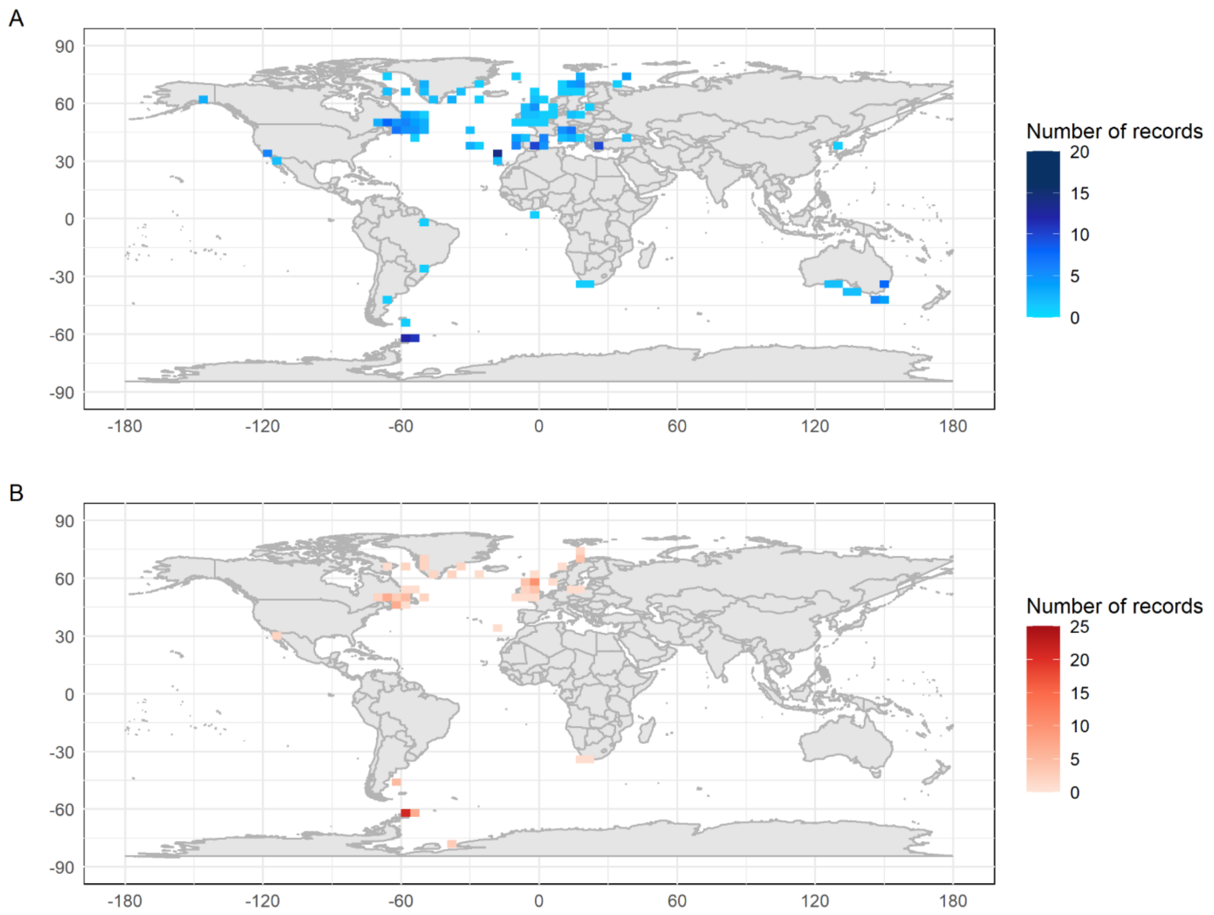


Figure S1.1: The records of both *Anisakis* spp. (A) and *Pseudoterranova* spp (B) in the prey species (intermediate hosts) that marine mammals eat.

(a)

(b)

Figure S1.2: (a) A heatmap of the relative effect of FAO region on *Anisakis* and *Pseudoterranova* spp. burden (the mean number of nematodes) and influence (the slope of the relationship between the number of nematodes and time). Burden was calculated by obtaining the standardized random effect estimate for each FAO region. The relative effect of burden shows

whether the data from specific FAO regions have higher or lower anisakid abundance compared to the mean. Influence was calculated as the relative effect of including the region in the model. Positive relative effect of influence indicates that the region increased the slope of the temporal effect in the model, while negative relative effects decreased the slope. Black boxes indicate that the region did not include data on that genus. (b) A map of the FAO regions overlaid with the data-points used in our analysis.

Figure S1.3: A heatmap representation of the effect of each intermediate host species on the burden and influence of *Anisakis* spp. Positive and negative values can be interpreted similarly to Figure S1.2.

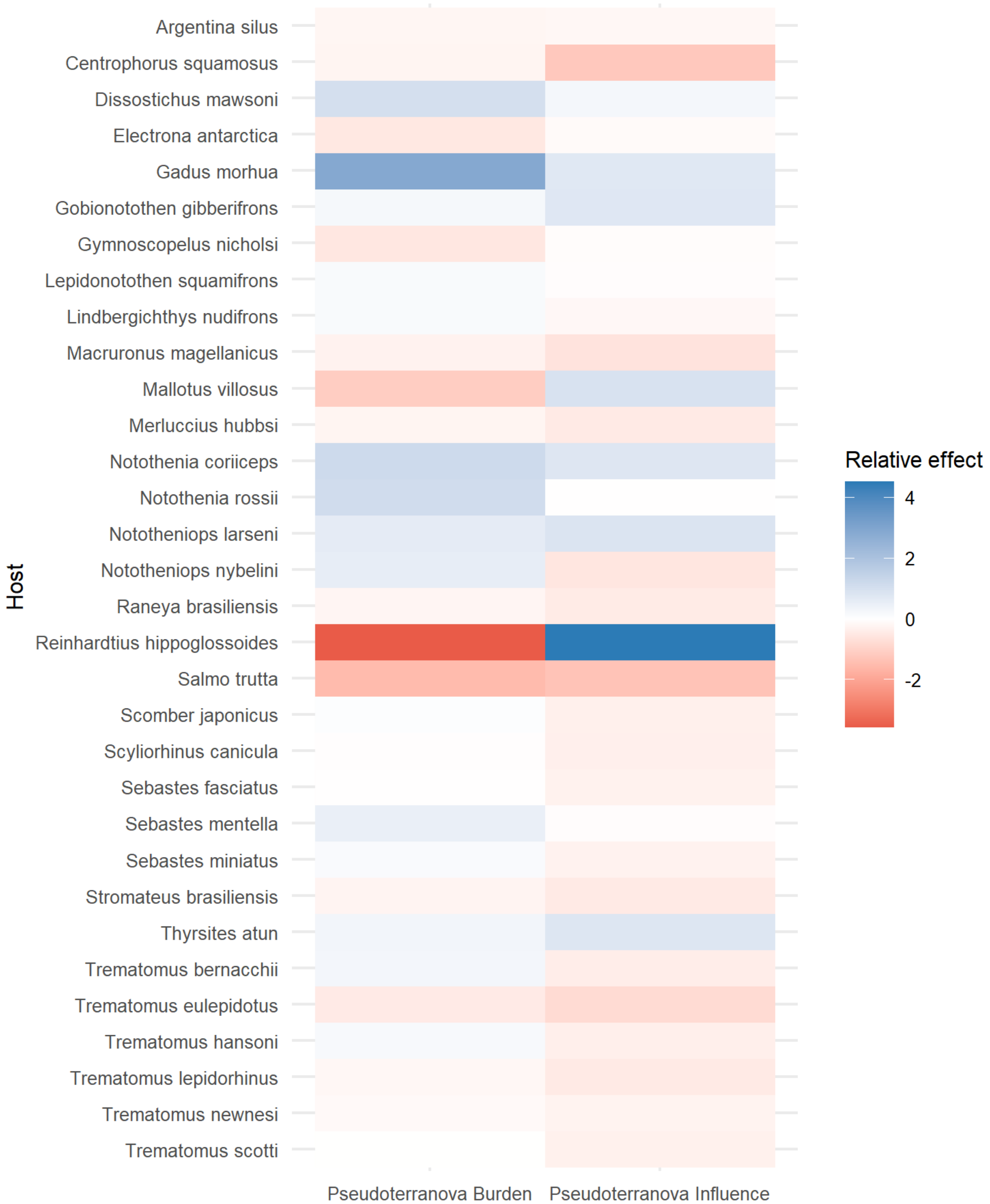


Figure S1.4: A heatmap representation of the effect of each intermediate host species on the burden and influence of *Pseudoterranova* spp. Positive and negative values can be interpreted similarly to Figure S1.2.

APPENDIX CH. 1 REFERENCES

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Chapter 2: Marine mammal recovery is associated with the resurgence of a nematode parasite

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ABSTRACT

As the oceans change, the abundance of parasites and risk of infection to marine mammals may be changing. Nematodes in the family Anisakidae can harm marine mammals, and recent studies revealed a global increase in these parasites, but trends are unknown in the marine mammal rich northeastern Pacific Ocean. Rising anisakid burdens could disproportionately affect marine mammals facing multiple stressors, like endangered southern resident killer whales in Puget Sound, Washington, USA. We sought to determine how anisakid risk in Puget Sound has changed over 98 years by dissecting museum specimens of prey species: Pacific herring, Walleye pollock, Surf smelt, Pacific hake, and Copper rockfish. The larval anisakid *Contracaecum* spp. was most abundant, but declined over time with a recent increase since 1989 that was most correlated with increasing harbor seal abundance. Marine mammals in Puget Sound are probably less burdened by anisakids than they were historically, but the recent recovery of anisakids could impact the health of these hosts.

INTRODUCTION

In the early 20th century, anthropogenic activities disrupted the marine ecosystem of Puget Sound, WA by removing marine mammals. Harbor seals (*Phoca vitulina*), California sea lions (*Zalophus californianus*), and Steller sea lions (*Eumetopias jubatus*) were culled to reduce competition with fisheries, and their populations were driven to low numbers in the first half of the 20th century (Newby 1973; Jeffries et al. 2003). Commercial whaling removed a majority of baleen whales from the region, effectively extirpating humpback whales (*Megaptera novaeangliae*) from Puget Sound by the early 1900s (Calambokidis et al. 2017; Calambokidis et al. 2018), and the aquarium live trade removed approximately 40% of southern resident killer whales (*Orcinus orca,* SRKW) from the population in the 1960s and 1970s (Balcomb and Goebel 1976).

To conserve the remaining US marine mammal populations, the Marine Mammal Protection Act (MMPA) was enacted in 1972 and prohibited the continued exploitation of marine mammals throughout the United States. Following the implementation of the MMPA, many Puget Sound marine mammal species have recovered, including harbor seals, California and Steller sea lions, humpback whales, and harbor porpoise (*Phocena phocena*)—with some nearing historic population abundances (Calambokidis and Baird 1994; Calambokidis et al. 2017; Elliser and Hall 2021; Jefferson et al. 2021; NOAA 2017). An exception to these recovery successes is SRKW, which is currently listed as Endangered under the Endangered Species Act, with only 74 whales in the population in 2021 (Marine Mammal Commission 2021).

While the threat of direct exploitation has declined, contemporary marine mammals face other threats, including vessel collisions (Schoeman et al. 2020), fisheries interactions and bycatch (Read et al. 2006; Read 2008), harassment (Spradlin et al. 2001), collapse of prey populations (e.g., Chinook salmon for SRKW, NMFS 2008), vessel noise (Erbe et al. 2019),

climate change (Learmonth et al. 2006), accumulation of pollutants (Reijnders and de Ruiter-Dijkman 1995), and entanglement and ingestion of man-made materials (Poeta et al. 2017). Stressors can act synergistically, resulting in declines in health and reproductive capacity – and sometimes mortality (Wright 2012; Kellar et al. 2017; Simmonds 2018). In Puget Sound, SRKW face compounding stressors of prey loss, pollutant bioaccumulation, and noise pollution, and while the population may be able to overcome these individually, in synergy they result in population declines (Lacy et al. 2017). These stressors may also amplify other underlying persistent stressors, like parasite infections.

Some of the most common parasites found in marine mammals are intestinal nematodes in the family Anisakidae, or anisakids (Dailey 2001). Anisakids are generally thought to have minor impacts on marine mammal host health, though they can cause peritonitis, gastritis, ulceration, secondary bacterial infections, hemorrhaging, and sometimes death (Cattan et al. 1976; Dailey 2001; Dailey and Stroud 1978; Stroud and Roffe 1979; Dailey 1985; Smith 1989). The family Anisakidae includes the genera *Contracaecum* spp., *Anisakis* spp., and *Pseudoterranova* spp. Some *Contracaecum* spp. use pinnipeds as their definitive hosts, while other species exploit seabirds; *Anisakis* spp. use cetaceans; and *Pseudoterranova* spp. use pinnipeds. Each parasite species can accidentally infect a host of a different clade (Dailey 2001). For example, *Contracaecum* can infect cetaceans (Cavallero et al. 2011; Luque et al. 2010) and *Anisakis* can infect pinnipeds (Nagasawa et al. 1999), and both can cause pathology, but cannot reproduce in these non-target hosts (Dailey 2001). Anisakids have complex life cycles that follow a general pattern (Figure 1): eggs are released into the water column in the feces of their definitive host, develop into their first larval stage before being consumed by a small invertebrate, then pass through one or several intermediate hosts (Pravetoni et al. 2012) before

reaching their definitive host through ingestion (Klimpel and Palm 2011). The parasite reaches its adult stage and reproduces while inhabiting the definitive host's gastrointestinal tract.

We sought to determine whether the risk of anisakid infections has changed over the past century for Puget Sound's marine mammals to better identify underlying health risks to vulnerable marine mammals like SRKW. In Chapter 1, I determined that the global burden of *Anisakis* spp. nematodes in the fish prey of marine mammals have increased since the 1970s. However, this meta-analysis was conducted without data from the northeast Pacific. Therefore, we wanted to know whether this pattern would hold in Puget Sound. We suspected that parasite risk, measured through anisakid abundance in marine mammal prey species, might have changed for a few reasons, including variable definitive host abundance and changing environmental conditions.

Parasites like anisakids are sensitive to changes in host population density (Combes 2001). While many seabird species have declined in Puget Sound, known seabird hosts of *Contracaecum* spp., including cormorants and herons (Deardorff and Overstreet 1980), have increased over the past 20 years (Vilchis et al. 2015), possibly increasing the abundance of *Contracaecum* spp. Similarly, MMPA associated increases in marine mammal definitive hosts could drive increases in transmission (e.g., Chandra and Khan 1988). In other systems, increases in pinniped abundance have led to increases in *Contracaecum* spp. in intermediate hosts (Buchmann and Kania 2012; Haarder et al. 2014). Because anisakids require multiple hosts to carry out their life cycles, an increase in anisakid abundance could reflect some ecosystem recovery, indicating that all requisite hosts are abundant enough for anisakids to not only survive, but increase. However, because not all marine mammal populations in Puget Sound are recovering, this would imply that the recovery of some marine mammal species could

compromise the health of other, more vulnerable species by increasing parasite prevalence and potentially transmission.

Parasites are also sensitive to environmental change (Lafferty and Kuris 1999; Wood et al. 2023a). Wood et al. (2023a) examined museum specimens of eight fish species in Puget Sound and found widespread declines in parasites with life cycles requiring three or more hosts, corresponding to increasing sea surface temperature. Complex life cycle parasites are especially likely to be impacted by climate change both indirectly through range shifts and phenological mismatches with hosts, and directly by reducing parasite virulence (Carlson et al. 2017). Puget Sound has been shaped by anthropogenic changes for over a century. The region has become one of the most populated estuarine environments, resulting in nutrient input and harmful algal blooms, toxic pollutant and sewage input, and extensive nearshore habitat alteration (Brandenberger et al. 2008; Anderson et al. 2021; Fresh et al. 2011). In addition, sea surface temperatures in Puget Sound increased by 1°C between 1950 and 2005 (Snover et al. 2005). Anisakids rely on the health of multiple host species, and these species are affected by increasing temperatures and pollution in variable ways. For example, increasing temperatures can put physiological stress on fish and reduce their ability to cope with added stressors (Alfonso et al. 2021). Pollutants can either increase parasitism by reducing host immune function or limit parasitism by directly affecting the parasite (Sures et al. 2006). Changes in pollutants and temperature may counteract any increases in anisakids resulting from increasing definitive host density, possibly resulting in a decrease or no change in anisakid abundance and infection risk.

There are no data on long-term change in the parasite burden of marine mammals, and accumulating historical data is challenging. Few preserved historical specimens of marine mammal gastrointestinal tracts or feces are available to measure parasite burdens from the past.

Because anisakids are transmitted to marine mammals via their prey (Figure 1), we examined the anisakid abundance of historical specimens of common marine mammal prey as a proxy for infection risk in marine mammal hosts. We estimated changes in anisakid abundance in fish intermediate hosts in Puget Sound over the past 98 years (1920–2018), which might decrease due to the negative impacts of climate change on parasite populations (Wood et al. 2023a) or increase in anisakids due to increases in definitive hosts since the 1970s, corresponding to the local protection of definitive hosts. To test these hypotheses, we assessed the effect of some definitive host abundance and environmental factors on the abundance of anisakids.

METHODS

Species and specimen selection

To assess change in parasite abundance across a 98-year period, we dissected fluid-preserved natural history specimens of five marine mammal prey species: Pacific herring (*Clupea pallasii;* n = 114), walleye pollock (*Gadus chalcogrammus;* n = 98), surf smelt (*Hypomesus pretiosus;* n = 80), Pacific hake (*Merluccius productus*; n = 69), and copper rockfish (*Sebastes caurinus;* n = 87; Table 1). These species are preyed upon by many marine mammals in the region, including minke (*Balaenoptera acutorostrata*) and humpback whales, Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), Steller and California sea lions, harbor and northern elephant seals (*Mirounga angustirostris*), and harbor porpoises (Appendix Ch. 2: Table S2.1, Figure S2.1). The fish species were selected based on their availability in natural history collections, how evenly the species was represented in natural history collections across the 20th century, and prevalence in marine mammal diets. Under ideal conditions, our analysis would have included a higher trophic level fish, like Chinook salmon (*Oncorhynchus tshawytscha*), the preferred prey of SRKW, but unfortunately, adult salmon specimens are rare in natural history collections.

However, salmon eat many species of smaller forage fish, including Pacific herring (Daly et al. 2009; Duffy et al. 2010), suggesting that Pacific herring should reflect the anisakid risk for salmon, and hence the anisakid risk for killer whales.

We used fluid-preserved specimens from natural history collections across North America, but primarily from the University of Washington Fish Collection at the Burke Museum of Natural History and Culture. We dissected specimens with a modified methodology to minimize destruction to specimens while maximizing parasite detectability (Fiorenza et al. 2020; Wood et al. 2023b). We dissected a mean of 8.2 (range 0–15) for each host species per decade from 1920–2018 (Table 2.1). Additionally, there were a few fish available from 1880–1920 that we opportunistically dissected (Table 2.1). We selected fish from the Puget Sound region (Figure 2.2). For each specimen, we recorded the collection date and locality obtained from natural history collection databases or specimen record cards and measured the host's total length in millimeters.

In cases when a range of years was reported in the accession data associated with a specimen, we used the average of the minimum and maximum years. In instances where latitude and longitude were not reported but a specific location was, we estimated the latitude and longitude using Google Maps. If neither a specific location nor set of coordinates were reported for a specimen, we recorded both latitude and longitude as not available (NA).

Dissections

Each specimen was dissected and examined under a microscope for parasites both externally and within the internal organs, as described in Welicky et al. (2021) and Wood et al. (2023b). All parasites found were collected, identified to the lowest taxonomic level, counted, and vouchered.

Nematodes were cleared (Cable 1963) and identified to the genus level morphologically by JMK, RLW, and NCM. All the anisakids we found were in their larval stages. In cases when a nematode could not be identified to the genus level, it was identified as "unknown" and excluded from further analysis (461 nematodes were unknown). Finally, we calculated the abundance of each parasite genus per host individual.

Selecting drivers

To assess what might drive observed differences in anisakids over time, we compiled data on the abundance of potential definitive hosts and on environmental drivers. Because availability of estimates of marine mammal abundance varies in space and time, we used harbor seals abundance estimates collected consistently from the San Juan Islands between 1978 and 2013 as a proxy (Jeffries et al. 2003; Chasco et al. 2017). Since the San Juan Islands have a greater harbor seal abundance than elsewhere in Puget Sound (Jefferson et al. 2021), these estimates represent the upper range of regional seal abundances. To proxy the abundance of bird definitive hosts of *Contracaecum*, we used annual estimates for the great blue heron (*Ardea herodias*) and double-crested cormorant (*Phalacrocorax auritus*) from the Audubon Christmas Bird Count for Washington State from 1968–2018 (Butcher 1990). To proxy climate, we aggregated sea surface temperature data collected from 1921–2019 at Race Rocks lighthouse (48.2980°N, 123.5315°W; British Columbia Lightstation Sea-Surface Temperature and Salinity Data), extracting average monthly temperature in °C and removing any years that lacked more than one month of data (n = 2, 1940 and 1941) before taking the annual average for each year. To proxy pollution, we compiled and averaged pollutant data from a continuous record of lead, arsenic, zinc, nickel, vanadium, chromium, copper, barium, beryllium, and lignin and soil biomarker concentrations obtained through coring Puget Sound sediments near Tacoma and Seattle, WA (Brandenberger et

al. 2008). Because some of the pollutants were collinear with one another, we ran a principal component analysis and found that the first two principal components explained 81% of the variation. We performed a LOESS smoother on both principal components to account for measurement error. Finally, we fit a correlation matrix to the estimates of all potential drivers (Appendix Ch. 2: Figure S2.2). Both cormorant and blue heron counts were highly collinear with harbor seal abundance, as was the pollutant time series (Appendix Ch. 2: Figure S2.2). Therefore, we omitted seabird abundances and sediment pollutant levels as predictor variables to avoid multicollinearity. This left harbor seal abundance and temperature as the possible drivers in our final model.

Statistical analysis

State-space model

We fit dynamic models to the available data on anisakid counts in preserved specimens, harbor seal abundance, and temperature to estimate the "true" state of each variable across the entire time period for each of the three time series. Then we used these models to ask whether anisakid dynamics were associated with harbor seal abundance and temperature. Because each of the time series contained missing data (e.g., data were not available for all years and for each of the three time series), and because observations were subject to observation error, we fit state-space models to each of these three time series. The advantage of state-space models is that they allow for both observation error and process error (e.g., variability in the underlying process), which therefore permits us to estimate the true "state" of each of the three variables for all years of the time series.

As a first step, we described the changes in anisakid abundance in Puget Sound fish, harbor seal abundance, and temperature presuming that each time series was independent from

each other. For anisakids, we fit a state-space population model to the observed parasite counts from all available data (1880–2018) to evaluate the underlying temporal dynamics of parasite abundance after accounting for potential confounding effects related to fish specimen characteristics. This core model was a density-independent population dynamic model with autocorrelated process error:

1. $N_{t+1} = N_t e^{r_t}$

2. $r_t = \eta_t$

3. $\eta_t = \rho\eta_{t-1} + \sqrt{1-\rho^2}\upsilon_t$

$\upsilon_t \sim N(0, \sigma)$

Where N_t is the true anisakid population level in year t, r_t is the population growth rate in year t, η_t is the deviation from the expected population growth rate in year t, ρ is the autocorrelation parameter, and υ_t is a random variable with mean 0 and variance 2. This model presumes that on average, annual population growth rates equal 0 over the time period, so that all variation in N_t is due to stochastic autocorrelated process error. We separated r_t and η_t in the formulation so that we could later expand the model to include an influence of harbor seals and temperature on annual growth rate.

We estimated the N_t by assuming that observations of parasite counts in sample i (y_i) depend on the population abundance in the corresponding year ($N_{t[i]}$), modified by the fixed and random effects:

4. $\log(\hat{y}_i) = \log{(N_t)}_{[i]} + \boldsymbol{X}_i\beta + \boldsymbol{U}_i\gamma$

$y_i \sim N(\hat{y}_i, \sigma^2_{obs})$

Where $\boldsymbol{X}_i$ and $\boldsymbol{U}_i$ are the vectors of fixed and random effect covariates for observation i, respectively, β and γ are the vectors of fitted fixed and random effect parameters, respectively, and k governs the observation error variance. The fixed effects account for the potentially confounding influence of fish taxonomic group, size, and location of capture on anisakid counts. The model likelihood was calculated by integrating over all possible values of v_t.

For both sea surface temperature and harbor seal population counts, we first scaled each time series so that they had a mean of 0 and standard deviation of 1, and then fit the following random walk state-space model to each scaled time series Z_i:

5. $Z_{i,t} = Z_{i,t} + \omega_{i,t}$

$\omega_{i,t} \sim N(0, \sigma_i)$

$Z_{i,t,obs} \sim N(Z_{i,t}, \sigma_{i,obs})$

Where $\omega_{i,t}$ is the annual change in Z_i, $X_{t,t,obs}$ is the observation of time series i in year t, and σ_i and $\sigma_{i,obs}$ are the process and the observation error in Z_t, respectively. As above, the model estimates Z_t by integrating over all possible values of ω_{it}.

We then sought to relate anisakid dynamics to temperature and harbor seal abundance, by making the anisakid population growth rate a function of either harbor seal or temperature:

6. $r_t = Z_{i,t}\theta_i + \eta_t$

Where θ_x is the effect of Z_i (either harbor seal or temperature) on the population growth rate of anisakids.

We fit three variations of the above model. The first where r_t is unrelated to either temperature or harbor seal population size. The second related r_t to harbor seal population size, and the third related r_t to temperature. We did not consider a fourth model that included temperature and harbor seal, because these two variables were highly correlated over the time period where data were available for both covariates. We evaluated the strength of evidence for each model using AIC weights. All models were fit using Template Model Builder (TMB 1.9.4 in R 4.2.3; Kristensen et al. 2023).

RESULTS

A total of 2,052 anisakid nematodes from two genera (*Contracaecum* and *Anisakis*) were identified in the five fish host species (n = 439). *Contracaecum* spp. were detected in every fish species, with a total of 1,906 *Contracaecum* individuals detected and a prevalence of 47.8%. *Anisakis* spp. were only detected in walleye pollock, Pacific hake, and surf smelt. A total of 146 *Anisakis* nematodes were found across 11 host individuals, at a prevalence of 2.5%. This was an insufficient sample size for statistical analyses, therefore, the state-space analysis was run on *Contracaecum* spp. only.

State-space model

The base state-space model of anisakid abundance revealed decadal-scale variability from 1920 to 2019. The counts of *Contracaecum* spp. in fish museum specimens suggest a roughly 4-fold decline in overall population levels in Puget Sound from 1920 until the early 1970s, with most of the decline occurring between late 1940s and through the 1960s. Population levels appeared to stabilize for several decades before beginning to rise again starting in the late 1990s and continuing until the end of our data time series in 2019.

Our average temperature data spanned from 1920–1939 and 1942–2019. Because we did not have temperature data prior to 1920, we only estimated *Contracaecum* counts in the subsequent models from 1920–2019 (Figure 2.4). Harbor seal abundance data from the San Juan Islands were limited; we had estimates from 1978, 1983–1999, and 2013. However, the harbor seal state-space model was able to converge over the same period (1920–2019), and predicted abundance for the period prior to 1978 and in intervening years (1979–1982, 2000–2012, 2014–2019) (Figure 2.5b). These harbor seal abundance estimates were relatively low prior to the late 1970s with wide confidence intervals, after which the population increased rapidly from the late 1970s to mid-1990s, and then stabilized.

Model selection indicated marginal support of models that related anisakid dynamics to either temperature or harbor seal abundance over the null model where anisakid dynamics were related to other unobserved variables (Table 2.2). The strength of evidence (i.e., AIC weight) was not overwhelming, reflecting the limited duration of the harbor seal time series, and the limited ability to capture inter-annual dynamics of anisakid populations at the same time scale as our temperature data.

For the harbor seal model, the model predicts similar decline and partial recovery in anisakid counts as described above, while also hindcasting unobserved harbor seal densities prior to 1978. This hindcast estimates a modest decline in harbor seals from 1920–1966. However, without abundance observations, these estimates are very imprecise. The harvest of seals was not consistently documented over the study period to incorporate estimates based on cull data into the model. Overall, this model estimates that anisakid population growth rates switch from negative to positive after 1990, when San Juan Island harbor seal densities reach 3,510.

For the temperature model, the model explains the rise in anisakid dynamics with respect to average sea surface temperature observations collected from 1920–2016. This model predicts that anisakid growth rate was mostly negative from 1920 to around 1980, after which this model predicts a greater increase in the parasites than in the previous two models, coinciding with temperatures consistently exceeding 9°C.

DISCUSSION

Conservation measures may facilitate the resurgence of Contracaecum spp.

While the models were similarly supported, we found that *Contracaecum* spp. abundance most closely mirrored the population trajectory of harbor seal definitive hosts in Puget Sound. *Contracaecum* spp. declined in the five fish hosts we assessed across Puget Sound between 1920 and the early 1990s. Since the 1990s, there has been an increase in *Contracaecum* in the fish hosts examined, correlated with an increase in harbor seal abundance, suggesting a recovery driven by an increase in definitive host abundance. Our analysis provides support for the hypothesis that the recent resurgence of *Contracaecum* in Puget Sound fishes might have been driven by conservation of marine mammal definitive hosts.

Definitive host drivers

Our findings support the hypothesis that *Contracaecum* spp. abundance is positively correlated with marine mammal abundance, as seen elsewhere following marine mammal protections (Chandra and Khan 1988); Buchmann and Kania 2012; Haarder et al. 2014). While harbor seal abundance prior to 1970 was unknown, our model was able to generate relatively low estimates with wide confidence intervals for harbor seals. This is consistent with expectations for harbor seal abundance over this period, due to active control measures in the region from 1940–1963,

but may not accurately represent total population abundance at the beginning of the study period (Jeffries et al. 2003). Harbor seals increased following the implementation of the MMPA, then reached a saturation point in the 1990s (Jefferson et al. 2021). We found that *Contracaecum* spp. abundances in prey species correspondingly increased following a lag period of approximately 17 years. It is unclear if this lag period is consistent with other systems, as no other studies have assessed change in *Contracaecum* over a similar continual temporal period. For example, in the Baltic Sea, a significant increase in *Contracaecum osculatum* was detected in Baltic cod (*Gadus morhua*) by comparing parasitological observations from 2012 to a dataset from 1982–1983 (Haarder et al. 2014); it is unknown when the increase began, because only two discrete time periods were compared. Our abundance estimates of harbor seals come from the San Juan Islands, where harbor seals have generally been more abundant than in other sub-regions of Puget Sound; therefore, our analysis may not have captured fine-scale relationships between local harbor seal populations and *Contracaecum* spp. abundance. However, it is likely that other marine mammals have contributed to *Contracaecum* spp. abundance in fish hosts; for example, since the 1970s, California (Calambokidis and Baird 1994) and Steller sea lions (Wiles 2014), and northern elephant seals (*Mirounga angustirostris;* Gaydos and Pearson 2011) have also increased in Puget Sound. It is likely that increases in other pinniped species that we were unable to quantify (which we believe are well-proxied by harbor seal abundance) may have contributed to this increasing trend in *Contracaecum* spp. as well.

While the recent increase in pinnipeds in this region could explain the recent increase in *Contracaecum* spp., without consistent abundance estimates of harbor seals or other pinnipeds in the region dating farther back in the 20th century, it is impossible to determine if pinniped culls caused the observed decline in *Contracaecum* from 1920–1990. Pinnipeds have been actively

hunted in this area since the early 1900s (Figure 3a) (Jeffries et al. 2003), and from 1943–1960 extensive bounty hunting of harbor seals drove the population down to fewer than 2,000 individuals (Newby 1973), which coincides with the period of the steepest decline in *Contracaecum* in our dataset. The harbor seal population trajectory prior to this bounty is unclear; the abundance of harbor seals in all of Washington State before 1940 was estimated by one group to be 5,000 to 6,000 (Newby 1973), while others estimate the abundance was closer to 10,000 seals (Scheffer and Slipp 1944), which matches the estimate of harbor seals in the much smaller region of Washington's inland waters today (Jefferson et al. 2021). Harassment could have also contributed to the decline of seals in this area; harbor seals are sensitive to noise and human disturbance, which can cause abandonment of traditional haulouts (Divinyi 1971), and the amount of human activity in Puget Sound increased over the 20th century (Newby 1973). Due to the uncertainty surrounding harbor seal abundances throughout the 1900s, it is possible that a decline in other hosts contributed to the decline in *Contracaecum* that we observed.

Importantly, due to the preservation method of fish hosts, we were unable to genetically identify *Contracaecum* worms to the species level. Larval nematodes are difficult to identify morphologically, and the DNA of the parasites was damaged during the formalin fixation of the hosts, making any genetic sequencing to resolve species-level identity challenging. Because of these limitations, we could not infer which definitive host species may be the most important driver of the observed change. Because cormorant and heron abundance estimates were strongly correlated with harbor seal estimates, we excluded seabird hosts from our analysis, but seabirds could have influenced the relationship observed in *Contracaecum* spp. as well. Globally, there are over 100 species of *Contracaecum*, some of which infect marine mammals, while others infect seabirds (Shamsi 2019). Without knowing which parasite species we discovered in our fish

hosts, we cannot determine whether the change in abundance reflected multiple *Contracaecum* species, which could result from increases in both seabird and pinniped hosts, or if it was driven by one parasite species and accordingly, one host group in particular. The correlation between *Contracaecum* spp. abundance and marine mammal abundance might mask a more specific parasite–seabird host relationship. Just as increases in pinniped populations have driven changes in *Contracaecum* abundance in intermediate hosts, increases in seabird abundance have correspondingly influenced the abundance of *Contracaecum* spp. in other systems following seabird protection (Švažas et al. 2011; Chukalova 2008). There is reason to suspect this would be the case in Puget Sound as well. Seabirds are at risk of entanglement and bycatch, threats that have been reduced by bird-friendly net requirements enacted in 1997 (Melvin et al. 1999) and the removal of derelict fishing gear starting in 2002 (Good et al. 2009; NWSC 2008). Seabird species that host *Contracaecum* have increased in the region (Vilchis et al. 2015) and this could have contributed to the recent increase in *Contracaecum* spp. in Puget Sound.

Environmental and intermediate host drivers

Though not supported as the best-fit model, the model with temperature as a driver also showed a correlation with *Contracaecum* spp. abundance, and we cannot rule out temperature as an explanation for the rise we observed in *Contracaecum* spp. Sea surface temperatures were relatively stable until the 1970s, after which temperatures began increasing rapidly (Figure 2.3). This turning point for SST occurred around the same time that harbor seal abundance began increasing, as evidenced by their high positive correlation (Appendix Ch. 2: Figure S2.1). The observed correlation of increasing *Contracaecum* spp. with increasing temperature suggested that in warm years, *Contracaecum* spp. were more abundant. These warmer-than-average years may represent pulse warming events, or heat waves (Claar and Wood 2020). While pulse

warming events are not expected to favor complex-life cycle parasites, they could increase parasite abundance through increases in vital rates (i.e., growth and reproduction), or by decreasing host immunity (Claar and Wood 2020). Additionally, increased temperatures speed up the development of anisakids (Measures 1996), and their protective cuticles allow for them to withstand increasing temperatures (Hynes and Nicholas 1963). This potential increase in *Contracaecum* spp. development rate could have led to a higher prevalence and/or parasite loads in warmer years. As the period of increasing mean temperature overlapped with efforts to reduce pollution and increase populations of definitive hosts, these factors could have worked synergistically to increase *Contracaecum* spp. abundances in later years.

We were unable to disentangle the effect of pollutants from the other potential drivers on anisakid abundance in our analysis. Pollutants were highly correlated with harbor seal abundance, probably because pollutant regulations enacted in the 1970s (i.e., the U.S. Clean Water Act) coincide with the MMPA. Because we were more interested in the role of marine mammal abundance as a driver for parasite abundance, we excluded the collinear pollutant variables from our models. However, pollutants can influence parasitism in several ways – for example, by increasing host susceptibility, decreasing the abundance of hosts, or increasing the mortality of hosts or parasites (Lafferty and Kuris 1999; Vidal-Martínez et al. 2010). Trace metals are known to generally have negative effects on intestinal helminths, resulting in mortality or reduced infectivity (Lafferty 1997; Pietrock and Marcogliese 2003; Vidal-Martínez et al. 2010), and pollutants may cause mortality in free-living stages (Evans 1982; Lafferty 1997; Pietrock and Marcogliese 2003). Additionally, *Contracaecum* spp. have complex life cycles that leave them vulnerable to each host’s response to pollution (Lafferty and Kuris 1999). As heavy metals bioaccumulate in a food web, this could result in mortality in several trophic levels of

their hosts (Poulin 1992; Garai et al. 2021; Das et al. 2002; Sures 2006). While we could not assess whether pollutants were a driver of parasite abundance in this analysis, they could have played a role in the long-term decline followed by the resurgence in *Contracaecum* spp. that we detected.

In addition to environmental factors, the decline we detected could have resulted from changes in intermediate host abundance, but there were a lack of data on host abundances across the study period. Reducing fish or marine mammal host abundance below a threshold level has been shown to reduce anisakid abundance in other systems (Odense 1978; Des Clers and Wootten 1990; Lafferty and Kuris 1999; McClelland et al. 1983). *Contracaecum* spp. can use a wide range of fish as intermediate hosts, and in Puget Sound, fish intermediate host populations have changed over the past century both through habitat loss and overfishing that left several of our study species depleted (Gaydos and Brown 2009; Palsson et al. 2009; Simenstad et al. 2011; Greene et al. 2015; Buchmann and Mehrdana 2016; Shamsi 2019; Essington et al. 2021). While we were unable to test fish population abundances as a potential driver, Wood et al. (2023) tested the effect of fish population on parasite counts and found it was not significant. Therefore, it is unlikely that declines in intermediate fish host abundances significantly contributed to the observed decline in *Contracaecum* spp.

Implications

The recent recovery of *Contracaecum* spp. has the potential to impact definitive host health in the Puget Sound. For pinniped and seabird definitive hosts, *Contracaecum* spp. infections can result in pathology and decreases in fitness; in seabirds, *Contracaecum* infections can result in ulcerative gastritis at the point of attachment in the gastrointestinal tract (Rokicki et al. 2011), and they are also known to cause gastric ulcers in the stomachs of pinnipeds (Liu and Edward

1971; Spraker et al. 2003; Hrabar et al. 2021). While not the definitive hosts for *Contracaecum* spp., cetaceans have also been infected with larval forms (Cavallero et al. 2011; Luque et al. 2010; Dailey 2001). Though the resulting pathology does not typically result in mortality, it can be a consequential stressor for hosts (Dailey 2001). A recent meta-analysis found that sub-lethal helminth infections reduce host energetic condition, which can have knock-on effects, including depressed immune response (Shanebeck et al. 2022). In already-stressed populations like SRKW, this increase could be consequential. If *Contracaecum* spp. continue to increase with definitive host populations, *Contracaecum* infections may become more of a threat to definitive pinniped and seabird hosts, and accidental cetacean hosts.

Implications for marine mammal health aside, the observed recent increase may be an indication that *Contracaecum* prevalence is beginning to return to a previous baseline. Previous studies have found that parasite biodiversity is directly tied to host biodiversity; for example, Hechinger and Lafferty (2005) found that in a salt marsh in California, species richness, heterogeneity, and abundance of definitive host bird species were positively associated with the same factors in trematodes in intermediate snail hosts. Other systems have exhibited an increase in disease burdens to previous baselines with a restoration of biodiversity. Wood and Lafferty (2013) suggest that the tick-borne pathogen that causes Lyme disease, *Borrelia burgdorferi*, labeled an "emerging disease" after its discovery in the northeastern US in the 1970s, is actually returning to a previous baseline. In prior centuries, agricultural conversion and hunting had driven out the white-tailed deer hosts on which tick populations rely. When agriculture moved out of the northeast, habitat, deer, ticks, and Lyme disease returned (Wood and Lafferty 2013). As anisakid nematodes rely on a diverse suite of hosts from multiple trophic levels (Klimpel and Palm 2011), any increase may be a signal of ecosystem recovery, as all requisite hosts are present

and abundant enough for *Contracaecum* spp. to not only carry out their life cycle, but also increase in abundance (Hudson et al. 2006).

CONCLUSION

Our study used historical specimens from natural history collections to detect a change in parasite burden that would have otherwise gone unnoticed. We found a significant decline in *Contracaecum* spp. nematodes from 1920–1989 across five fish species in Puget Sound, followed by an increase from the 1990s to 2018. This trend was correlated with the population trajectory of harbor seals in Puget Sound, but changes in temperature, environmental regulations, pollution, and seabird hosts may have also influenced the increase. The earlier decline in *Contracaecum*, beginning in the 1920s, is not explained by our data, and could be attributable to other definitive host declines in this region. However, the recent increase may be a cause for concern among at-risk marine mammal hosts, like SRKW. While *Contracaecum* spp. abundance, and therefore infection risk, may be below their historical baselines, marine mammals today face many more sublethal stressors than they did 100 years ago. As gastrointestinal parasites can work in tandem with other stressors to reduce marine mammal health, this recent increase in *Contracaecum* spp. in Puget Sound suggests that parasite infections may put recovering marine mammal populations at greater risk today than they would have been through most of the 20th century.

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CH. 2 TABLES

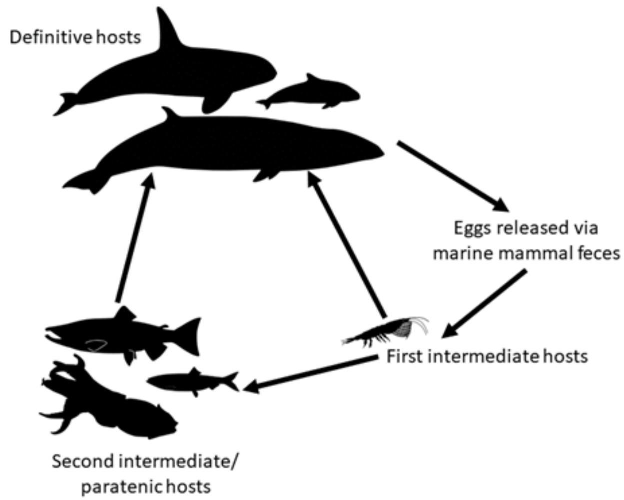
Table 2.1: The number of fish from each decade available for dissection, removing any specimens collected in years that did not have temperature data available.

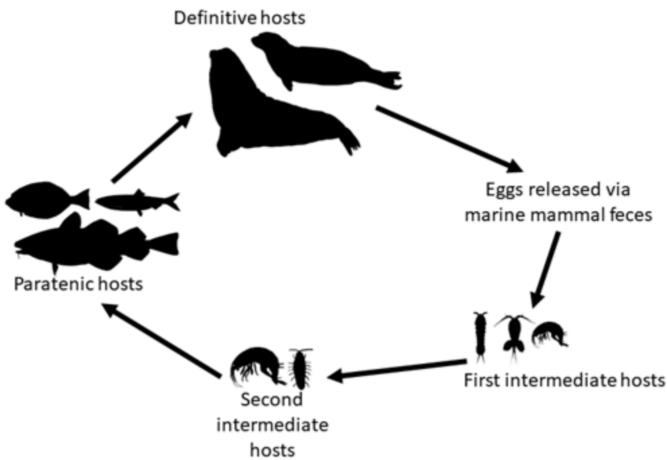
Species	**1880**	**1890**	**1900**	**1910**	**1920**	**1930**	**1940**	**1950**	**1960**	**1970**	**1980**	**1990**	**2000**	**2010**
Pacific hake	0	0	0	0	0	14	3	1	9	2	14	14	0	11
Surf smelt	1	0	2	0	15	13	13	0	15	5	4	0	0	3
Walleye pollock	0	0	4	0	2	14	14	5	3	12	4	12	12	14
Pacific herring	0	0	10	0	14	13	5	2	14	10	13	14	0	10
Copper rockfish	0	2	0	0	14	14	14	14	14	7	3	0	5	0

Table 2.2: The results from our model selection for the state space models investigating the relationship between *Contracaecum spp*. abundance and potential drivers. We used Akaike information criterion weight (AIC weight) to determine model fit.

Model	**Predictors**	**AIC**	**ΔAIC**	**AIC weight**
1	No predictors	1911.166	1.230	0.160
2	Temperature only	1910.308	0.372	0.246
3	Seals only	1909.936	0	0.296

CH. 2 FIGURES





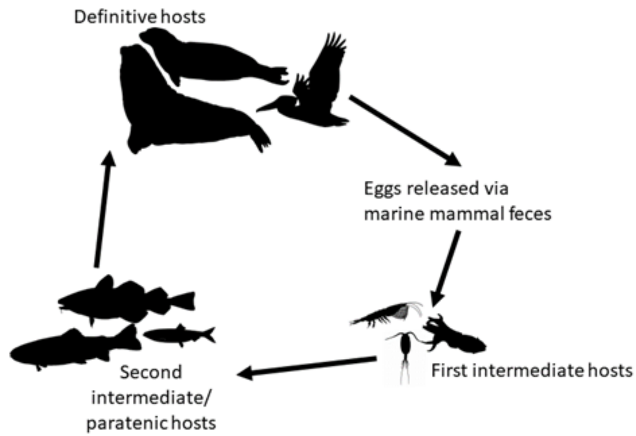


Figure 2.1: Generalized life cycle of anisakids– *Anisakis* spp. infect cetaceans as their definitive hosts, *Pseudoterranova* spp. infect pinnipeds, and *Contracaecum* spp. infect pinnipeds and seabirds. Vector images of cetaceans courtesy of Chris Huh under the Creative Commons Attribution-ShareAlike 3.0 Unported license.

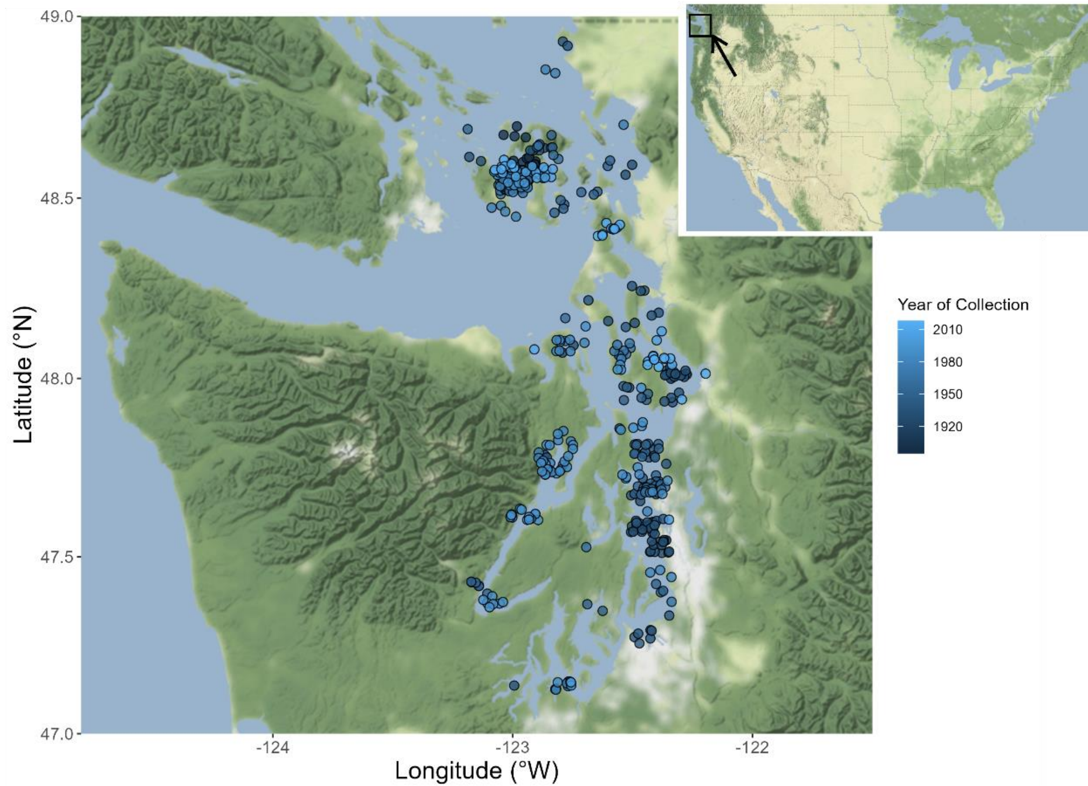
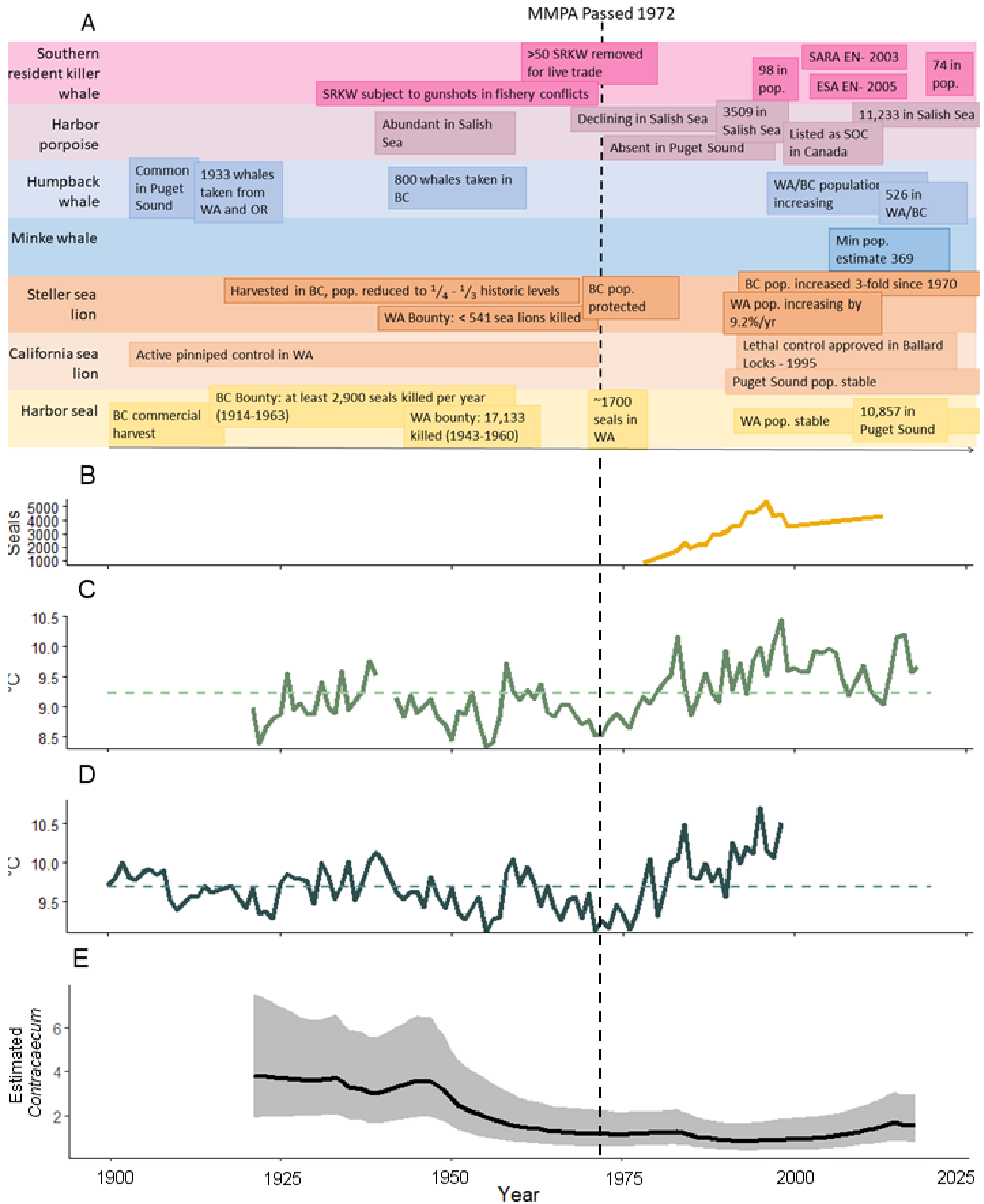


Figure 2.2: A map of the sampling location and year of collection for the fish specimens dissected for parasitological analysis. Darker colors indicate older specimens. The black rectangle represents where our sampling region is located within the United States.



A
MMPA Passed 1972
Southern resident killer whale
>50 SRKW removed for live trade
SRKW subject to gunshots in fishery conflicts
98 in pop.
SARA EN- 2003
ESA EN- 2005
74 in pop.
Harbor porpoise
Abundant in Salish Sea
Declining in Salish Sea
3509 in Salish Sea
Absent in Puget Sound
Listed as SOC in Canada
11,233 in Salish Sea
Humpback whale
Common in Puget Sound
1933 whales taken from WA and OR
800 whales taken in BC
WA/BC population increasing
526 in WA/BC
Minke whale
Min pop. estimate 369
Steller sea lion
Harvested in BC, pop. reduced to 1/4 - 1/3 historic levels
WA Bounty: < 541 sea lions killed
BC pop. protected
BC pop. increased 3-fold since 1970
WA pop. increasing by 9.2%/yr
California sea lion
Active pinniped control in WA
Lethal control approved in Ballard Locks - 1995
Puget Sound pop. stable
Harbor seal
BC commercial harvest
BC Bounty: at least 2,900 seals killed per year (1914-1963)
WA bounty: 17,133 killed (1943-1960)
~1700 seals in WA
WA pop. stable
10,857 in Puget Sound
B
Seals
C
°C
D
°C
E
Estimated Contracaecum
1900
1925
1950
1975
2000
2025
Year

Figure 2.3: A) A timeline of the exploitation of commonly found marine mammals in Puget Sound, with underlying plots showing the trajectory of B) harbor seal abundance in the San Juan Islands from Chasco et al. 2017; C) annual average sea surface temperature (SST) in °C collected from Race Rocks from 1928 to 2019 (solid line), compared to the average temperature of the entire period (dashed line); D) SST in °C of the North Pacific reconstructed from 1900-1999 through geoduck shells (solid line, Strom et al. 2004), compared to the average temperature of that period (dashed line); and E) the mean *Contracaecum* count predicted by Model 1, the state-space model without any drivers.

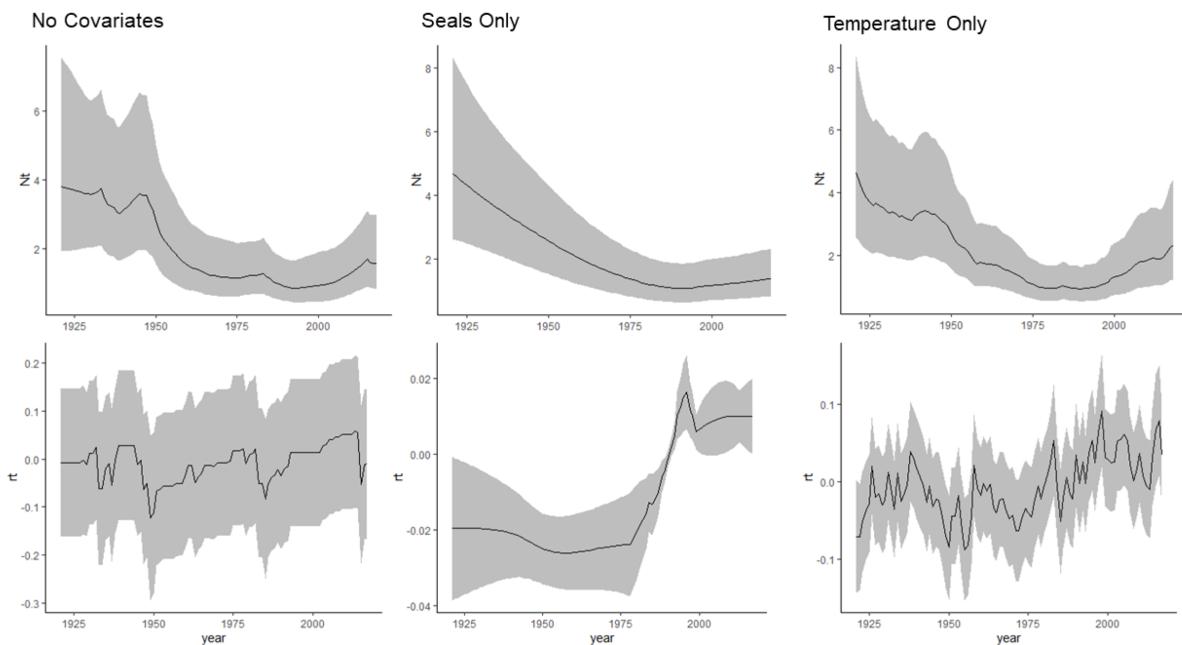
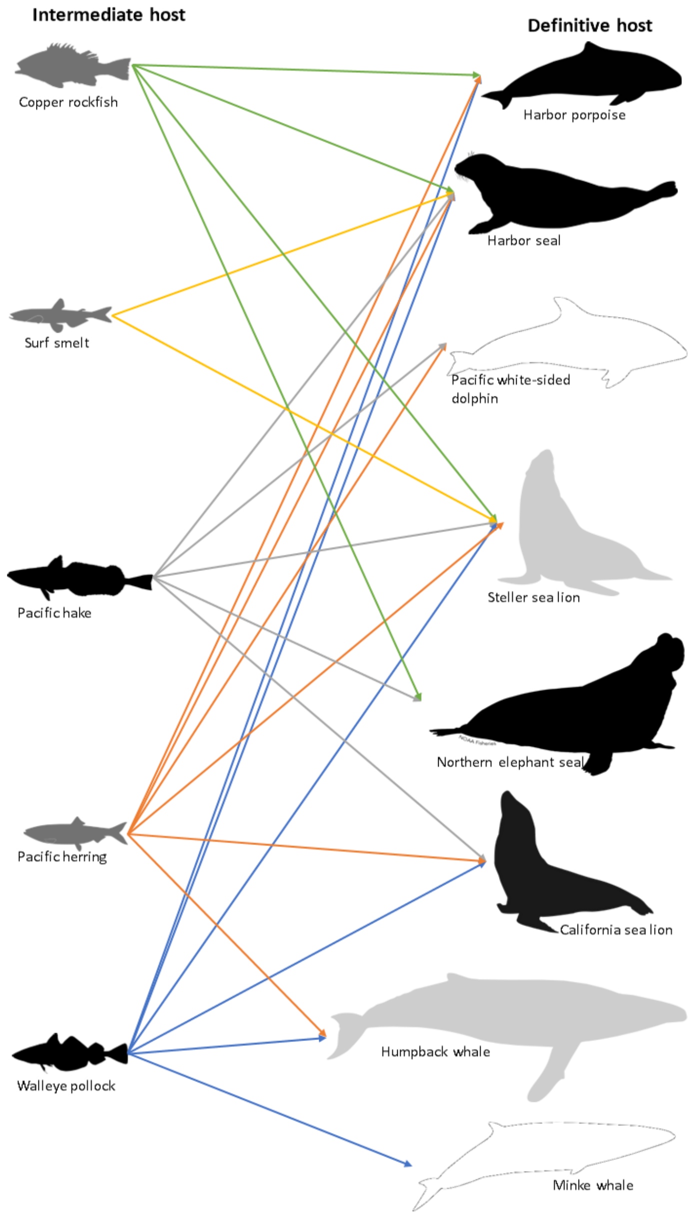


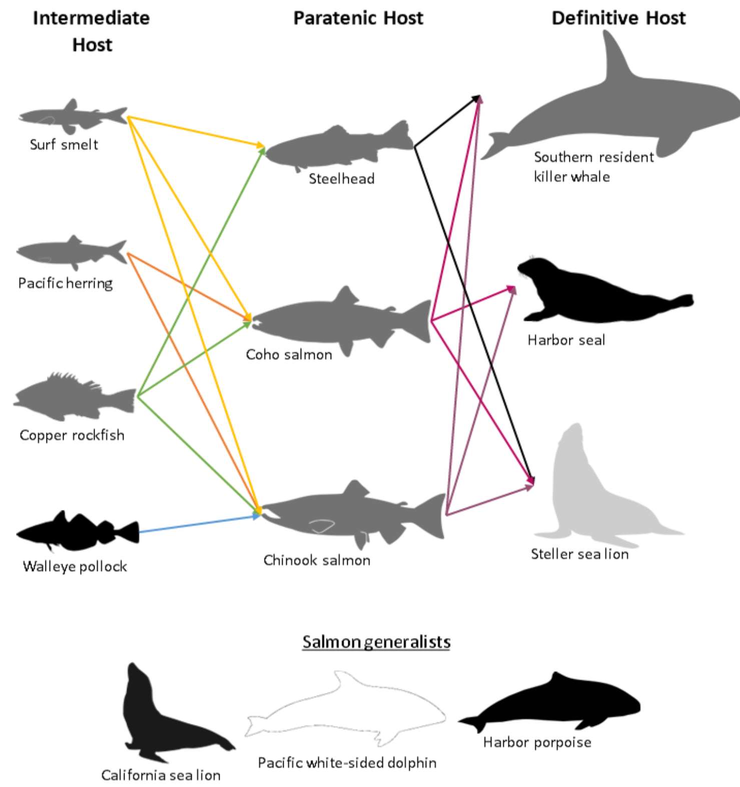
Figure 2.4: The candidate models, with the effect of (A) no covariates, (B) seals only, and (C) temperature only on the estimated abundance of *Contracaecum* abundance (N_t) and growth rate (r_t).

APPENDIX CH. 2

Table S2.1: Marine mammals and selected prey fish species. Data were reviewed for marine mammals in Washington, British Columbia, Alaska, or the entire Northeast Pacific. When possible, we included data from the Puget Sound and Salish Sea.

Fish intermediate host species	**Marine mammals who prey upon it**	**References**
Pacific herring *(Clupea pallasii pallasii)*	Humpback whale, Pacific white-sided dolphin, harbor porpoise, Harbor seal, Steller sea lion, northern fur seal	Witteveen et al. 2005, 2008, Ford 2014, Heise 1997b, Morton 2000, Nichol et al. 2013, Walker et al. 1998, Wilke and Kenyon 1952, Lance et al. 2012, Everitt et al. 1981, Sinclair and Zeppelin 2002, Womble and Sigler 2006, Gelatt and Gentry 2017
Surf smelt (*Hypomesus pretiosus)*	Harbor seal	Lance et al. 2012
Pacific hake (*Merluccius productus)*	Pacific white-sided dolphin, harbor porpoise, Harbor seal, California sea lion, Steller sea lion, northern fur seal, northern elephant seal	Heise 1997b, Morton 2000, Nichol et al. 2013, Walker et al. 1998, Lance et al. 2019, Everitt et al. 1981, Bailey and Ainley 1981, Sinclair and Zeppelin 2002, Womble and Sigler 2006, Zeppelin and Orr 2010, Antonelis et al. 1987, Condit and LeBoeuf et al. 1984
Copper rockfish (*Sebastes caurinus)*	Harbor porpoise, Harbor seal, northern fur seal, Steller sea lion, and northern elephant seal (all known to eat *Sebastes* sp).	Walker et al. 1998, Everitt et al. 1981, Kajimura et al. 1980, Antonelis et al. 1987, Condit and LeBoeuf et al. 1984, Pike 1958, Spaulding 1964
Walleye pollock (*Gadus chalcogrammus)*	Minke whale, humpback whale, harbor porpoise, Harbor seal, California sea lion, Steller sea lion, northern fur seal	Tamura et al. 2016, Witteveen et al. 2005, 2008, Ford 2014, Nichol et al. 2013, Walker et al. 1998, Lance et al. 2012, Everitt et al. 1981, Gelatt and Gentry 2017


Intermediate host
Definitive host
Copper rockfish
Harbor porpoise
Harbor seal
Surf smelt
Pacific white-sided dolphin
Steller sea lion
Pacific hake
Northern elephant seal
Pacific herring
California sea lion
Walleye pollock
Humpback whale
Minke whale


Intermediate Host
Paratenic Host
Definitive Host
Surf smelt
Steelhead
Southern resident killer whale
Pacific herring
Coho salmon
Harbor seal
Copper rockfish
Walleye pollock
Chinook salmon
Steller sea lion

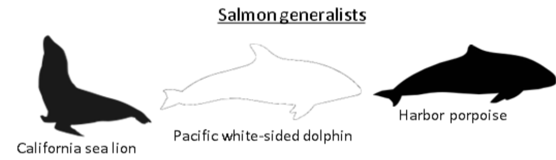

Salmon generalists
California sea lion
Pacific white-sided dolphin
Harbor porpoise

Figure S2.1: The trophic relationships between the selected fish species and (a) the marine mammal species and (b) salmon species in Puget Sound, WA. Species in black represent stable populations in this region, while dark gray indicate declining populations, light gray represent increasing populations, and outlined species are data deficient and therefore population trends are unknown. Paratenic hosts are hosts in which the parasite does not undergo any development, but that help the parasite reach their definitive host. The arrow color indicates which species is consumed by which host.

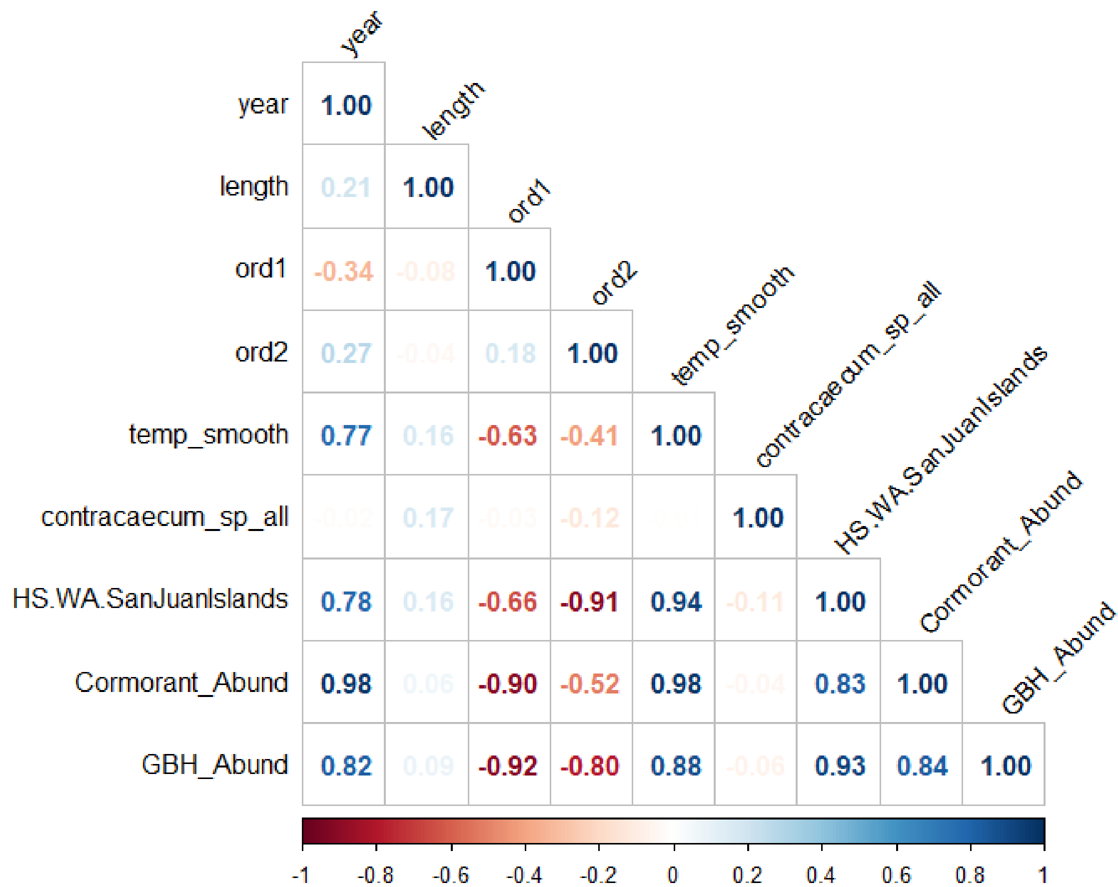


Figure S2.2: A correlation plot including all variables considered for analysis. Pollutant data were compiled from a continuous record of lead, arsenic, zinc, nickel, vanadium, chromium, copper, barium, beryllium, and lignin and soil biomarker concentrations obtained through coring Puget Sound sediments (Brandenberger et al. 2008). We extracted and averaged annual values collected from Puget Sound near Tacoma and Seattle, WA. Because some of the pollutants were collinear with one another, we ran a principal component analysis and found that the first two principal components ("ord1" and "ord2") explained 81% of the variation. We performed a LOESS smoother on both principal components to account for measurement error.

APPENDIX CH. 2 REFERENCES

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Chapter 3: Opening a can of worms: Archived canned fish fillets reveal 40 years of change in parasite burden for four salmon species

Authors: Natalie Mastick, Rachel Welicky, Aspen Katla, Bruce Odegaard, Virginia Ng, and Chelsea Wood

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ABSTRACT

How has parasitism changed for Alaskan salmon over the past several decades? Parasitological assessments of salmon are inconsistent across time, and though parasite data are sometimes noted when processing fillets for market, those data are not retained for more than a few years. The landscape of parasite risk is changing for salmon, and long-term data are needed to quantify this change. Parasitic nematodes of the family Anisakidae (anisakids) use salmonid fishes as intermediate or paratenic hosts in life cycles that terminate in marine mammal definitive hosts. Alaskan marine mammals have been protected since the 1970s, and as populations recover, the density of definitive hosts in this region has increased. To assess whether anisakid burden has changed in salmonids over time, we used a novel data source: salmon that were caught, canned, and thermally processed for human consumption in Alaska, USA. We examined canned fillets of chum (*Oncorhynchus keta*, *n* = 42), coho (*Oncorhynchus kisutch*, *n* = 22), pink (*Oncorhynchus gorbuscha*, *n* = 62), and sockeye salmon (*Oncorhynchus nerka*, *n* = 52) processed between 1979 and 2019. We dissected each fillet and quantified the number of worms per gram of salmon

tissue. Anisakid burden increased over time in chum and pink salmon, but there was no change in sockeye or coho salmon. This difference may be due to differences in the prey preferences of each species, or time spent in marine systems. Canned fish serve as a window into the past, providing information that would otherwise be lost, including information on change over time in the parasite burden of commercially, culturally, and ecologically important fish species.

INTRODUCTION

Species that are difficult to study, including small and inconspicuous species like parasites, can experience changes in abundance that go entirely unnoticed. For example, a highly visible parasite, *Clavinema mariae*, experienced an 8-fold increase in English sole (*Parophrys vetulus*) in Puget Sound, Washington, USA, that went undetected for almost a century, even though sole were the subject of both a major recreational fishing industry and government research (Howard et al. 2019). For marine parasites, there are few data sources that can be used to reconstruct information about past abundances (Harmon, Littlewood, and Wood 2019; Wood and Vanhove 2023). Comparing contemporary ecosystems to well-studied ecosystems of the past by sampling the same sites with the same methodology has been effective (e.g., Quinn et al. 2021), but is constrained by the available data, which is contingent upon the interests of past researchers, sufficient documentation, and data retention. Meta-analyses of existing scientific literature can be a useful tool to detect trends in parasite abundance (e.g., Fiorenza, Wendt, et al. 2020), but are limited by the temporal constraints of online literature repositories (Wood and Vanhove 2023). Natural history collections are a promising source for reconstructing parasite population data (Harmon, Littlewood, and Wood 2019; Howard et al. 2019; Fiorenza, Leslie, et al. 2020; Welicky et al. 2021; Wood et al. 2023a, 2023b), but their use is limited to what host species are available in collections.

Limitations on the techniques, datasets, and resources available to reconstruct long-term trajectories of parasite abundance are a cause for concern, because parasites are influential in ecosystems — for better and for worse. Parasites can affect host populations and the communities and ecosystems in which they are embedded (Hudson, Dobson, and Lafferty 2006; Lafferty 2008; Wood and Johnson 2015) and as a result they can pose a threat to fisheries (Lafferty et al. 2015). It is particularly important to understand how the burden of parasitism is changing for economically and commercially important hosts, given that parasites can influence these populations (Lafferty et al. 2015; Shinn et al. 2015; Timi and Poulin 2020). Salmon (*Onchorhynchus* spp.) are culturally, economically, and ecologically important in Alaska (Carothers et al. 2021; Alaska Department of Fish and Game 2023), with an ex-vessel commercial value of $786 million in 2021 (Alaska Department of Fish and Game, 2023). Salmon also make up a large part of the diet of many marine and terrestrial predators (Quakenbush et al. 2015; Morton 1990; Sigler et al. 2009; Helfield and Naiman 2006; Stanek et al. 2017). An increase in parasites in salmon could impact Alaskan residents and wildlife, as well as the state's economy, but without historical data on the parasite burden in salmon, change is undetectable.

Unfortunately, there are few pathways for quantifying change in the parasite burden of salmon. We can identify no long-term datasets of parasite abundance for any adult salmon species globally (Fiorenza et al. 2020); the longest-term datasets on parasites in salmon that exist include parasites in sockeye salmon smolts (41 years; Bennett et al. 1998) and parasites in juvenile sockeye salmon (12 years; Bentley and Burgner 2011), but these datasets do not reflect the burden in adult salmon from marine systems. Museum specimens of adult salmon are rare, as they would occupy a substantial amount of space in space-limited collections (Wood et al. 2023b). Processors sometimes record parasite presence, especially nematodes in the musculature,

but these data are usually not stored for more than a few years (Joe Logan, Alaska Seafood Industry Professional, personal communication). Meta-analysis would be an option, but there are few papers on salmon parasites in the Northeast Pacific, where many native salmon runs exist (Fiorenza et al. 2020). Salmon are common intermediate hosts to parasitic nematodes of the family Anisakidae, or anisakids (Deardorff and Kent 1989). These parasites infect salmon and other fish to reach their definitive hosts, marine mammals (e.g., Klimpel and Palm 2011), and infections in the musculature of fish can inhibit swimming ability and increase susceptibility to predation, as well as cause other pathogenic effects (Buchmann and Mehrdana 2016). Should anisakid infections be increasing, this could add to the list of stressors that salmon face, including habitat loss and degradation (Schoen et al. 2017), climate change (Bryant 2009), and increasing predation from recovering marine mammal populations (Chasco et al. 2017). For salmon, many of the more recent advances in historical parasite reconstruction are unavailable, which means changing parasite burden cannot be effectively assessed.

Propitiously, we came across a data source maintained by an industry organization that could be used to detect a change in parasite burden for salmon in the Northeast Pacific: over four decades of canned Alaskan salmon, preserved to eventually determine how can integrity was maintained over time. Four species of salmon were canned consistently across the Gulf of Alaska and into Bristol Bay from 1979 to 2021: chum (*Oncorhynchus keta*), coho (*Oncorhynchus kisutch*), pink (*Oncorhynchus gorbuscha*), and sockeye (*Oncorhynchus nerka*). We were able to find anisakid worms in this material, providing a novel data source to test whether there has been a change in anisakid burden in salmon over the past 42 years.

Many factors could affect anisakid abundance over time. Climate change could impact complex life cycle parasites negatively (Carlson et al. 2017; Wood et al. 2023a), or positively, as

many anisakids are resilient to warming temperatures (Measures 1996). Increasing abundances of some marine mammal definitive hosts (Muto 2021; Lowry et al. 2008; Matkin et al. 2014; Towers et al. 2015, 2019; Fisheries and Oceans Canada 2009) could increase anisakid prevalence in salmon (Buchmann and Kania 2012; Haarder et al. 2014; Zuo et al. 2017). We expected the magnitude of the change might differ by salmon species, as salmon species have distinct life histories and habitats that could result in differing levels of exposure (Kaeriyama et al. 2004; Brodeur et al. 2007; Johnson and Schindler 2009). For example, pink and chum spend a greater proportion of their lives in marine systems than do sockeye and coho, potentially increasing their exposure to marine parasites (Salo 1991; Heard 1991).

We dissected canned salmon samples and extracted, counted, and morphologically identified any nematodes present, acquiring a measure of anisakid abundance per gram of fillet. We then ran generalized linear mixed effect models to determine if anisakid abundance changed over time for each species. We found a significant increase in anisakid abundance in chum and pink salmon over the study period, suggesting that salmon that spend more time in marine systems may be more exposed to anisakid infections, especially as marine mammals in this region increase.

METHODS

There are several constraints to using historical data, including biases and inconsistencies in reporting, as well as temporal and spatial gaps or patchiness (Swetnam, Allen, and Betancourt 1999; McClenachan, Ferretti, and Baum 2012; McClenachan et al. 2015). However, these shortcomings do not invalidate the data (Swetnam, Allen, and Betancourt 1999; McClenchan et al. 2015), provided that any biases are recognized and addressed (Swetnam, Allen, and Betancourt 1999; McClenchan et al. 2015). To our knowledge, this paper is the first to use

canned salmon for historical reconstruction of past parasite abundance. To address the biases related to this dataset, we needed to account for potentially confounding variables, including the location of the cannery, chilling practices used onboard vessels, and fishing region.

Selection and dissection of archived specimens

In 2020, BO and VN approached CLW, NM, and RLW to ask whether the University of Washington research team might be able to detect parasites in an extensive collection of cans amassed by the Seafood Products Association, an industry association which BO and VN lead. The Seafood Products Association had retained samples of product from the 1970s onward for the purpose of assessing degradation of canned product, but in 2020 they learned about the historical parasite ecology studies our lab was conducting and offered cans to us. From this collection, BO identified 502 cans of salmon, including fillets from chum, coho, pink and sockeye salmon originating from Alaska and Washington, produced by various companies and canneries, and canned between 1979 and 2021. There were very few cans from Washington, so we decided to focus exclusively on Alaska. Our goal was to analyze 15 cans per species for each decade. We selected cans from well-represented cannery locations when available (i.e., more than 1 can available from that location). For coho, there were few can samples available, so all cans available were used for analysis. We did not include cans that had unknown dates of packaging, or unknown species contents. Some cans did not include a specific year, and were only classified as “pre-1982”, and these were excluded from analysis. After these exclusions, our dataset contained pink (n = 62), sockeye (n = 52), chum (n = 42), and coho (n = 22) cans spread as evenly as possible across decades (Figure 3.1). Can sizes varied, including cans that were 3.75 oz, 7.5 oz, and 14.75 oz. We did not weigh the salmon fillet, as the cans contained varying amounts of liquid. Instead, we accounted for fillet size with the can size converted to grams.

Additionally, it is possible that multiple fish could be included in a single can (Bruce Odegaard, Seafood Products Association, pers. comm.).

After method testing (Appendix Ch. 3A), we found that dissecting the fish into small pieces with two, 4.25 inch fine-point forceps was the most efficient way of detecting and extracting nematodes. Nematodes formed pockets in the musculature of the fillet, which were easily detectable as we dissected. When we found a nematode, we carefully extracted the worm and preserved it in 70% ethanol. We validated our detection method by having a second observer check the work of the dissector until we achieved 100% agreement, after which we chose 6% of cans at random to check to ensure consistent agreement. Fillets in older cans were more fragile, as degradation of the fillet had occurred over time, but nematodes were still easily detectable.

We expected that all nematodes detected in the fillets would belong to the family Anisakidae, because anisakids are the only parasite family known from the muscle of Alaskan salmon (Moles 2007). We attempted to confirm the identity of the nematodes to the genus level in two ways: (1) by clearing the vouchered nematodes with lactophenol solution according to standard nematode identification procedure (Cable 1963) and identifying based on internal anatomy, and (2) by morphologically identifying the vouchered nematodes under a stereomicroscope using external anatomy only. Often, nematodes were broken or too tightly coiled to identify. The nematodes did not clear entirely (Figure 3.2b) and the cuticle remained cloudy, therefore the internal anatomy was difficult to identify. Therefore, we identified nematodes based on external anatomy where we were able. When the nematodes were not broken or too tightly coiled, we identified the worms as anisakids by the presence of a larval tooth and ventral excretory pore, which appeared as a dimple on the anterior end (Figure 3.2c), indicative of a nematode in the family Anisakidae (Hurst 1984). We were able to definitively

identify 34% (127 / 372) of the nematodes we found; of these, 100% were anisakids. Therefore, we assumed that all detected nematodes were anisakids, including those that could not be definitively identified.

Geographical predictors

To quantify differences in anisakid burden among geographical regions, we classified the region where each can was sourced, and therefore, where the fish was caught. We used four regions, adhering to the fishing regions commonly described by seafood companies in this area: Bristol Bay, which included all canneries north of the Aleutians; Western, which included canneries on the Aleutians over to Kodiak, AK; Central, which included canneries between Homer and Cordova, AK; and Southeast, which included canneries from Excursion Inlet south to Ketchikan, AK. To incorporate cannery location as a proxy for area of capture, we collected the latitude and longitude of the town where each cannery was located using Google Maps (Figure 3.3). Differences in geography made it necessary for us to split our analysis into two statistical models: one for pink, chum, and coho salmon, and another for sockeye salmon.

Pink, chum, and coho salmon decay quickly in transport, and are therefore almost always processed in the region where they are caught, typically within a 50-mile radius of the cannery (John Daly, industry expert, pers. comm.). To account for this, we included in our statistical model for parasite burden in pink, chum, and coho salmon a random effect of cannery ID nested within region (see *Statistical analysis*, below).

Conversely, sockeye is more robust to decay and therefore more transportable. Sometimes sockeye were canned in regions adjacent to where they were caught, though this varied by year of collection (John Daly, industry expert, pers. comm.). Sockeye in the more southern areas of their range faced declines from the 1990s to mid-2000s (Rand et al. 2012),

while Bristol Bay populations increased (Hilborn 2006; Rand et al. 2012; Salmone et al. 2017). This led to sockeye being transported outside of Bristol Bay to be canned in other regions, starting roughly in the 2000s (John Daly, industry expert, pers. comm.). In 2018, two major canneries closed in Bristol Bay, so fish caught in this region were more commonly shipped outside of Bristol Bay for canning (John Daly, industry expert, pers. comm.). Because of these differences in catch region, we created a time period variable representing periods in which transport patterns were consistent: pre-2000s (local canning), 2000–2018 (some non-local canning), and 2018–2021 (common non-local canning). We included this variable in our statistical model for parasite burden in sockeye salmon, where the model calculated a slope for the interaction between time period and region as a random effect (1 | region*time period).

Onboard chilling practices

By the mid-1990s, all fishing vessels were strongly encouraged to have chilling capability onboard, usually ice or a tank of seawater cooled to 32–36°F degrees for vessels fishing near the cannery, and 28°F for vessels transporting the catch a greater distance (John Daly, industry expert, pers. comm.). Not only does this keep the fish fresh longer, but it also may slow the migration of anisakids into the muscle of the fish (Cipriani et al. 2016). Anisakids can inhabit the viscera of intermediate hosts, and often will attempt to escape the host when the host dies by migrating to the muscle (Smith and Wootten 1975). The migration of anisakids from the viscera to the fillet may be tied to an increase in temperature, so reducing the temperature at which the fish is stored results in fewer nematodes in the fillet (Cipriani et al. 2016). To account for this change in preservation method, we incorporated an additional fixed effect of chilling method in our models (see *Statistical analysis*, below). We did not know the exact date that chilling practices were adopted for each vessel, but assumed that cans dated before 1995 did not have on-

board chilling, while cans during and after 1995 did, based on expert opinion (John Daly, industry expert, pers. comm.). While canning in this region has become less popular over the duration of our study (e.g., 30–40 canneries operated in Alaska in the 1970s, but there were only 16 in 2023; John Daly, industry expert, pers. comm.), the poorer quality fish, classified as No. 3 grade, were always selected for canning (John Daly, industry expert, pers. comm.). Poor quality could include softer or bruised fish, seal bites, blemishes, older catches (i.e., more time on ice), or smaller body sizes. The specifications for a No. 3 grade vary slightly from company to company but have not changed systematically over the study period (John Daly, industry expert, pers. comm.).

Statistical analysis

We tested whether nematode counts per can changed over time with generalized linear mixed effect models (GLMMs). First, we wanted to ensure that our statistical outputs would not be biased by spatial or temporal autocorrelation. We used a simplified model (anisakid count ~ year) and tested for spatial autocorrelation with the testSpatialAutocorrelation() function in the DHARMa package (Hartig 2022) in R (R Core Team, 2021). We tested for temporal autocorrelation with a Durban-Watson test using the dwtest() function in the lmtest package (Zeileis and Hothorn 2002) in R. We used the glmmTMB() function in the package of the same name (Brooks et al. 2017) in R to run both GLMMs. We detected that the data from sockeye salmon were spatially autocorrelated, so we included region in the corresponding model to account for spatial differences.

We fit a global GLMM, and then performed model selection using the dredge() function in the R package MuMIn (Bartoń 2023), which generated a table of candidate models using combinations of fixed term effects from the global model (Appendix Ch. 3B: Tables S3.1 and

S3.2). We determined which model was best supported by the data using AIC values. After model selection, we ran two GLMMs to determine how the number of anisakids changed over time. We could not determine how many individual fish were in each can, so we quantified the number of anisakids per can as the response variable. We tested for the effect of can size because we did not know if can size would be correlated with anisakid count. We ran two models to account for differences between the salmon species; since sockeye salmon were transported differently than chum, coho, and pink salmon (see above), we ran a separate model for sockeye salmon.

Model 1 – tests whether anisakid abundance changed in coho, chum, and pink salmon, accounting for geographical, cannery, and management company differences, and incorporating can size and chilling practices. We used a negative binomial distribution to account for zero-inflation in parasite counts. Can size and year were scaled using the scale() function in R. Scaled can size, chilling practice, and the interaction between scaled year and salmon species were included as fixed effects. Region nested within factory and region nested within company were included as random effects.

Equation 3.1:

$$NAnisakids_{ijkl} \sim Negative\ binomial\ (\mu_{ijkl})$$

$$E(NAnisakids_{ijkl} = \mu_{ijkl}$$

$$\log(\mu_{ijkl}) = ScaledCanSize_{ijkl} + ScaledYear_{ijkl} \times FishSpecies_{ijkl} + ChillingPractice_{ijkl} + Factory_j/Region_k + Company_l/Region_k$$

$$Factory_j \sim N(0, \sigma^2)$$

$$Region_k \sim N(0, \sigma^2)$$

$Company_l \sim N(0, \sigma^2)$

Where the response variable$_{ijkl}$ represents a measurement of nematode abundance from the *i*th can from the *j*th factory where the sample was canned, in the *k*th region where the factory was located, by the *l*th company that the factory was run by.

Model 2 – the sockeye model, which is the same as Model 1 except that it incorporates the effect of region interaction with time period. Because sockeye were transported long distances for canning, and their factory location may not be representative of where they were caught, especially following the closure of two canneries in Bristol Bay, we needed to incorporate time period (pre-2000s, 2000–2018, and 2018–2021) into the model. Scaled can size, factory, company, and the interaction between region and time period (pre-2000s, 2000–2018, and 2018–2021) were included as fixed effects.

Equation 3.2:

$$NAnisakids_{ijklm} \sim Negative\ binomial\ (\mu_{ijklm})$$

$$E(NAnisakids_{ijklm}) = \mu_{ijklm}$$

$$\log(\mu_{ijklm}) = ScaledCanSize_{ijklm} + ScaledYear_{ijklm} + Region_j \times TimePeriod_k + Factory_l + Company_m$$

$$Region_j \sim N(0, \sigma^2)$$

$$TimePeriod_k \sim N(0, \sigma^2)$$

$$Factory_l \sim N(0, \sigma^2)$$

$$Company_m \sim N(0, \sigma^2)$$

Where the response variable$_{ijklm}$ represents a measurement of nematode abundance from the *i*th can from the *j*th region that the factory was located interacting with the *k*th time period of collection, from the *l*th factory, by the *m*th company.

RESULTS

We dissected 178 cans collected from Alaska from 1979–2021, including 42 cans of chum, 22 of coho, 62 of pink, and 52 of sockeye. Of these cans, 50.6% contained nematodes, including 57.1% of chum cans, 27.3% of coho, 46.8% of pink, and 59.6% of sockeye. A total of 372 nematodes were extracted from the cans. Among all nematodes detected, 37.7% (140 / 372) had a larval tooth consistent with the anatomy of nematodes of the family Anisakidae, and of those, 90.3% (127 / 140) had a visible dimple consistent with the location of the excretory pore on members of the family Anisakidae (Hurst 1984). Of all nematodes extracted, 34% (127 / 372) were confirmed as belonging to the family Anisakidae by the identification of both a larval tooth and an excretory pore. The remaining nematodes were unidentifiable because they were broken (anterior end), the cuticle was too thick to identify morphological features, the nematode was too tightly coiled to identify the anterior end without damaging the specimen, or the vouchered specimen dried up.

Model 1

Our best fit model included scaled can size, and the interaction between salmon species and scaled year, excluding the effect of chilling practice. However, the model with chilling practice as a fixed effect was the second best fit (ΔAIC = 0.77), and we were interested in correcting for the effect of chilling, so we used this model. We found different trajectories of change over time in anisakid burden across host species (Table 3.1). Anisakids were estimated to be increasing in

chum (estimate = 1.254, p = 0.004), and in pink salmon (estimate = 0.800, p = 0.015), but the other species did not display significant trends by year (Table 3.1). As we expected, there was an increase in the number of anisakids with increasing can size (estimate = 0.6112, p = 0.00357); the can size effect controlled for differences in the mass of fish tissue evaluated among cans. Chilling practice did not have a significant effect on anisakid burden in the fillet.

While we detected an increase over time in chum, the trend was driven by a single count of 115 nematodes in a can from 2019. We were certain that this count was accurate, but it was also highly influential: removing this data point resulted in a non-significant trend. We sought to understand the likelihood that this result arose due to random chance by running a permutation test on a simplified model of anisakid count ~ year for chum salmon data. For 5,000 iterations, we randomly resampled year without replacement for each data point, repeated the analysis, and record the estimate for the effect of year. The resulting p-value (i.e., the proportion of permutations with an estimate greater than the observed estimate) was 0.0136, indicating that it was statistically unlikely that the observed increase over time arose due to random chance.

Model 2

Following model selection, the best fitting model for sockeye did not include year as a fixed effect (Appendix Ch. 3B: Table S3.2). This suggests that year is not an informative predictor for anisakid prevalence in sockeye salmon. The best fitting sockeye model included can size as a fixed effect and random effects of the interaction of region and time period, company, and factory ID. For the sockeye-only Model, there was a significant increase in the number of anisakids with increasing can size (estimate = 0.529, p= 0.0147; Table 3.2).

DISCUSSION

We found a significant increase in anisakid burden in chum and pink salmon over the 42-year study period. We found no significant change in the number of anisakids in coho and sockeye. The variability we detected among salmon species in change in anisakid infection could be attributable to differences in the life history of these fishes. The increase in anisakids we detected in chum and pink salmon in this study agrees with observations from other systems. Meta-analysis has detected a global increase in *Anisakis* spp. in intermediate hosts since the 1960s (Fiorenza et al. 2020; Chapter 1, this dissertation). More locally, parasitological dissection of museum specimens revealed a recent increase in *Contracaecum* spp. in Puget Sound fish, following a precipitous decline from 1920–1995, probably due to fluctuations in harbor seal abundance (Chapter 2, this dissertation). Though our study made use of a novel data source, the trend detected is consistent with previous studies from other regions, and reveals a previously undetected increase in anisakid nematodes in Alaskan salmon over the past 40 years.

What could drive an increase in anisakids?

The increase we observed in parasite burden across time may reflect an increase in one or more definitive host species. Marine mammals are the definitive hosts of anisakids, and these species have had varying population trends throughout Alaska. While there have been regional declines in Cook Inlet beluga whales (*Delphinapterus leucas*) and the western stock of Steller sea lions (*Eumetopias jubatus*), most species have been increasing in Alaskan waters since the implementation of the Marine Mammal Protection Act in 1972 including: Northern fur seals (*Callorhinus ursinus*) on Bogoslof Island, harbor seal (*Phoca vitulina*) populations in Bristol Bay and Kodiak (Muto et al. 2021), humpback whales (*Megaptera novaeangliae*), Bristol Bay belugas, southern Alaska resident killer whales, Northern resident killer whales, and West Coast

transient killer whales (*Orcinus orca*) (Muto et al. 2021; Lowry et al. 2008; Matkin et al. 2014; Towers et al. 2015, 2019; Fisheries and Oceans Canada 2009).

With increases in marine mammal hosts, there can be corresponding increases in the anisakids detected in intermediate fish hosts. Such trends have been observed in the Baltic Sea with gray seals and Baltic cod (Buchmann and Kania 2012; Haarder et al. 2014; Mehrdana et al. 2014), and in Puget Sound with harbor seals (Chapter 2). In the Baltic Sea, an increase in gray seals (*Halichoerus grypus*) resulted in an increase in the prevalence in Baltic cod (*Gadus morhua*) of *Contracaecum* spp. (prevalence = 22% in 1980s, 55.1% in 2012, 100% in 2014) and *Pseudoterranova* spp. (prevalence = 2% in 2011, >50% in large cod and 20% in small cod in 2014); Haarder et al. 2014; Mehrdana et al. 2014). In Puget Sound, WA, USA, there has been a recent increase in the number of *Contracaecum* spp. found in five fish species, possibly due to increasing harbor seal abundances in the area (Chapter 2). As juveniles, pink and chum salmon spend several months living in nearshore marine and estuarine regions before moving offshore (Levings 2016; Howard, Miller, and Murphy 2017). Coastal regions and estuaries in Alaska are frequently used by marine mammals, including pinnipeds, belugas, and humpback whales (Muto et al. 2020), which could increase the prevalence of anisakids in the environment. This could result in an increasing risk to salmon of exposure to infected prey if salmon feed more prevalently in coastal areas nearby seal haulouts or areas frequented by marine mammals.

The change over time we observed could also be attributable to increases in sea surface temperatures (SST). Though climate change could have negative effects on complex-life cycle parasite transmission (Marcogliese 2008; Carlson et al. 2017; Wood et al. 2023a), there are some scenarios that could increase anisakid prevalence with increasing temperatures. Warming temperatures could increase parasite abundance by increasing parasite vital rates or decreasing

host immunity (Macnab and Barber 2012; Claar and Wood 2020). Increased temperatures have been found to speed up the development of anisakid eggs (Measures 1996). Anisakids are generally protected from changing environmental conditions by their hosts, but they have a brief, free-living stage in the marine environment (Klimpel and Palm 2011). During this phase, their protective cuticles allow for them to survive in a variety of environmental conditions (Page 2013). For one species, *Pseudoterranova decipiens,* though the optimal temperature for larval survival is 5°C, they can survive up to 45 days in 15°C temperatures (Measures 1996), which is slightly greater than the average annual SST highs in Alaska (National Centers for Environmental Information 2023). However, this narrower window of survival could result in a greater temporal mismatch between anisakid larvae and their requisite hosts (Paull and Johnson 2014). Additionally, climate change will impact the distribution of anisakid hosts (Marcogliese 2008). Even if climate change negatively impacts some intermediate host species in Alaska, as ocean temperatures in northern latitudes increase, warm water hosts may move northward (Marcogliese 2008). This range expansion is expected to increase the prevalence of anisakids in northern regions (Klimpel and Palm 2011). An increase in growth and an expanded range of anisakid hosts with increasing temperatures could have contributed to the increase we observed in anisakid infections over our study period, although it does not explain why we observed increases in some salmon species but not others.

Differences across salmon hosts

Salmon species differ in the amount of time they spend in marine systems, where anisakids are found. Pink and chum are known to have short freshwater stages and more extensive marine life stages (Salo 1991; Heard 1991). Pink salmon make little use of freshwater environments, instead migrating to marine systems as fry where they mature after 2 years (Heard 1991; Quinn 2018).

Chum salmon typically stay in freshwater for several days or weeks before migrating to the marine environment, where they mature after 3–5 years (Salo 1991; Quinn 2018). In contrast, sockeye fry tend to reside in lakes for 1–2 years immediately after emergence, before migrating to sea, where they spend 2–3 years (Quinn 2018). Coho typically stay in streams for 1–2 years before migrating to sea where they spend 1 year before returning to spawn (Quinn 2018). Fish that spend a greater proportion of their lives in marine systems are more likely to be infected with marine parasites than those that spend more time in freshwater systems. This might explain why those salmon species with more marine life styles (pink and chum) experienced increases in anisakid burden, while those with more freshwater life styles (sockeye and coho) did not. While not assessed in this study, we expect that Chinook would experience similar increases in anisakid abundance because they spend most of their lives in coastal marine systems (Healy 1991). This would put marine mammal predators that forage on Chinook, like endangered southern resident killer whales (Ford and Ellis 2006), at higher risk of anisakid infection.

The salmon species we assessed are opportunistic foragers, but have different diets (Kaeriyama et al. 2004; Brodeur et al. 2007; Johnson and Schindler 2009). Coho are piscivorous, while chum, pink, and sockeye feed on lower trophic level prey, consisting mostly of zooplankton (Kaeriyama et al. 2004; Brodeur et al. 2007; Johnson and Schindler 2009). Salmon diet shifts annually with variability in environmental conditions (Kaeriyama et al. 2004), and may change with increasing temperatures (Coyle et al. 2011; Beamish, Sweeting, and Neville 2004). In the Bering Sea, pink, chum, and sockeye have shifted their diets in periods of warming temperatures, consuming more fish in warm years and more euphausiids in cooler years (Coyle et al. 2011). If the fish preyed upon are a trophic level higher than euphausiids, they may be more likely to have accumulated anisakid parasites (Lester and McVinish 2016). In the Strait of

Georgia, rather than a diet shift, warmer periods resulted in increased feeding intensity and frequency, as well as increased size and survival (Beamish, Sweeting, and Neville 2004). Larger fish in general have more parasites (Poulin 2000). An increase in feeding intensity could increase the likelihood of consuming infected prey, which would increase anisakid prevalence in salmon feeding in marine systems. If anisakids are increasing in the marine environment, and warming conditions lead to increased feeding intensity and frequency, those salmon species that spend more time in the marine environment would exhibit a greater increase in anisakid burden.

Implications

The increase in anisakids observed in Alaskan pink and chum salmon over our study period shows that these parasites are on the rise in commercially and ecologically important salmon species. Anisakids, if consumed alive, can cause anisakidosis in humans (Measures 2014), which generally results in symptoms that resemble food poisoning (Deardorff, Kayes, and Fukumura 1991; Bouree et al. 1995). Anisakids are sensitive to extreme temperatures and can be killed by freezing or cooking the fillet (Buchmann and Mehrdana 2016), therefore anisakids will be killed by the canning process. Additionally, preparing salmon according to U.S. FDA guidelines will kill any anisakids present. But consumers who have been previously exposed to anisakids can develop sensitivities to anisakid antigens (Alonso-Gómez et al. 2004). Allergens from anisakids can remain in the fish product even if the worms are dead and cause allergic reactions to sensitized consumers (Audicana et al. 1997), though this is true for all methods of cooking infected fish. An increase in these parasites could result in more instances of these allergic reactions in sensitized consumers.

From an ecosystem perspective, this increase in anisakids may result in higher parasite prevalence across a suite of hosts, including salmon, but it can also be considered an indication

of ecosystem recovery. Parasites can be used as a proxy of ecosystem health (Hudson et al. 2006; Lafferty 2008). We do not have enough temporal data to say what anisakid burden in salmon might have been over a century ago, prior to colonization, commercial fishing, and whaling in this region. But in other regions, increases in anisakids have reflected a return to historical abundances that were driven by the high availability of marine mammal definitive hosts in pre-industrial ecosystems (Chapter 2). The presence of these nematodes indicates that their requisite hosts are abundant enough for the completion of their life cycles (Hudson et al. 2006; Lafferty 2008). The previous decline of marine mammals in the region due to commercial whaling and fur pelt industries may have bottlenecked anisakid populations, and the rise we observe here may merely reflect a return to more natural conditions.

Although the increase in anisakids that we observed might represent a return to more "natural" conditions, that does not mean that it is an unmitigated good. While anisakids may be returning to a previous baseline, their hosts face different threats than they did historically. Alaskan salmon face numerous threats that could impact populations, including increasing stream temperatures (Bryant 2009; Shanley et al. 2015; Mauger et al. 2017), increasing precipitation and streamflows (Shanley and Albert 2014; Sloat, Reeves, and Christiansen 2017), invasive species and range expansions of competitor species (Jalbert et al. 2021), and landscape change (Schoen et al. 2017). Similarly, though marine mammals in this region are recovering, they face numerous other threats, including vessel collisions (Schoeman, Patterson-Abrolat, and Plön 2020) and noise (Erbe et al. 2019), fisheries interactions and bycatch (Read, Drinker, and Northridge 2006; Read 2008), climate change (Learmonth et al. 2006), accumulation of pollutants (Reijnders and de Ruiter-Dijkman 1995), and entanglement and ingestion of man-made materials (Poeta et al. 2017). Populations that face multiple threats may be particularly

vulnerable to increases in parasite burden, as the impacts of these threats can act synergistically (Marcogliese 2008; Wright 2012; Kellar et al. 2017; Simmonds 2018). The increase in anisakids that we observed suggests that both salmon and marine mammals in this region are more likely to become infected than they were 40 years ago, which may be a cause for concern for endangered or at-risk Alaskan salmon and marine mammals.

Caveats

Chilling practices are intended to preserve catches longer, and a co-benefit of this practice is that it may reduce the migration of nematodes into the fillet of the fish. However, we found that chilling practices did not have a significant effect on parasite abundance in salmon cans. This could be due to the temperature of refrigeration. Cipriani et al. (2016) showed that anisakid abundance in the fillet is lower when stored at a low temperature (2°C / 35.6°F), as migration of worms into the fillet increases after capture when stored at a higher temperature (5 or 7°C). Salmon in Alaska are chilled between 28–36°F, so anisakids should be prevented from migrating into the fillet, resulting in fewer worms than in unrefrigerated fish. However, chilling practices are not always strictly enforced (Bruce Odegaard, Seafood Products Association, pers. comm.). Additionally, we used the pre- and post-1995 time frame as an estimate, but there was not a specific date when every fishing vessel began chilling their fish. So, it is perhaps more likely, because not every vessel chills their catch and because the implementation of onboard chilling occurred over an unknown time frame, that our chilling metric did not effectively capture the nuanced differences in vessel chilling practices across vessels. However, if there were an effect of chilling that we were not able to detect in this study, the increase in anisakid abundance over time may be an underestimate of the true increase in anisakid burden—that is, had the chilling

effect not been implemented in the middle of our time series, the observed increase in anisakids might have been much greater.

Despite our attempts to morphologically identify the nematodes to the genus level, the worms we extracted from the canned fish were often too damaged to identify. We found that nematodes were easily broken in the cooking and extraction process, the cuticles became more opaque, and the bodies were often too tightly coiled to allow visualization of key morphological features. Although we could not confirm that all nematodes were anisakids, we assumed that they were, because anisakids (specifically *Anisakis* spp., *Pseudoterranova decipiens*, and *Pseudoterranova* spp.) are the only parasitic nematodes found in the muscle of Pacific salmon in Alaska (Moles 2007). In past studies in the Puget Sound, salmon hosts have had high prevalence of *Anisakis simplex* – reaching 100% prevalence in sockeye (Deardorff and Kent 1989) and chum (Myers 1979). Similarly, in Bristol Bay and Prince William Sound, Alaska, all chum, pink, and sockeye salmon sampled were found to have *Anisakis simplex* infections (Karl et al. 2011). We were able to positively identify 34% of the nematodes extracted, and 100% of those were identified as belonging to the family Anisakidae. Though we could not identify all nematodes morphologically, molecular identification may be possible. Based on the successful identification of some of the nematodes, and previous research on anisakids in salmon in this region, we are confident that our findings represent changes in the prevalence of Anisakidae.

Though we demonstrated that parasite detection was possible in canned salmon, we have no validation study to confirm that the detectability of nematodes is not affected by can age. Canned salmon have a commercial shelf life of five years, and the oldest cans in our collection were over 40 years old. Older cans were found to degrade over time—cans bulged at the seams, formed black blisters known as “sulfite blackening” along the inside, fizzed when opened, and

showed visible signs of rot in the canned fillet. Rot included cloudy liquid within the can, softened fillets, and green, glassy cyst-like orbs around the spine that are likely attributable to chemical reactions inside the can over time. It is possible that the trends we detected in anisakid burden were due to degradation with can age, and that more worms were detectable in recent cans because they had had less time to decay. However, we believe that this is unlikely for a few reasons. First, we do not expect that nematodes would have degraded differently across the salmon species, as they were all treated to the same thermal processing procedure and storage. If the pattern we detected were due to worms degrading over time, we would expect to have seen the same rate of degradation across the four salmon species. Second, we never detected any material that resembled part of a nematode; we only ever found whole nematodes. If anisakids were degrading in the cans over time, we would have expected to detect some partially degraded nematodes. Finally, anisakid cuticles consist of multiple layers that allow them to withstand both varying environmental conditions and the acidic environment of a host's gastrointestinal tract (Myers 1960; Page 2013). This also makes them resistant to degradation over time. For example, although anisakids have not been reported in archaeological studies (Arriaza et al. 2010), other parasitic roundworms have been found during examination of coprolites that were thousands of years old (Reinhard and Araujo 2012; Reinhard and Bryant 2008). We cannot rule out the possibility that the pattern reported here is an artifact of nematode degradation with time, but we believe that this is unlikely.

It is possible that the selection of fillets for canning changed over time, influencing the worm burden. For example, preferentially selecting fish with more visible nematodes present in later years could have resulted in an increasing trend in anisakids burden. However, salmon canning already uses the least visually appealing fish, a practice that has not systematically

changed over time (John Daly, industry expert, pers. comm.). Selecting progressively older fish could also contribute to a more parasitized can, as parasite burden generally increases with fish length (Poulin 2000), however this is unlikely, as average body size has been declining in North Pacific salmon (Bigler et al. 1996). Additionally, through our conversations with salmon canning industry professionals, we have learned that the selection of salmon for cans varies more from company to company than over time, which would only result in a trend over time if every cannery changed its practices in the same way over time. If the demand for canned salmon was much greater at the start of our study period, then a greater proportion of all fish caught may have gone to canning, regardless of condition or size, which would have resulted in a concentration of the most heavily infected fish in canning during later years with lower demand. However, that does not appear to be the case — demand was low in the 1970s and canned salmon markets grew from 1980 to the 1990s (U.S. National Park Service 2023). Therefore, it is unlikely that changes in salmon selection over time resulted in the observed trend of increasing anisakids in canned salmon.

CONCLUSION

We used a novel data source—archived canned salmon—to detect an increase in anisakid nematodes in Alaskan chum and pink salmon. We did not detect similar trends in sockeye or coho. We hypothesize that, because pink and chum salmon spend a greater proportion of their lives in marine systems, they are more likely to be exposed to this marine parasite. Increasing burdens of anisakids in these two species is probably attributable to increasing marine mammal abundance in this region, though climate change may also contribute by speeding up anisakid growth and reproduction.

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CH. 3 TABLES

Table 3.1: Results from Model 1, incorporating data from pink, coho, and chum, with significant effects are in bold. We ran the analysis three times, changing the reference salmon species to obtain the estimate, standard error, Z value, and P value for each species. Random effect values were calculated with chum in the reference position.

Variable	Estimate	Std. Error	Z Value	P Value
Can size (g)	0.611	0.210	2.914	**0.004**
Chilling practice	0.789	0.593	1.330	0.183
Year * Chum	1.258	0.306	4.115	**3.88e-05**
Year * Coho	-0.191	0.752	-0.253	0.800
Year * Pink	0.800	0.328	2.438	**0.015**
Random effects	**Variance**	**Std. Dev**		
Factory/Region	7.284e-10	2.699e-10		
Company/Region	7.605e-10	2.758e-05		

Table 3.2: Results for Model 2, with sockeye-only data. Significant effects are in bold.

Variable	Estimate	Std Error	Z Value	P Value
Can size	0.529	0.217	2.439	**0.015**
Random effects	**Variance**	**Std Dev.**		
Region*Time Period	0.335	0.579		
Factory	0.483	0.695		
Company	0.187	0.432		

CH. 3 FIGURES

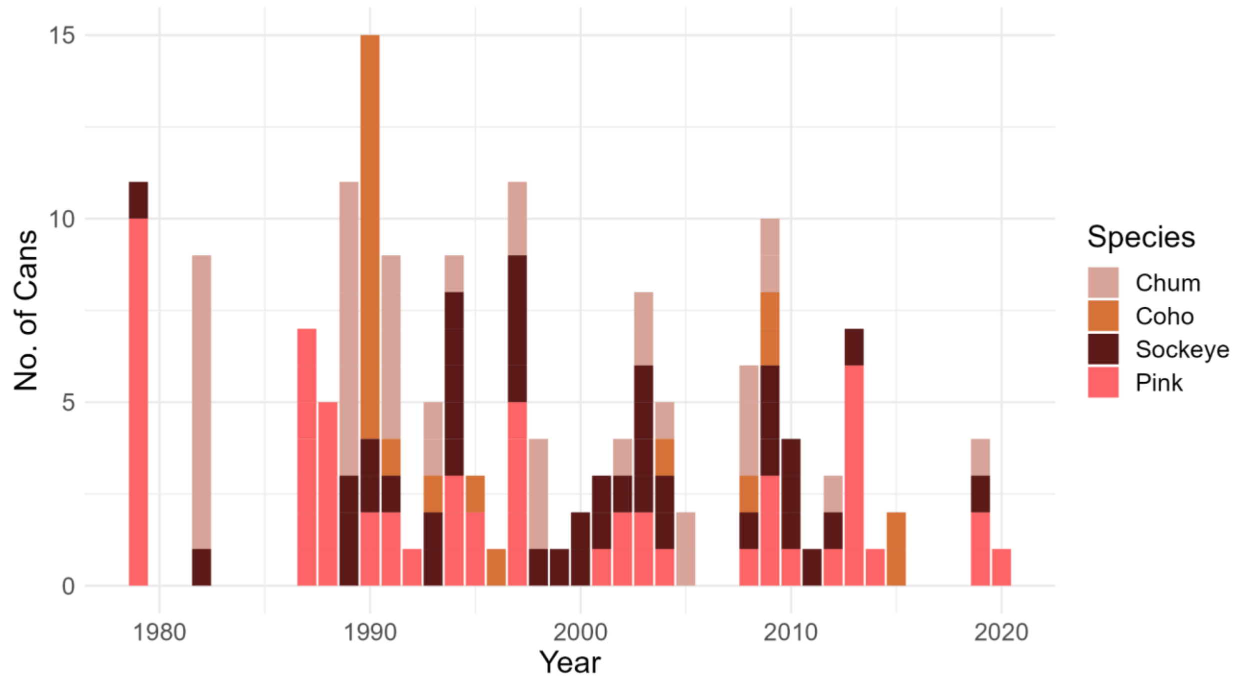

Figure 3.1. The distribution of canned salmon samples available for each salmon species in each decade.

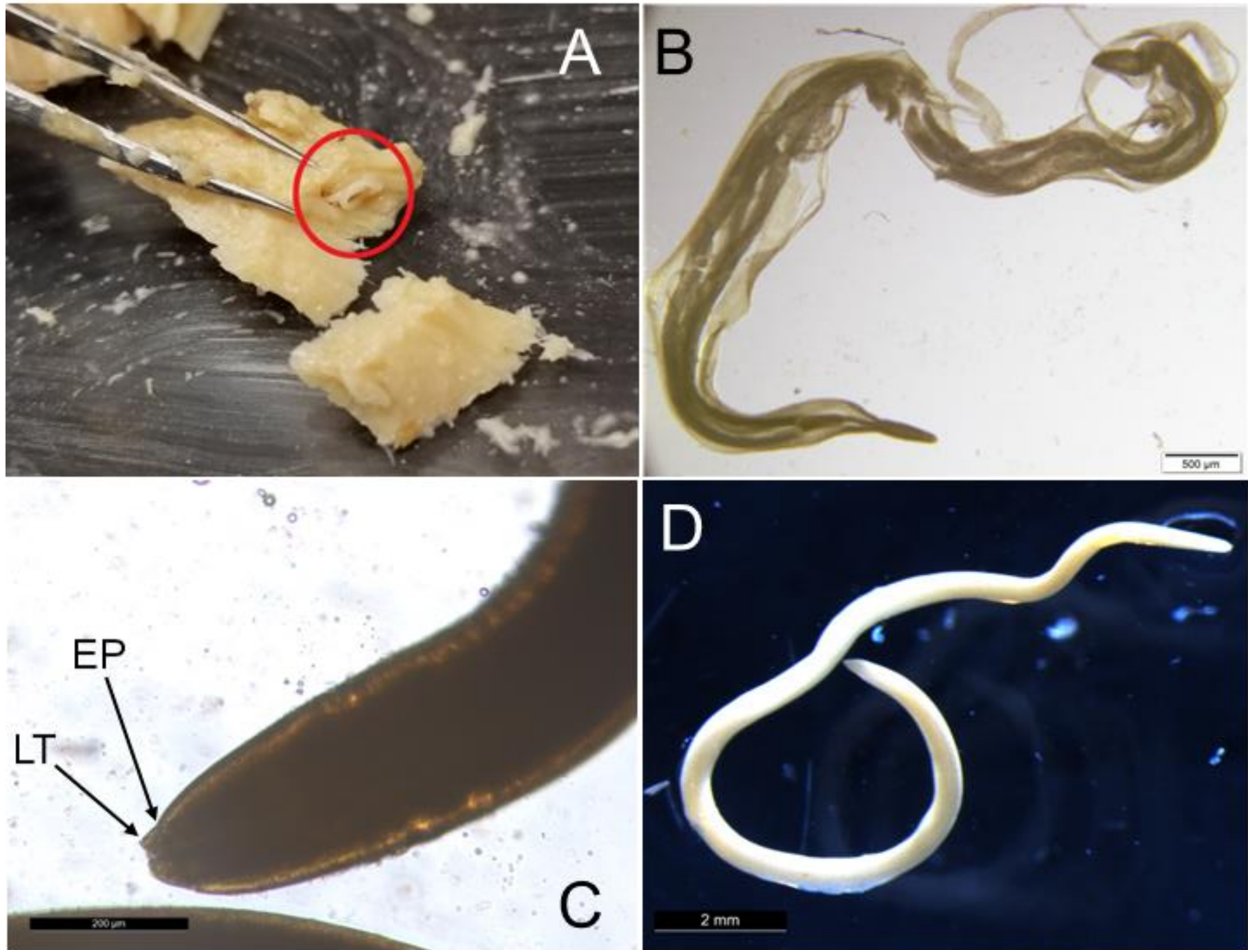

Figure 3.2. A) A photo of a nematode (red circle) in a canned salmon fillet. The nematodes coil within the muscle and form pockets that are easily detected when dissecting with forceps. (B) A nematode recovered from canned salmon, cleared with lactophenol solution. Nematodes were highly degraded during the canning process, to the extent that even clearing the recovered specimen could not give us sufficient information to form an accurate genus-level identification. (C) An ethanol-preserved, uncleared anisakid nematode, identified to the family level by the presence of a larval tooth (LT) and an excretory pore (EP) ventral to it, as described by Hurst et al. 1984. (D) A preserved, uncleared anisakid. The cuticle is very cloudy, and internal organs are not visible.

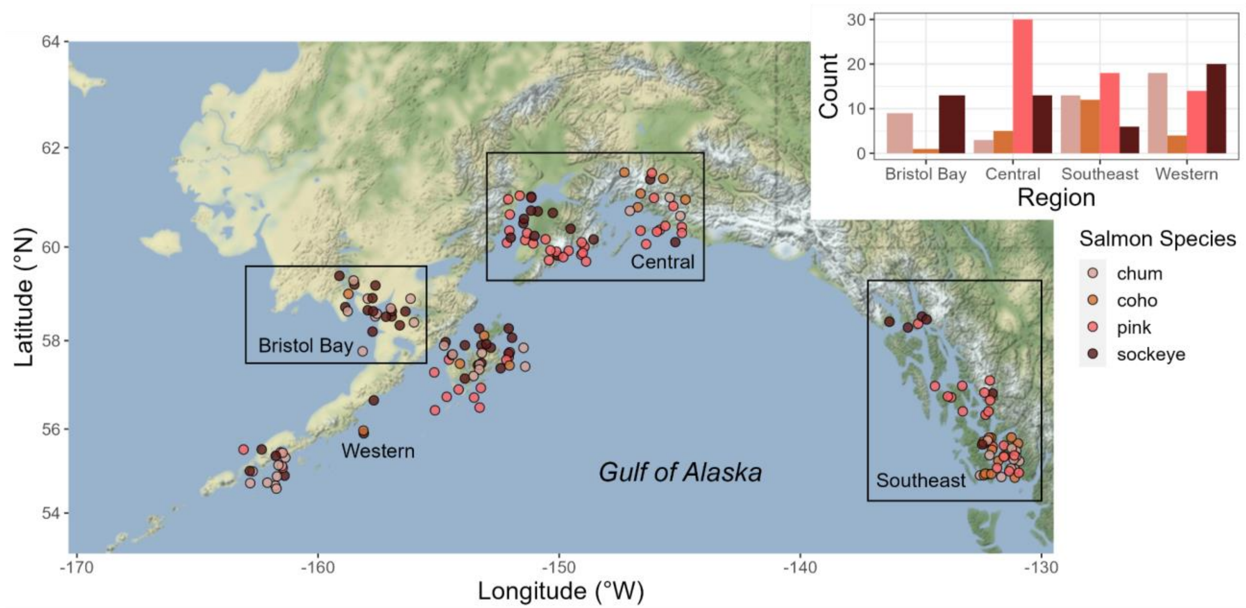

Figure 3.3. A map of the canning locations for each canned salmon sample, where each dot is a can that we dissected, color reflects the species of salmon canned (chum in light pink, coho in orange, pink in hot pink, and sockeye in burgundy), and the points are jittered to avoid overlap. The inset figure shows the number of cans of each species collected in each region. Most cans came from the Gulf of Alaska (Western, Central, and Southeast regions), though some chum, sockeye, and coho were collected from the Bristol Bay region.

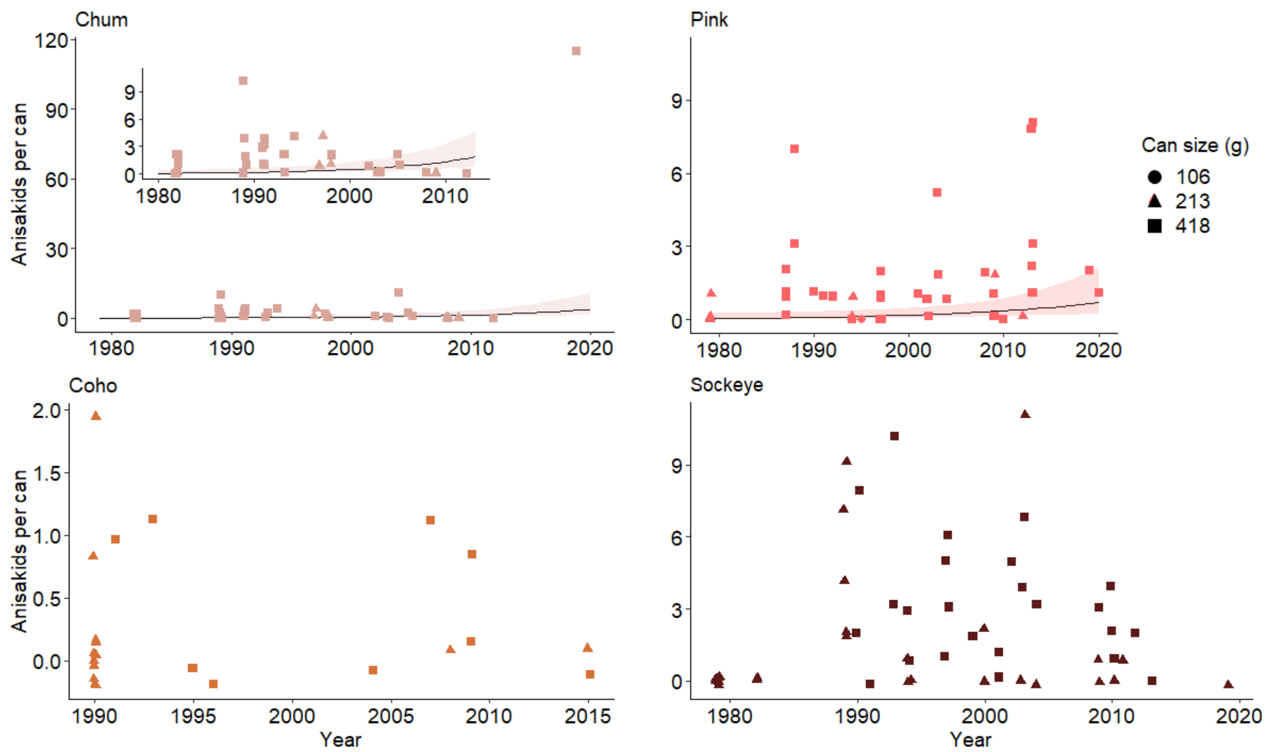

Figure 3.4: The predicted effect of time on anisakids for chum and pink, which experienced significant increases, in Model 1, and the raw data points for coho and sockeye, which did not experience a significant change over time. In chum and pink, predictions are based on the region with the most observations (Southeast), a random factory, the most sampled company, chilling practices in effect (i.e., post-1995 group), and the median can size. Points represent raw count data jittered, which resulted in variability in the graphed points. The inset graph for chum is the same graph with axes truncated to exclude the high influence observation of 115 nematodes in 2019.

APPENDIX CH. 3A

Testing methods for detecting nematodes in canned fish fillets

Using cans of unknown origin dates or species contents, we tested three methodologies of dissecting fillets: the UV-Press method (Karl and Leinmann 1993), the candling method (Valdimarsson et al. 1985), and manual dissection with forceps. The UV-Press method is commonly used to detect anisakids in fresh or frozen fillets, in which a fillet is either flattened between two acrylic sheets or vacuum-pressed to 2-3 mm thick (Karl and Leinmann 1993; Gomez-Morales et al. 2018). A 366 nm UV light is shined at the flattened fillet in a darkened room and the nematodes fluoresce and can easily be counted (Gomez-Morales et al. 2018). The candling method involves placing a fillet (with skin removed) on a backlit surface and examining the fillet for coiled nematodes (Valdimarsson et al. 1985). Thinly slicing the fillets or pressing the fillets to 3-4 mm thickness before candling can improve nematode detectability (Karl and Leinmann 1993). To test the UV-Press and candling methods, we pressed a section of the canned fillet known to have at least one nematode present between two plexiglass plates using an industrial press, flattening the fillet to a thickness of 2-3 mm. When subjected to a UV light, the nematodes did not fluoresce, possibly due to degradation of proteins during cooking. When candled, worms were detectable, but the high moisture content of the can and the softness of the cooked fillet made it difficult for the nematode's position to be maintained when the plates were separated, as the flattened muscle came apart once the top plexiglass plate was removed. We tested dissecting each fillet manually with forceps, and found that this was the cleanest, most effective way to detect, count, and preserve nematodes without damaging the parasite. We found that the nematodes formed pockets in the cooked fillet that were detectable when using forceps,

but that were not retained when the fillet was pressed. Therefore, we used the forceps dissection method for all cans.

We developed our search image on spare cans, during which time we had a second observer check each fillet for any remaining nematodes. Once our dissectors reached 100% agreement, we began dissecting our sample cans. Dissections were conducted by one trained observer (AK or NM), and a second observer checked cans at random. We opened each can and noted the can size as a metric of fillet mass. If the fillet appeared mostly intact, we then drained the can of most of its liquid. If the fillet appeared to have degraded into the liquid, we did not drain the can prior to examining the fillet. We scooped a portion of the fillet onto a clear acrylic sheet atop a black benchtop to maximize contrast. Using two pairs of forceps (4.25-inch, fine point), we carefully dissected the fillet into small pieces (less than 1 cm^2). When we came across a nematode pocket, we carefully extracted the nematode from the fillet and placed it in a vial of 70% ethanol for preservation. We tallied the number of worms extracted for each can.

APPENDIX CH. 3A REFERENCES

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Karl, H. & Leinemann, M. 1993. A fast and quantitative detection method for nematodes in fish fillets and fishery products. *Arch. Lebensmittelhyg* 44: 124-125.

Valdimarsson, G., Hjalti, Einarsson, H. & King, F.J. 1985. Detection of parasites in fish muscle by candling technique. *Journal of the Association of Official Analytical Chemists* 68:549-551.

APPENDIX CH. 3B

Table S3.1: Top ten models for Model 1, assessing change in anisakids for chum, coho, and pink salmon. The model used for analysis is represented in bold.

Intercept	**Chilling practice**	**Salmon species**	**Can size**	**Year**	**Salmon* Year**	**DF**	**AICc**	**ΔAICc**
+		+	+	+	+	12	376.7	0
+	**+**	**+**	**+**	**+**	**+**	**13**	**377.4**	**0.77**
+	+	+	+	+		11	378.2	1.49
+		+	+	+		10	379.5	2.77
-	+	+		+		10	380.9	4.21
+	+	+		+	+	12	383.4	6.70
+		+		+		9	384.7	8.03
+		+		+	+	11	385.8	9.12

Table S3.2: Top fitting models for Model 2, including sockeye-only. The model used in the analysis is represented in bold.

Intercept	**Chilling practice**	**Can size**	**Year**	**DF**	**AICc**	**ΔAICc**
+		+		8	212.6	0
+		**+**	**-**	**9**	**213.3**	**0.76**
+	+	+		9	214.1	1.55
+				7	216.2	3.63
+	+	+	-	10	216.4	3.82
+			-	8	218.3	5.69
+	+			8	218.8	6.26
+	+		-	9	221.0	8.45

Chapter 4: Parasite infections in living killer whales of the Northeast Pacific Ocean

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ABSTRACT

Multiple populations of resident killer whales (*Orcinus orca*) inhabit the Northeast Pacific, but southern residents (SRKWs) are the most at-risk. SRKWs were listed as Endangered in the United States in 2005 and have since shown little sign of recovery. Several factors have been identified as threats to this population, including depletion of their preferred prey, toxic contaminants in their food and water, and an increase in physical and acoustic disturbance by vessels, all of which have contributed to an energetically stressed population. When accounting for these stressors, population viability analyses estimate that the SRKW population should be stable, but, contrary to these predictions, SRKWs continue to decline in abundance. Underlying health risks, like parasitism, may be contributing to this population's failure to recover, but little is known about persistent parasite infections in living killer whales. To assess parasite infections in Northeastern Pacific killer whales, we tested scat from SRKWs and compared infection status against two populations of "resident" killer whales that are not in decline, northern resident killer whales (NRKWs) and southern Alaskan resident whales (ARKWs), and "offshore" killer whales (OKW), whose status is largely unknown. We analyzed 35 fecal samples collected from 28 killer whales (25 SRKW, seven ARKW, two NRKW, and one OKW) and tested for parasites in two

ways: counting the parasites through microscopic identification of parasite eggs and identifying parasites through DNA metabarcoding analysis of the feces. We used photogrammetry to determine whether parasite infection status was associated with the body condition of whales. We found that the detection of parasite infection through DNA was a more reliable approach to detect infection compared to using morphological identification of eggs. Most individuals sampled (94%) were positive for *Anisakis* spp. infection. These infections were equally common across populations, and were not correlated with whale body condition, though our body condition analysis only had sufficient statistical power to detect a strong correlation. These results suggest that *Anisakis* infection is nearly ubiquitous among killer whales of the northeast Pacific, implying a need for further research into the fitness impacts of intestinal parasites for these hosts.

INTRODUCTION

Multiple ecotypes of killer whales inhabit the Northeast Pacific Ocean: “transient,” or Bigg’s, killer whales, which prey on marine mammals, offshore killer whales (OKW), which feed primarily on high trophic level fish (Ford et al. 2011), and “resident” killer whales which are piscivorous. Little is known about the range, diet, and abundance trends of OKW (Ford et al. 2014; Schorr et al. 2022), though much research has been conducted on resident killer whales in this region. There are several populations of resident killer whales, including southern Alaska (ARKWs), northern (NRKWs), and southern resident killer whales (SRKWs). These populations overlap in ranges that span the west coast of the United States and Canada, from the Gulf of Alaska to California, and have similar diets consisting of mostly *Oncorhynchus* spp. (Bigg 1982; Krahn et al. 2004; Matkin 2011; Ford and Ellis 2006; Hanson et al. 2021). While they overlap in range and diet, ARKW and NRKW populations have increased in size while the southern

resident population have declined over the past two decades (Olesiuk et al. 2005; Fisheries and Oceans Canada 2018; Murray et al. 2021; Matkin et al. 2014; Lacy et al. 2017). During 1962–1973, NRKW and SRKW populations were depleted by the live aquarium trade, which resulted in the death or removal of 62 whales (Bigg and Wolman 1975). Though the capture of killer whales was prohibited in Washington State in 1976, SRKWs continue to be threatened by an increasing number of stressors, including declines in abundance of their preferred prey, Chinook salmon (*Oncorhynchus tshawytscha*; Hanson et al. 2010; Hanson et al. 2021); high concentrations of pollutants in their food and water (Krahn et al. 2009); and acoustic and physical disturbance from small boats and ships in the Salish Sea (Lusseau et al. 2009; Williams et al. 2014; Holt 2008). The SRKW population was listed as Endangered in Canada in 2001, and in the U.S. in 2005 when the population consisted of 88 individuals, yet the population declined to only 73 individuals in 2022 (Center for Whale Research, 2023).

Declines in the abundance of SRKWs are likely attributable to the cumulative effects of multiple stressors. SRKWs, and killer whales in general, have a relatively low intrinsic population growth rate, which leaves them particularly susceptible to stress (Stark et al. 2004). While an individual might be able to withstand the impacts of one sublethal stressor, the effects of multiple stressors can compound one another, leading to greatly diminished fitness and fecundity (Williams et al. 2016; Wright, 2012). Lacy et al. (2017) modeled the cumulative effects of the known stressors of SRKWs, and predicted a stable population — a prediction that contrasts the declines observed since 2016 (Marine Mammal Commission 2023). However, the stressors included in the model may not be the only sublethal stressors acting upon SRKWs.

Parasites can work in tandem with other stressors to reduce the health of their hosts. Parasites both reduce the energy available to hosts (Shanebeck et al. 2022), and can modulate the

immune response indirectly through diverting energy away from the immune system and by directly manipulating the immune system (Schmid-Hempel 2008). Together, these impacts can result in a host that is more vulnerable to other infections, exacerbating the impact of additional stressors (Beldomenico et al. 2008; Marcogliese and Pietrock 2011). This can lead to a negative feedback loop, in which killer whales are unable to resist infection after being subjected to stress from multiple factors. As a result, their condition is reduced, leaving them more vulnerable to stress (Beldomenico and Begon 2010). A study on harbor porpoises (*Phocena phoca*) found that nutritionally stressed individuals were more likely to have parasite infections, suggesting that parasites may have more of an impact on fitness in unhealthy animals (Ten Doeschate et al. 2017). Additionally, host characteristics may influence susceptibility to parasite infections, meaning that parasitism could disproportionately affect some members of the population based on age or sex (Marcogliese and Pietrock 2011). If, for example, young whales are particularly susceptible to parasitism, then SRKWs may not recover at the predicted rate (Lacy et al. 2017). Parasitism can lead to significant and long-lasting energy loss in an already nutritionally stressed population (Shanebeck et al. 2022), which can subsequently negatively impact reproductive success and population growth (Irvine 2006; Riordan et al. 2007).

The prevalence and impact of parasitism as a sublethal stressor is unknown in SRKWs. Modern parasitological examinations are limited to analyzing difficult-to-obtain fecal samples from wild animals or necropsies of deceased animals, the latter likely being unrepresentative of healthy wild individuals (Dailey and Stroud,1978; Aguilar and Borrell 1994; Ten Doeschate et al. 2017; Hermosilla et al. 2018; Raverty et al. 2020). Due to the difficulty of assessing parasite infections in wild, living animals, we lack knowledge on parasitism in most wild marine mammals, including the prevalence and intensity of infections (Table 4.1). Through necropsies,

we know that marine mammals can host high loads of parasites (Dailey and Stroud 1978; Dailey 1980; Stroud and Roffe 1979), but the parasites of killer whales are largely unknown (e.g., Raverty et al. 2017; Raverty et al. 2020; Lehnert et al. 2023).

One of the most common parasites found in the intestinal tract of marine mammals are nematodes (i.e., roundworms) in the family Anisakidae (anisakids; Dailey 2001). There are three prominent genera that use marine mammals as their definitive hosts: *Anisakis* spp., which infect cetaceans, *Pseudoterranova* spp., which infect pinnipeds, and *Contracaecum* spp., which infect pinnipeds and seabirds (Køie et al. 1995; Klimpel and Palm 2011). Thirty-four species of cetaceans are known to harbor *Anisakis* spp., including killer whales (Mattiucci and Nascetti 2007; Klimpel and Palm 2011; Raverty et al. 2020). The *Anisakis* spp. life cycle involves multiple larval phases and takes place mainly in the pelagic environment (Klimpel and Palm 2011; Figure 4.1). Eggs are deposited into the ocean through cetacean feces, where they develop into larvae and are transported up the food web via ingestion, infecting crustacean and fish intermediate hosts (Klimpel and Palm 2011). Cetaceans become infected by ingesting infected intermediate hosts, at which time the larvae develop into adults and reproduce within the definitive host gastrointestinal tract. Anisakids cause both direct and indirect fitness costs in their marine mammal definitive hosts. *Anisakis* spp. can cause gastritis and ulceration (Cattan et al. 1976; Haebler & Moeller 2021), and they can cause peritonitis that ultimately leads to hemorrhaging and death (Dailey and Stroud 1978; Stroud and Roffe 1979; van Beurden et al. 2015). Though they rarely cause mortality, anisakids likely have an underestimated effect on host health as an energy sink (Shanebeck et al. 2022).

In Chapter 1, I showed that there has been an increase in *Anisakis* spp. abundance in prey species of marine mammals globally. In Puget Sound, *Contracaecum* spp. abundance in fish has

increased with increasing marine mammal abundance (Chapter 2, this dissertation). Rising rates of anisakid infections may pose a threat to many marine mammal species, but those whose populations are already declining because of cumulative stressors could be particularly at risk. With an increase in the abundance of anisakids in intermediate hosts, are SRKW facing increasing infection prevalence? And does this threatened population have a higher infection prevalence than sympatric resident killer whale populations in the Northeast Pacific?

Here, we present results on the parasite infections of killer whales in the Northeast Pacific Ocean, with a focus on endangered SRKWs. To assess the extent and potential impact of parasite infections, we combined data collected from several field efforts, including photographic identification, body condition assessments, and fecal samples. Specifically, we asked: (1) What parasites infect SRKWs? (2) Are more SRKWs infected than other resident killer whales in this region? (3) What factors influence parasite infection in SRKWs? And (4) is body condition associated with the presence of parasites? Our findings constitute the first survey of parasites in living SRKW and other killer whales in the Northeast Pacific.

METHODS

Killer whales in the Northeast Pacific have been photo-identified and monitored since the early 1970s, making them one of the most well-studied cetacean species (e.g., Center for Whale Research, Northern Gulf Oceanic Society, Fisheries and Oceans Canada's Pacific Biological Station). Routine monitoring and health assessments of SRKWs have allowed for the collection of long-term health data of SRKW, including fecal samples and body condition estimates (Fearnbach et al. 2020, 2018; Ford et al. 2016; Hanson et al. 2010). These long-term health assessments provided the SRKW samples used in this analysis.

Creating a parasite identification guide for North Pacific wild killer whales

To determine which parasites could infect killer whales, we reviewed the literature for documentation of intestinal parasites that infect members of the family Delphinidae in the Northeast Pacific ocean, which resulted in our list of probable species (Table 4.1). This list was used to assemble an identification guide, in which we compiled photos or written descriptions of parasite eggs (Appendix Ch. 4B). This list was also run through GenBank to determine which parasites had sequence data available (Table 4.1) and was used to determine which primers to use in DNA metabarcoding analysis.

Scat sample analysis

Fecal samples from SRKW have been collected regularly as part of NOAA Northwest Fisheries Science Center long-term monitoring of the SRKW population. SRKW fecal samples were collected using methodology previously described by Hanson et al. (2010) and Ford et al. (2016). Samples were assigned by the field team to individual whale identities via photo-identification using a long-term catalog, and identities were confirmed by genetic sequencing (Ford et al. 1996). NRKW samples were collected by the Marine Ecology and Telemetry Research group, and ARKW samples were collected by the Northern Gulf Oceanic Society using protocols similar to NOAA. We acquired 1-mL fecal subsamples from SRKW (25), ARKW (7), NRKW (2), and from one OKW. Each SRKW scat sample is linked to an individually identified whale. ARKW, NRKW, and OKW samples are from unknown individuals. All samples were associated with a specific date and location of collection.

For each sample, we performed both fecal floatation and sedimentation. Each fecal sample was partially thawed, and a subsample was collected with a flame-sterilized metal spoon

and weighed. We aimed to take 0.5-g samples, but if the fecal sample was less than 1 g, we took approximately half of the sample and recorded the exact mass. We subjected each subsample to standard fecal sedimentation and floatation protocols (Girard et al. 2016). Briefly, each sample was reconstituted with a soap solution (30 ml of Dawn soap in 1 gallon tap water) and mixed until thoroughly emulsified. The solution was strained using gauze into a 50 mL conical tube, and centrifuged at 1300 rpm for 10 minutes in a fixed-head centrifuge. Several drops of the resulting pellet were subsampled for sedimentation. We then decanted the soap solution from the pellet, and reconstituted the pellet with zinc sulfate solution (1.18 specific gravity). The mixture was transferred to a 15-mL conical tube and filled to 14.5 mL, then centrifuged for 1300 rpm for 10 minutes. The tube was carefully removed from the centrifuge and filled until there was a slight positive meniscus. We placed a coverslip on top of the tube and waited 10 minutes before removing the coverslip for immediate analysis. Both sedimentation and floatation slides were examined under a Leica DM2500 compound microscope to count and photograph parasite eggs. We later identified the eggs to the best of our ability using the only published marine mammal parasite egg identification key – *Diagnostic Key to the Parasites of Some Marine Mammals* (Dailey et al. 1980) – and when available, images of parasite eggs that infect other marine mammals in the northeast Pacific (Appendix Ch. 4B).

If eggs were present in a sample, we classified the infection status as a binomial response — uninfected or infected. To assess how egg counts differed by individual, we divided egg counts by the sample mass to get a measure of eggs per gram. If multiple slides were analyzed, egg counts were divided by the number of slides to obtain a consistent measure of eggs per slide prior to dividing by the sample weight. In one instance, the weight of the sample was not recorded, but it was noted that the sample was less than 0.5 g. For that sample, we

precautionarily assumed that the sample was 0.5 g, so as to not overestimate the egg count. However, because eggs can vary greatly depending on the number of nematodes present in the gastrointestinal tract and the reproductive stage of the nematodes (Ugland et al. 2004), egg counts were not a reliable metric of parasite burden (Davey et al. 2021), and thus were not used for quantitative analysis.

DNA metabarcoding analysis

To determine if there were additional parasites or pathogens present in the fecal samples, we sent the remaining portion of each fecal sample to Jonah Ventures environmental genetics lab in Boulder, Colorado, for library preparation and sequencing. Frozen samples were thawed for 1 to 2 hours before processing. Sample barcodes were recorded and assigned a well within the 96 well plate or numbered extraction tube. Under a laminar flow hood, sterile cotton swabs (Fisher, cat# 22-363-173) were coated with fecal matter, and the swabs were placed in the corresponding extraction plate or tube. Sterile tweezers and pliers were used to handle cotton swabs and remove the wooden ends of the cotton swab before extraction. Plates or tubes were immediately processed or stored in –20°C until the extraction process could be performed.

Genomic DNA was extracted from samples using the DNeasy 96 PowerSoil Pro Kit (384) (Cat # 47017) according to the manufacturer's protocol. Genomic DNA was eluted into 100 µl and frozen at –20°C. To determine amplicon size and PCR efficiency, each reaction was visually inspected using a 2% agarose gel with 5 µl of each sample as input. Amplicons were then cleaned by incubating amplicons with Exo1/SAP for 30 minutes at 37°C followed by inactivation at 95°C for 5 minutes and stored at –20°C.

We used a published primer for detecting eukaryotic organisms, nucLSUDf1 (5'-CGTCTTGAAACACGGACCAAG-3') and nucLSUDr1 (5'-GCATAGTTCACCATCTTTCGGG-3'), to amplify the forward and reverse segments of the 28S region of ribosomal RNA (Sonnenberg et al. 2007; Cabodevilla et al. 2022). This primer was selected based on its likelihood of amplifying all but one (*Campula* spp., for which no reference sequence was available) of the parasite species likely to be found in these populations of killer whales (Table 1), and ability to differentiate to the genus or species level for all of the possible parasites but apicomplexans. Both forward and reverse primers also contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Each 25-µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5 µL Master Mix, 0.5 µL of each primer, 1.0 µL of gDNA, and 10.5 µL DNase/RNase-free H2O. DNA was PCR amplified using the following conditions: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 57°C, 1 minute at 72°C, with a permanent hold at 4°C.

A second round of PCR was performed to complete the sequencing library construct, appending the final Illumina sequencing adapters and integrating a sample-specific, 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µLof template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95°C for 30 sec, 55°C for 30 seconds, and 72°C for 30 seconds. Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). 25µl of PCR amplicon was purified and normalized using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol.

Samples were then pooled together by adding 5 µL of each normalized sample to the pool. Sample library pools were sent for sequencing on an Illumina MiSeq (San Diego, CA) at the Texas A&M Agrilife Genomics and Bioinformatics Sequencing Core facility using the v2 500-cycle kit (cat# MS-102-2003). Necessary quality control measures were performed at the sequencing center prior to sequencing, including assessing the size distribution of the pool and DNA concentration.

Raw sequence data were demultiplexed using *pheniqs* v2.1.0 (Galanti et al. 2021), enforcing strict matching of sample barcode indices (i.e, no errors). *Cutadapt* v3.4 (Martin 2011) was then used to remove gene primers from the forward and reverse reads, discarding any read pairs where one or both primers were not found at the expected location (5') with an error rate < 0.15. Read pairs were then merged using *vsearch* v2.15.2 (Torbjorn et al. 2016), discarding resulting sequences with a length of < 300 bp, > 450 bp, or with a maximum expected error rate > 0.5 bp (Edgar and Flyvbjerg 2015). For each sample, reads were then clustered using the *unoise3* denoising algorithm (Edgar 2016) as implemented in *vsearch*, using an alpha value of 5 and discarding unique raw sequences observed fewer than 8 times. Counts of the resulting exact sequence variants (ESVs) were then compiled and putative chimeras were removed using the *uchime3* algorithm, as implemented in *vsearch*. For each final ESV, a consensus taxonomy was assigned using a custom best-hits algorithm and a reference database consisting of publicly available sequences (GenBank, Benson et al. 2005) as well as Jonah Ventures voucher sequences records. Reference database searching used an exhaustive semi-global pairwise alignment with vsearch, and match quality was quantified using a custom, query-centric approach, where the percent match ignores terminal gaps in the target sequence, but not the query sequence. The consensus taxonomy was then generated using either all 100% matching reference sequences or

all reference sequences within 1% of the top match, accepting the reference taxonomy for any taxonomic level with > 90% agreement across the top hits.

We processed and analyzed the data using the *phyloseq* package in R (McMurdie and Holmes 2013). We filtered out any sequences that were matched with less than 98% confidence, and any sequences that made up less than 1% of one or more samples. Although it is common to remove samples with read counts below a certain threshold, this is a practice that is largely aimed at ensuring unbiased representation of rare sequences in a sample. Our species of interest were among the most abundant sequences in all samples; therefore we elected to include all samples in an effort to maintain a representative sample size. Once initial quality filtering was completed, we grouped sequences by genus, and estimated the relative abundance of each genus in a sample as the genus-specific read count divided by the total read count for all sequences in the sample.

Photogrammetry data

Aerial images of SRKWs were collected using a helicopter in 2013 and a remotely operated drone between 2015–2021 (Fearnbach et al. 2011, 2018, 2020; Durban et al. 2015). Photogrammetry data were assigned to individual whale identities using an established aerial catalog showing markings that are visible from the air, allowing measurements to be linked to whales of known age and sex (Fearnbach et al. 2011, 2020; Durban et al. 2015). The eye patch ratio, a ratio of two pixel measurements taken between the two eye patches on the head of a killer whale, was used as a quantitative metric of body condition (e.g., Fearnbach et al. 2020). Body condition was classified into one of five categories based on measurements, as described by Stewart et al. (2021). To link parasite infection status with body condition, we compiled body

condition data collected within a month of each scat sample. This resulted in 19 fecal to body condition pairs for 14 unique individual whales.

Statistical analysis

We had three objectives in our statistical analysis: 1) Determine whether parasite infection status differed across the four populations; 2) determine whether parasite infection status was correlated with any temporal (i.e., month or year) or demographic (i.e., age, sex, or pod) factors; and 3) identify whether body condition varied with parasite infection status.

Between-population differences

To characterize broad-scale differences in the composition of gut DNA among populations, we calculated the Shannon diversity index for each killer whale population using the estimate_richness() function in phyloseq. We ran a differential sequence analysis to determine whether the read counts of any non-parasitic but potentially pathogenic sequences (e.g., pathogenic fungi; Joe Gaydos, marine mammal veterinarian, personal communication) varied by population using the DESeq() function in the DESeq2 package in R. Because differential sequence analyses can only compare two populations, we ran two tests to determine whether SRKW sequence counts differed from ARKW or NRKW. There were too few samples from OKWs to make comparisons.

To determine whether infection status differed by population, we ran a generalized linear mixed-effect model (GLMM) for each parasite detected in multiple whales through morphological identification. We ran the model using the glmmTMB() function in the R package of the same name (Brooks et al. 2017). Model 1 included all but one sample of available

sequence data (n = 33), as one sample (collected from an ARKW) was entirely composed of <98% matched sequences, and the sample size from OKW was too small to include in the model. We used parasite infection status detected through morphological identification (i.e., presence/absence) as the response variable. As infection status was a binomial variable, we fit the model to a binomial distribution. We included population (SRKW, ARKW, or NRKW) as a fixed effect, year as a random effect to account for any variation from year to year, and whale identity as a random effect to account for any individual variation.

Model 1:

$$InfectionStatus_{ijk} \sim Binomial(\mu_{ijk})$$

$$E\left(InfectionStatus_{ijk}\right) = \mu_{ijk}$$

$$\log\left(\mu_{ijk}\right) = Population_i + \left(1\middle|Year_{jk}\right) + (1|WhaleID_{jk})$$

$$Year_{jk} \sim N(0, \sigma^2)$$

Where the response variable$_{ijk}$ represents presence or absence of a parasite from the ith sample from the jth whale in the kth year when the sample was collected.

Correlated factors

To determine if parasite infection status was correlated with demographic or temporal factors, we ran a GLMM for each parasite group, using similar methods to Model 1 but focusing only on SRKW data (n = 23). We incorporated both demographic and temporal variables as fixed effects in the full model: age class (juvenile, subadult, or adult), sex, pod (J or L pod only, as there were too few whales sampled from K pod), month, and year. Year was scaled using the scale()

function in R prior to model fitting. The only months sampled were September and November. Whale ID was included as a random effect, to account for individual variation. We performed model selection, comparing the full model to models with every other combination of fixed effects using the dredge() function in the MuMIn package in R (Bartoń 2023). The model with the lowest AIC score was selected.

Model 2:

$InfectionStatus_{il} \sim Binomial(\mu_{il})$

$E(InfectionStatus_{ijk}) = \mu_{il}$

$\log(\mu_{ijklmno}) = Sex_k + AgeClass_l + Pod_m + Month_n + ScaledYear_o + (1|WhaleID_{jklm})$

$WhaleID_{jklm} \sim N(0, \sigma^2)$

Where the response variable$_{ilklmno}$ represents a measurement of parasite relative abundance from the *i*th sample from the *j*th whale of the *k*th sex and the *l*th age class in the *m*th pod sampled in the *n*th month of the *o*th year.

Body condition and parasite abundance

We ran a differential sequence analysis to determine whether the read counts of any potentially pathogenic sequences varied with body condition using the DESeq() function in the DESeq2 package in R. As differential sequence analyses are pairwise, to perform these analyses, we grouped body condition class into low (BC 1 or 2) or high (3 or 4).

To determine if infection status varied by body condition, we analyzed only SRKW samples that had a corresponding body condition metric (*n* = 19). Our response variable was the infection status for each parasite. We ran a generalized linear mixed effect model with a binomial distribution using the glmmTMB() function in R. Fixed effects were the body condition class (a categorical class between 1 and 5), pod, sex, and age class. Whale ID was included as a random effect. We performed model selection, comparing the full model to models with every other combination of fixed effects using the dredge() function in the MuMIn package in R (Bartoń 2023).

Model 3:

$$InfectionStatus_{il} \sim Binomial(\mu_{il})$$

$$E(InfectionStatus_{ijk}) = \mu_{il}$$

$$\log(\mu_{ijklmn}) = Sex_k + AgeClass_l + Pod_m + BodyCondition_n + (1|WhaleID_{jklmn})$$

$$WhaleID_{jklmn} \sim N(0, \sigma^2)$$

Where the response variable$_{ijklmn}$ represents the relative abundance of each parasite from the *i*th sample collected from the *j*th whale of the *k*th sex in the *l*th pod with the *n*th body condition class.

Because we do not know the directionality of the relationship between parasite infection and body condition, we also ran an ordinal regression model with body condition class as the response variable, parasite infection status, pod, and sex as fixed effects, and age class and whale

identity as random effects using the clmm() function in the ordinal package in R (Christensen 2022).

Power Analysis

We ran a power analysis for Model 3 to quantify statistical power for detecting effects of body condition on parasite infection status in two simulated datasets: one with our sample size (19 fecal samples from 14 whales), and one that sampled every extant individual with the same proportion of repeated individuals as was observed in our dataset (100 fecal samples from 74 whales). Because whales were sampled more than once, we generated our simulated data based on the same ratio of unique whales to fecal samples (14/19 = 74/100). We simulated our datasets based on the six years when both photogrammetry and fecal data were available. To inform the simulation, we used existing demographic data from the Orca Network. We created a dataset of the IDs, associated pods, and birth years of each individual in the population alive from 2008 to 2021. For each row, we assigned a random year from one of six, representing the six years in which our samples were obtained. We subtracted the birth year from the assigned year to get age at sampling and reassigned any negative values to 0. We assigned age classes to each individual, including 1 (calves, 0–4 years of age), 2 (subadults, 5–11 years of age), 3 (adults, 12+ years of age). Age class was treated as a factor. We simulated body condition data based on the true measurements in our dataset for each age class (i.e., all members of the same age class are assigned a body condition sampled from a distribution of the frequency of body conditions observed in our dataset for individuals in that group), and assigned measurements based on age class. We simulated a parasite presence/absence vector using a binomial distribution based on the presence/absence ratio from our data.

We built a simplified GLMM using lmer() that included scaled infection status as the response variable, scaled body condition as a fixed effect, and whale ID as a random effect. Both variables were scaled using the scale() function in R. By scaling the values of the predictor and response variables, we standardized the regression so that the beta coefficients each represent a correlation coefficient. We used the function powerSim() in the simr package in R (Green and MacLeod 2016), which ran the model 500 times and calculated the proportion of instances in which the p-value < 0.05. We tested a range of correlation coefficients, from 0.1 (very small correlation) to 0.9 (very large correlation) (Mukaka 2012) by manually changing the fixed effect coefficient in the model and running the simulation to determine how much power we had to detect an effect. We first did this with our simulated small dataset (19 fecal samples from 14 whales) and then repeated the analysis with our simulated large dataset (100 fecal samples from 74 whales). We ran the model through the powerCurve() function to determine what sample size is needed to detect a low (0.3), moderate (0.5), high (0.7) correlation on the large dataset.

RESULTS

Scat sample analysis

Species in the Anisakidae family were the most abundant parasites found in both fecal floatation and sedimentation, with 2,732 eggs identified (in 26/35 killer whale samples). This was also the only parasite taxon that we were able to identify with the diagnostic key (Dailey et al. 1980). Two other parasites were detected in low numbers, though we could not definitively identify them morphologically, and they were not detected genetically. One was most consistent with *Balantidium* spp. cysts (4 cysts detected), found in SRKW, and the other was identified as likely *Odhneriella* spp. (2 eggs detected), found in the single offshore killer whale (Figure 2). All other

possible eggs occurred in low numbers (1-20 eggs detected across all samples) and were not identifiable with the key or our supplementary identification guide (Supplementary Figure 2, Supplementary Table 1). Because these parasites did not occur at high frequencies, they were not included in quantitative analysis. Based on the diagnostic key by Dailey et al. (1980), we identified two anisakids in fecal floatation and sedimentation analysis: *Contracaecum spp.* (n_{ARKW} = 23, n_{SRKW} = 228) and *Anisakis* spp. eggs (n_{ARKW} = 105, n_{NRKW} = 1, n_{SRKW} = 2375; Figure 4.2). However, in the genetic analysis, no *Contracaecum* spp. were detected, though the selected primer should have been able to differentiate the two anisakid species. Though this could have occurred if *Contracaecum* spp. were rare enough that they were not sequenced, the life history of *Contracaecum* spp. provides support for this being a misidentification. *Contracaecum* spp. are not known to use cetaceans as definitive hosts, and are not expected to reach a reproductive adult stage within a killer whale (Klimpel and Palm 2011), therefore it would be unlikely to detect eggs in killer whale feces. This leads us to conclude that following the diagnostic key may lead to misidentification of eggs in killer whale fecal samples. Because of this discrepancy in morphological identification, we grouped eggs identified as *Anisakis* spp. and *Contracaecum* spp. into the family Anisakidae to report a single combined morphological egg count per float and a single combined egg count per sedimentation.

DNA metabarcoding analysis

Across all fecal samples, there were a total of 384,109 sequence reads. When the data were filtered to remove any sequences with less than 98% certainty of match, the total number of reads was reduced to 378,932 (min = 0, max = 42,806, mean = 10,827). When subset to only include *Anisakis* spp., the number of reads was 349,730 (min = 0, max = 43,242, mean = 9,992), or 92.3% of the total reads.

The only confirmed killer whale parasite in our samples was *Anisakis* spp. (Figure 4.3). Other potential eukaryotic pathogens we detected included the fungi *Aspergillus penicillioides*. Other species in this family cause fungal infections in odontocetes (Gaydos et al. 2004), and the species documented here can cause respiratory disease in humans (Klich, 2009), but it is not known to cause the same pathology in odontocetes (Joe Gaydos, marine mammal veterinarian, pers. comm.). The sequence was only detected in two whales at a low level (Figure 4.4c). *Candida* spp. were also detected in two whales at similarly low levels (Figure 4.4c). The fungi *Candida* spp. have caused fungal infections in odontocetes including killer whales, though we could not identify the sequence to the species level, so we cannot be sure that this specific species causes similar pathology in killer whales (Gaydos et al. 2004). Several ciliates and copepods were detected, but not the species that have been associated with skin disease in killer whales (Schulman and Lipscomb, 1999; Vecchione et al. 2014).

In addition to detecting species that could be pathogenic to killer whales, we also detected parasites that infect salmon. *Henneguya* spp., a myxozoan parasite known to cause disease in salmon (Fiala et al. 2015; Fish, 1939), but not odontocetes (Joe Gaydos, marine mammal veterinarian, pers. comm.) was detected in six samples from three individuals. This suggests that some of the salmon eaten by killer whales were infected with parasites that could be detected through the whale's feces. Additionally, the algae *Heterosigma akashiwo* was detected in one sample at a relatively low level (Figure 4.3b). *H. akashiwo* causes red tides that can be lethal to fish (Khan et al. 1997; Taylor and Haigh 1993), and occurs in areas of the SRKW range (Hard et al. 2000; O'Halloran et al. 2006). It is possible, but unlikely that this detection came from DNA in the water surrounding the sample when collected.

We also detected a few sequences that suggested there may have been contamination following collection of the fecal samples. For example, in one sample we detected *Allium cepa* sequences (onion, relative abundance in sample = 0.003), and in another we found *Musa acuminata* (banana, relative abundance in sample = 0.004). These may have come from contamination upon collection or storage in the field.

Comparing methodologies

There was only one instance in which the number of anisakid eggs identified in the fecal floatation exceeded the number identified in the fecal sedimentation (1 egg detected in the floatation, 0 in the sedimentation). Otherwise, fecal sedimentation proved to be a better method for detecting anisakid eggs in killer whale fecal samples than fecal floatation. Based on the egg counts obtained from killer whale samples analyzed through sedimentation, 76.5% (26/34) had anisakid eggs present in their fecal samples (Table 4.2). In comparison, *Anisakis* was detected in 94.1% (32/34) of fecal samples through genetic analysis. We also compared the anisakid egg counts from sedimentation for each sample to the *Anisakis* relative abundance from sequencing (Figure 4.4b), and found a weak linear relationship within each of the pairs of variables. Sequencing was better at detecting *Anisakis* presence than laboratory fecal sedimentation, given the number of samples that had zero eggs but some amount of *Anisakis* DNA detected (Figure 4.4b). However, as egg presence/absence is a regularly used metric for assessing parasite infection (Ten Doeschate et al. 2017) and because the prevalence of *Anisakis* DNA was too high to effectively run binomial models, we used morphological presence absence as our response variable in our models. Additionally, two SRKWs were sampled multiple times in the same day, which allowed us to make qualitative observations on parasite detection between samples. Notably, the egg counts varied by sample, even when collected from the same individual on the

same day (Table 4.3). Both J42 and L86 were sampled multiple times in one day, and relative abundance of *Anisakis* reads varied slightly for both individuals (J42: 0.999 vs. 0.846; L86: 0.938 vs. 0.737), while egg counts were much more variable (J42: 0 vs. 92; L86: 135 vs. 458).

Statistical analysis

Between-population differences

The diversity of the species detected through molecular analysis differed among the four populations. NRKW had the highest mean diversity (though sample size was low at *n* = 2 individuals), followed by SRKW (Appendix Ch. 4A: Figure S4.1). ARKWs and the OKW whale had similarly low diversity (Appendix Ch. 4A: Figure S4.1). The only marine mammal parasite identified by molecular analysis was *Anisakis* spp., and the remaining sequences identified varied from marine hydrozoans to terrestrial plants (Figure 4.3). *Anisakis* was detected in 85.7% (6/7) of ARKWs, 50% (1/2) of NRKWs, 92% (23/25) of SRKWs, and 100% (1/1) of OKWs.

Our DESeq analysis of potentially pathogenic non-parasites indicated a significant difference in *Henneguya* spp., a fish parasite, between SRKWs and ARKWs (base mean = 23.1, log two-fold change = 3.598, P_{adj} = 0.0255), with more reads found in SRKWs. This was the only potentially pathogenic species that had a significantly different read count across the populations.

Because *Anisakis* was the only marine mammal parasite found in molecular analysis, and only anisakids were identified in morphological analysis, we only analyzed anisakid infections. Our first GLMM assessed whether there was a difference between infection status among the three resident populations of killer whales sampled: ARKW (*n* = 6), NRKW (*n* = 2), and SRKW (*n* = 25). This analysis used all available sample data except for one ARKW sample, which only

consisted of <98% matched reads, and the one OKW sample (*n* = 33). We tested whether infection status differed by population. We found that anisakid infection status was not significantly different among populations (Table 4.3).

Correlated factors

Our second model assessed whether anisakid infection status was influenced by temporal or demographic variables. Model selection provided the most support for the model that included month as the only fixed effect, and whale ID as a random effect (Table 4.4). Month had a marginally significant effect on the relative abundance of *Anisakis* in the sample, with samples collected in September having significantly higher parasite infection status than November those collected in (estimate = 2.43, P = 0.078; Figure 4.5).

Body condition and parasite abundance

Our differential sequence analysis revealed a read count difference between animals in poor and good body condition in *Heterosigma akashiwo* (base mean = 211.6, log two-fold change = -12.31, P_{adj} = 0.001), which was observed in one SRKW sample.

Our third model assessed whether SRKW body condition class influenced anisakid infection status. Our sample size to address this question was limited to the samples that had a body condition measurement collected in the same month as the scat sample (n = 19). We first ran a model with every variable we thought might impact body condition: this included relative *Anisakis* abundance as the response variable, body condition class, sex, age class, and pod as fixed effects, and whale ID as a random effect. There was no difference in parasite infection status across body condition (Table 4.5; Figure 4.5). However, the model containing body

condition was not the best fit model (ΔAIC = 2.46). To assess if parasite infection status had a significant effect on body condition, we also ran an ordinal regression model with body condition class as the response variable. Similarly found no effect of parasite infection status on body condition (estimate = 2.153, SE = 1.617, Z = 1.331, P = 0.183).

Power analysis

The power analysis demonstrated that at our current sample size, we would be able to detect a high correlation (0.8) between body condition and relative parasite abundance 80% of the time. If there was a low correlation (0.3), we would have only a 17.2% probability of detecting it at our current sample size. We would have a 42.8% chance of detecting a moderate correlation (0.5; Table 6). If we were to sample every whale in the population with the same level of replication as observed in our data (n = 100), we would be able to detect even a low (0.3) correlation between relative parasite abundance and body condition 80.8% of the time.

DISCUSSION

Prior to our study, the only published documentation of the parasitic fauna of killer whales in the Northeast Pacific came from necropsies. Our study successfully documented which parasites exist in living, wild whales. Our analysis showed not only that there are parasites present in wild, living SRKWs, but also that they are nearly ubiquitous in all populations sampled. *Anisakis* spp. were detected in most whales sampled, including southern, Alaska, and northern resident killer whales, and the offshore killer whale sampled. This finding is consistent with what is known about *Anisakis* infections in other odontocetes (Colón-Llavina et al. 2009; Margolis and Dailey 1972). The widespread infection of killer whales in the Northeast Pacific is a new discovery: in previous necropsies of killer whales, only two of the seven residents analyzed were found to be

infected with *Anisakis* spp. (Raverty et al. 2020). Our findings show that *Anisakis* infections are prevalent in SRKWs, and may be an additional stressor on this already stressed population.

Parasites detected

Each method of parasite detection resulted in the identification of possible pathogens that were not captured by the other methodologies. Through fecal floatation, we detected what was likely *Odhneriella* spp. from the OKW sampled. Through fecal sedimentation, we found cysts consistent with *Balantidium* spp. in SRKW samples. *Balantidium* spp. was not included on our list of possible parasites that we used to choose a suitable primer, and would not have been amplified by the selected primer. *Balantidium* spp. are protozoan parasites that have previously been detected in fin whales in Portugal (*Balaenoptera physalus*; Hermosilla et al. 2016), but to our knowledge this group has not been described in delphinids in the Northeast Pacific. The only species of this family known to be pathogenic for mammals is *Balantidium coli* (Ponce-Gordo et al. 2011), which commonly infects terrestrial animals (Schuster and Ramirez-Avila 2008), and has only been detected in one marine mammal species, the Chilean sea lion (*Otaria flavescens*; Hermosilla et al. 2013). We could not definitively identify the cysts using morphological keys, so it is unclear whether the cysts identified in our analysis are *B. coli* and thus, potentially pathogenic. While *Odheriella* spp. was included in our list of possible parasites (Table 1), a genetic sequence was not available on GenBank to use for reference in molecular identification. From our molecular analysis, we did not detect any additional parasite species of whales (Table 1), but we did detect a few other potentially pathogenic species of the fish they consume. The detection of *Henneguya* spp. suggests that some of the prey consumed by the whales sampled were infected with this parasite. Similarly, we detected *Heterosigma akashiwo*, which is a toxic algae that can cause pathology in fish (Khan et al. 1997). Several fungal species were also

detected, notably *Aspergillus penicillioides* and *Candida* spp., which may not represent a health risk unless they are able to opportunistically colonize an open wound (Joe Gaydos, marine mammal veterinarian, pers. comm.).

We detected several other eggs through sedimentation and floatation that we were unable to identify either through morphological identification or use of our selected primer (Appendix Ch. 4A: Figure S4.2); this is probably due to the limited scope of the marine mammal parasite taxonomic identification resources for parasite eggs of delphinids in this region, the availability of reference sequences for these species, or a combination of low read count per sample and the rarity of the species in the samples where they were detected.

The most prevalent parasite species found in all killer whale populations, and detected by all methodologies, were *Anisakis* spp. There were multiple instances in which the egg count was 0, but *Anisakis* DNA was detected through molecular analysis. This is probably due to the variability in egg shedding rates over time (Ugland et al. 2004). Anisakids live in the marine mammal hosts for 37 to 109 days before maturation, and eggs are shed in the last week of the nematode's life (Ugland et al. 2004). Individual nematodes can produce anywhere from 500,000 to over 1 million eggs, depending on their body length (Simard 1997; Ugland et al. 2004). They shed 85% of their eggs within the first 3 days of spawning, and that rate declines as spawning goes on (Ugland et al. 2004). Depending on when the fecal sample is taken, an infected individual might not have any eggs detected in the fecal sample, though they do have living adult worms detectable with DNA metabarcoding. Additionally, egg counts do not reflect the male anisakids or immature females present in the host.

While not used in our models, DNA metabarcoding detection may be a better indicator of infection status because this method can detect not only eggs in the fecal sample, but also DNA of adult nematodes still living in the gut tract (Berger and Aubin-Horth 2018). However, several important factors in the way metabarcoding data are generated will affect the interpretation of these data, and can lead to misleading results if not taken into account. The relationship between DNA quantity and organismal abundance is unknown (Davey et al. 2021), so we cannot infer parasite abundance through our metabarcoding analysis. Relative abundance of parasites in a DNA sample can give insight into the most common sequences in a sample, but are prone to amplification bias, in which the selected primer can have variable amplification efficiencies (i.e., amplify some sequences more than others) or fail to amplify rare sequences (Pinol et al. 2015; Gold et al. 2023). Additionally, relative abundance between individuals could be biased by the type of genetic material in the sample (worm vs. egg), how accessible DNA content was, and the composition of the subsample (Gold et al. 2023). Further research should use qPCR and/or ddPCR methods to improve upon our metabarcoding estimates of the relative quantity of DNA from *Anisakis* in SRKW fecal samples. Continued research comparing DNA metabarcoding with traditional egg counting methods and assessments of gastrointestinal parasite infections in necropsied whales will be important to understanding and interpreting the relationships between DNA metabarcoding data, egg counts, and parasite load.

There were clear indications that our samples had some level of contamination. While we did our best to sterilize our sampling tools before sample extraction, we were not working in a sterile environment. Additionally, it is likely that some contamination occurred in the field during sample collection, which would explain reads from banana and onion in our sequence data. Though we used a filter to remove any sequences with fewer than 1% reads, there may still be

some positive reads from contamination. Additionally, *Anisakis* does have a free-living stage, from 43–91 days (Measures 1996), so theoretically, some reads could have been attributable to DNA in the water.

Between-population differences

We expected there to be differences in the infection status among the four killer whale populations. Of the resident killer whales in the northeast Pacific, ARKWs and NRKWs populations are both larger and increasing, while the SRKW population is small, with continuous declines impeding recovery (Matkin et al. 2014; Fisheries and Oceans Canada 2018). OKWs are more abundant than SRKWs, but little is known about trends in abundance (Schorr et al. 2022; Ford et al. 2014). Because SRKWs face several cumulative stressors (Lacy et al. 2017) at higher levels, we expected their immune systems to be more compromised (Curry, 1999) compared to the other populations, making SRKW less equipped to fight parasite infections. Contrary to our expectations, we found that there was no difference in parasite abundance across the killer whale populations. Each population sampled had an *Anisakis* spp. infections, with prevalence of 50% or greater. This contrasts with the findings of Raverty et al. (2020), in which the authors only detected *Anisakis simplex* in one ARKW and 25% (1/4) of the SRKWs necropsied, although they did find that the *Anisakis* infection in a three-year old female SRKW was associated with gastritis, which had not been detected previously in killer whales with *Anisakis* infections (Raverty et al. 2020). As we detected *Anisakis* infections in most whales sampled, associated gastritis could be more common than previously suspected from necropsy data alone.

The similarity among populations may reflect similarities in the infection prevalence of their prey, and thus their risk of exposure. ARKW range from the Kodiak archipelago down to

Southeast Alaska (Matkin et al. 2014), NRKW range from Southeast Alaska to coastal Washington (Olesiuk et al. 2005), SRKW range from Southeast Alaska to central California (Hanson et al. 2021), while OKW range from the Aleutian Islands to Southern California (Dahlheim et al. 2008; Ford et al. 2014). This means that the three resident populations are consuming salmon and the offshores are consuming higher trophic level fish along a large portion of the Northeast Pacific coast (Schorr et al. 2022; Ford et al. 2014). In Chapter 3, I found that anisakid abundances in chum and pink salmon have increased in Alaska from 1979 to 2020. As marine mammal populations have increased along the inland and coastal waters of Washington (Calambokidis and Baird, 1994; Elliser and Hall, 2021; Jefferson et al. 2021; Calambokidis et al, 2017; NOAA 2017), Oregon (Brown et al. 2005; Derville et al. 2022), and California (Laake et al. 2018; Lowry, 2014) the prevalence of these nematodes are likely to increase in response. This has been observed in other systems (e.g., Buchmann and Kania 2012; Haarder et al. 2014; Chapter 2, this dissertation). Increased abundances of other marine mammal hosts may have contributed to high abundances of anisakids, and therefore to the high observed infection prevalence in the four killer whale populations included in this study.

We did not detect significant differences in the prevalence of any other marine mammal parasites across populations. We did find two eggs consistent with *Odhneriella* spp. in the OKW sample, a species that was not detected in any of the resident samples. This genus of trematodes has previously been found in several marine mammals, including ARKW (Skrjabin 1959; Raverty et al. 2020), long-finned pilot whale (*Globicephala melaena*; Balbuena et al. 1989); beluga whale (*Delphinapterus leucas*; (Delyamure et al. 1958) and walrus (*Odobenus rosmarus*; Dailey 1980). It belongs to the family Brachycladiidae, which occurs in the bile and pancreatic ducts, intestine, lungs, and sinuses of marine mammals (Gibson, 2005). One member of this

family is suggested to have caused whale strandings (Dailey and Walker, 1978), though *Odhneriella* spp. are not known to have any pathological effects (Balbuena et al. 1989). The life cycle of this trematode is largely unknown (Balbuena et al. 1989), though recent work on other members of this family suggests that they use gastropods of the family Naticidae as their first intermediate hosts and bivalves as second intermediate hosts (Kremnev et al. 2020). Without knowing more specifically what other hosts this genus uses, it is difficult to say why it was detected in the offshore killer whale and not any of the residents, but it could be due to the different diet of offshore killer whales (Krahn et al. 2007), which are known to consume sharks, opah, and other high-trophic level fishes (Ford et al. 2011; Morin et al. 2006), thus exposing them to a suite of different parasites than resident killer whales. Our analysis showed that *Henneguya* spp. detection was greater in ARKWs than SRKWs, suggesting that ARKWs consume more fish infected with this parasite than SRKWs, though *Henneguya* spp. was only detected in one ARKW sample. Notably, this genus was detected in six SRKWs samples as well, indicating that *Henneguya* spp. infect the prey of both populations.

Correlates with anisakid infection status

We found that month was the best predictor for anisakid infection status in SRKW. Infections were marginally greater in September than November (Table 4.4), suggesting that infection status was marginally greater in the summer than the fall, though we analyzed fewer samples collected in November ($n = 3$). This could be due to differences in SRKW summer and fall diets (Hanson et al. 2010. From June to August, Chinook salmon is the primary component of SRKW diet. In September, SRKW diet is made up of Chinook and coho salmon (Ford et al. 2016), while in the fall and early winter, their diet is a mixture of Chinook, coho, and chum salmon (Hanson et al. 2021). Because there is a lag of 37–109 days post-infection for *Anisakis* to reproduce (Ugland

et al. 2004), these higher *Anisakis* abundances in the late summer could indicate that the Chinook they consume earlier in the summer are more infected with these nematodes than the coho and chum that make up a greater proportion of their diet later in the summer and fall.

Anisakids and body condition

Notably, none of the demographic data included in our model were good predictors for parasite infection status. When we ran the full model that included body condition, we did not detect a significant correlation between body condition class and anisakid infection status. However, at our sample size, we only had the power to detect a strong correlation (0.8). For comparison, a similar study that found a correlation between body condition and parasite infection status in harbor porpoise stomachs used data from 97 necropsied porpoises to achieve the power needed to detect that association (Ten Doeschate et al. 2017). The fecal samples and the photogrammetry data each require extensive field efforts to collect. As we were only using fecal samples collected within a one-month window of photogrammetry data collection, the level of replication needed to detect a weak correlation would be difficult to achieve. This is a common problem with using noninvasive samples from rare species in the marine environment (Cossu et al. 2022; Smith and Wang, 2014). We estimate that, if we sampled the entire population at the same level of replication as in our dataset (i.e., 100 samples from all 74 whales that currently exist), we would have much higher power, and could detect even a weak correlation between parasite abundance and body condition. Additional ongoing collection of fecal samples and body condition data will increase the power to detect correlations between parasite infections and body condition.

While we were not able to detect a difference in *Anisakis* spp. abundance by body condition, we did detect a difference in *Heterosigma akashiwo*, with higher infection status in the

SRKW with better body condition. We interpret this result cautiously, as the difference detected is probably attributable to the one observation of the toxin at low abundance in an individual SRKW (Figure 4.4c). It is notable that the toxic algae was detected in fecal samples, however, indicating that at least one whale fed in an area with a red tide event or upon a fish affected by the toxin.

Implications

Though we could not determine whether body condition is correlated with anisakid infection status, nor anisakid infection status with body condition, we suspect that intestinal parasites contribute to energetic stress in these populations (Shanebeck et al. 2022). While SRKWs are the most at-risk resident killer whales in this region, NRKWs are also listed as threatened. Both populations have been exposed to high levels of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Hickie et al. 2007; Rayne et al. 2004), though southern residents have a higher intake rate of PCBs (Cullon et al. 2009). Pollutant exposure alone can lead to immune suppression (Martin et al. 2010), but SRKWs also face an elevated energy deficit (Couture et al. 2022; Wasser et al. 2017), which can cause less energy to be invested in immune response (Moore and Hopkins, 2009). A silver lining is that anisakid infections are not a permanent ailment. Our analysis showed that one of the whales sampled multiple times (J49) lost their infection between September 2018 and November 2019. *Anisakis* only have a maximum life span of 109 days within a marine mammal host (Ugland et al. 2004). However, given the increasing abundance of anisakids in salmon (Chapter 3, this dissertation) and in the Puget Sound (Chapter 2), as well as the immunocompromised nature of their preferred prey in their foraging region (Sures 2008), these infections may become more prevalent, and have a greater impact in the future. While *Anisakis* spp. are not considered to be a major disease

threat to the SRKW population (Gaydos et al. 2004), they are likely to have a larger energetic cost than previously assumed (Shanebeck et al. 2022) and should be considered as an additional sublethal stressor in the population.

Additionally, SRKW have recently been found to face inbreeding depression (Kardos et al. 2023), which could reduce immune functioning and make them more susceptible to parasite infections. Inbreeding can lead to decreased genetic diversity, which may be correlated to host susceptibility to parasite infection (Allendorf, 1986; O'Brien and Evermann, 1988). Studies on many systems have supported this hypothesis; inbreeding has led to higher infection prevalence (Stevens et al. 1997) and susceptibility to ectoparasites (Luong et al. 2007) in insects, higher parasite intensity and less ability to clear an infection in guppies (Smallbone et al. 2016), and higher infection severity in Chinook salmon (Arkush et al. 2002). Maternal inbreeding specifically resulted in weaker cell-mediated immune response resulting in a weaker immune response in song sparrows (Reid et al. 2003). In mammals, endangered gazelle species with high levels of inbreeding were found to have greater prevalence of gastrointestinal parasites (Cassinello et al. 2001). Parasite infections can alter population trajectories, and may be able to raise the extinction risk of inbred populations (McCallum and Dobson 1995; Smallbone et al. 2016), and should be considered in the management of endangered species (Cassinello et al. 2001).

Considerations

Our study is the first systematic assessment of parasites in living, wild killer whales in this region. Previously, the parasites that infected killer whales were known only from necropsies (Lehnert et al. 2023; Raverty et al. 2020; Reckendorf et al. 2018) and rare instances in which at-

risk calves were screened for parasites. We used three methods to quantify parasite infection in the samples analyzed, finding that molecular identification of parasites was more capable of detecting infection status than fecal floatation or sedimentation methods. However, there are limitations to both molecular and morphological methods. There are limited taxonomic resources available to aid in identifying marine mammal parasite eggs morphologically. We reviewed the literature and marine parasite taxonomic identification guides for photos or illustrations of the parasites we thought were likely to be found in killer whales, and there were several species for which we could not find reliable descriptions of egg morphology. Similarly, genetic databases for parasites are also incomplete. Comparing morphological data to our molecular findings, we found that, by following the identification guide for marine mammal parasites (Dailey et al. 1980), both *Contracaecum* spp. and *Anisakis* spp. were detected in the samples. The primer we selected for sequencing should have differentiated between the two species, but we found no evidence of *Contracaecum* by molecular analysis, which may have been due to false positives during morphological analysis, or a failure of our primer to amplify comparably rare *Contracaecum* DNA. Further research is needed to genetically identify parasite eggs detected morphologically to ensure accurate diagnoses.

Because we had variable success with the methods used, future monitoring efforts could make use of one or more methods, depending on the goal and budget. Fecal floatation and sedimentation are relatively inexpensive and fast methodologies that can provide an indication of whale infection status, with the caveat that it may miss non-reproductive parasites infecting the host (Ugland et al. 2004). A molecular approach can provide a more comprehensive assessment, detecting parasite eDNA from the fecal material even if the eggs are not present (Berger and

Aubin-Horth 2018), but it is more expensive and time consuming and detection depends on the primers used and availability of reference sequences for all parasites of interest.

CONCLUSION

We used existing fecal samples from four populations of living, wild killer whales in the Northeast Pacific Ocean to assess parasite infections. We found that a majority of the whales sampled, across all populations, were infected with *Anisakis* spp. Parasite infections are rarely monitored in SRKW, but given the impact of intestinal helminths on hosts (Shanebeck et al. 2022), and the energy deficiency (Couture et al. 2022; Wasser et al. 2017), high load of pollutants, and inbreeding depression impacting the immune function in the members of this population (Krahn et al. 2009; Kardos et al. 2023), we believe this is an oversight. There are so few remaining SRKWs that conservation measures implemented on the scale of the individual may be needed to ensure the persistence of the population. Intestinal parasitism is a treatable ailment, should individual intervention be needed going forward. Continued monitoring of parasite infections should be implemented in ongoing population monitoring efforts.

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CH. 4 TABLES

Table 4.1: List of parasites that infect Delphinidae in the Northeast Pacific, compiled from published necropsy reports. Parasites that were likely to infect the gastrointestinal tract are indicated with an asterisk.

Parasite	**Group**	**Host**	**Region**	**Citation**	**Availability on GenBank**
*Anisakis simplex**	Nematoda	*Orcinus orca* (AR, SR), *Balaenoptera borealis, Balaenoptera physalus, Berardius bairdi, Megaptera novaeangliae, Physeter catodon, Ziphius cavirostris, Pseudorca crassidens*	AK, WA, BC	Raverty et al. 2020, Margolis et al. 1954, Margolis and Dailey 1972; Mueller 1927, Cornwall 1928, Margolis and Pike 1955, Zam et al. 1971, Dailey and Brownell 1972, Baird et al. 1988, Colom-Llavina 2005, Klimpel and Palm 2011	Nucleotide
*Anisakis sp.**	Nematoda	*Balaenoptera borealis, Balaenoptera physalus, Berardius bairdi, Megaptera novaeangliae, Orcinus orca, Physeter catodon, Ziphius cavirostris, Phocoena phocena*	AK, BC, WA, CA	Scheffer and Slipp 1948, Margolis and Pike 1955, Kenyon 1961, Rice 1963; Margolis and Dailey 1972, Smith 1989, Heyning 1989, Mignucci-Giannoni et al. 1998, Colom-Llavina 2005	Partial sequence
*Anisakis typica**	Nematoda	Unidentified dolphin,	Pacific	Margolis et al. 1954, Zam et al.	Nucleotide

		Pseudorca crassidens		1971, Dailey and Brownell 1972, Baird et al. 1988, Colom-Llavina 2005	
*Anisakis pacificus**	Nematoda	*Orcinus orca*		Heptner et al. 1976, Raverty and Gaydos 2004	No
*Bolbosoma capitalum**	Acanthocephala	*Pseudorca crassidens*	Pacific	Dailey and Brownwell 1972, Colom-Llavina 2005	No
*Bolbosoma niponicum**	Acanthocephala	*Orcinus orca*		Heptner et al. 1976, Raverty and Gaydos 2004	Partial sequence
*Bolbosoma physeteris**	Acanthocephala	*Orcinus orca*		Heptner et al. 1976, Raverty and Gaydos 2004	No
*Braunina cordiformis**	Trematoda	*Tursiops truncatus*	CA	Johnston and Ridgway 1969; Margolis and Dailey 1972	Partial sequence
Brucella spp.	Bacteria	*Orcinus orca*	Northeastern Atlantic and Pacific	Jepson et al. 1997, Raverty et al. 2004, Raverty and Gaydos 2004	Partial sequence
*Campula palliate**	Trematoda	*Delphinus delphis*	Pacific	Cordes and O'Hara 1979, Colom-Llavina 2005	No
*Campula sp.**	Trematoda	*Orcinus orca*		Gibson et al. 1998, Raverty and Gaydos 2004	No
*Contracaecum sp.**	Nematoda	*Lagenorhynchus obliquidens*	CA	Martin et al. 1970	Partial sequence
*Corynosoma alaskensis**	Acanthocephala	*Phocoena phocoena*	AK	Golvan 1959	No
Edwardsiella tarda	Bacteria	*Orcinus orca*	NE Pacific	Ford et al. 2000, Gaydos et al. 2004	Nucelotide
*Hadwenius nipponicus**	Trematoda	*Phocoena phocena*	WA	Ching and Robinson 1959; Margolis and Dailey 1972	No

*Odhneriella subtila**	Trematoda	*Orcinus orca* (AR)	AK	Heptner et al. 1976, Raverty and Gaydos 2004, Raverty et al. 2020	No
*Oschmarinella albamarina**	Trematoda	*Orcinus orca*		Gibson and Bray 1997, Raverty and Gaydos 2004	No
*Phyllobothrium sp.**	Cestoda	*Orcinus orca*		Dailey and Brownwell, 1972; Raverty and Gaydos 2004	Partial sequence
*Pyramico-cephalus phocarum**	Cestoda	*Enhydra lutris, Erignathus barbatus, Phocea sp., Phocoena phocoena*	AK	Rauch 1953, Hilliard 1960, Kenyon 1962, Johnson et al. 1966, Rausch and Hilliard 1970; Margolis and Dailey 1972	Partial sequence
Salmonella	Bacteria	*Orcinus orca* (O)	CA	Raverty et al. 2020	Partial sequence
Sarcocystis neurona	Eucoccidiorida	*Orcinus orca* (T)	CA	Raverty et al. 2020	Partial sequence
Toxoplasma gondii	Eucoccidiorida	*Orcinus orca* (T)	CA	Raverty et al. 2020	Nucleotide
*Trigonocotyle spasskyi**	Cestoda	*Orcinus orca*		Dailey and Brownwell 1972; Raverty and Gaydos 2004	No

Table 4.2: The anisakid prevalence in each sample, from sedimentation (Anisakids/g), sequencing (*Anisakis* read counts), and the relative proportion of *Anisakis* reads to other identified sequences in each sample. Colors indicate individuals that were sampled more than once.

Whale ID	**Population**	**Pod**	**Date**	**Anisakids/g**	***Anisakis read counts***	**Relative Proportion of Anisakis reads**
ARKW_12	ARKW	UNK	2017	82.9	37579	0.999
ARKW_13	ARKW	UNK	2017	31.4	18	1
ARKW_15	ARKW	UNK	2017	165.9	38152	1
ARKW_28	ARKW	UNK	2018	0	0	NA
ARKW_27	ARKW	UNK	2018	6.5	43776	0.988
ARKW_03	ARKW	UNK	2018	2.3	451	0.982
ARKW_01	ARKW	UNK	2018	0	56	1
J26	SRKW	J	28 Sep 2011	20.2	142	1
K25	SRKW	K	30 Oct 2013	134.9	7963	0.996
UNK	SRKW	U	22 Sep 2015	2	49	1
J42	SRKW	J	14 Sep 2016	90.7	25490	0.999
L106	SRKW	L	10 Sep 2017	0	3767	0.422
J35	SRKW	J	19 Sep 2017	36	7786	0.999
L113	SRKW	L	22 Sep 2017	48.2	2265	1
L47	SRKW	L	23 Sep 2017	0	12	0.002
J42	SRKW	J	23 Sep 2017	0	3360	0.938
J42	SRKW	J	23 Sep 2017	91.2	4446	0.734

J42	SRKW	J	24 Sep 2017	2506.5	18622	1
J36	SRKW	J	24 Sep 2017	472.5	10622	0.982
J22	SRKW	J	24 Sep 2017	584.9	3594	0.948
L86	SRKW	L	26 Sep 2017	134.9	12966	0.848
L86	SRKW	L	26 Sep 2017	458.1	36187	0.999
J19	SRKW	J	26 Sep 2017	14.8	7099	0.991
L118	SRKW	L	30 Sep 2017	32.2	10401	0.987
J16	SRKW	J	13 Sep 2018	399.6	38929	1
J49	SRKW	J	25 Sep 2018	135	1347	0.814
UNK	NRKW	UNK	19 Aug 2019	2.7	9994	0.989
UNK	NRKW	UNK	19 Aug 2019	0	0	0
J49	SRKW	J	13 Nov 2019	0	0	0
J39	SRKW	J	13 Nov 2019	141.4	45	1
J27	SRKW	J	13 Nov 2019	0	0	0
L116	SRKW	L	11 Sep 2021	21.3	3516	0.979
L106	SRKW	L	12 Sep 2021	141.2	53	0.53
J26	SRKW	J	15 Sep 2021	17.2	16915	0.983

Table 4.3: Model 1 assessed differences in anisakid infection status among the resident populations sampled. This analysis used data from all samples with sufficient molecular data available from ARKW (n = 6), NRKW (n = 2), and SRKW (n = 25). Alaska residents were in the reference position.

	Estimate	**Std. Error**	**Z value**	**P value**
Intercept	1.609	1.095	1.469	0.142
NRKW	-1.609	1.789	-0.900	0.368
SRKW	-0.223	1.204	-0.185	0.853

Table 4.4: Model 2 tested whether demographic variables (age, sex, pod) or temporal variables (month or year) had an effect on anisakid infection status of the host. Model selection suggested a model with month as fixed effect was the best fit. The model was run with September in the reference position, and used all available data from SRKW with known identities and age classes (n = 24).

	Estimate	**Std. Error**	**Z value**	**P value**
Intercept	-0.693	1.225	-0.566	0.571
September	2.428	1.376	1.765	0.078

Table 4.5: Model 3 assessed whether body condition class had an effect on anisakid infection status. The best fit model was the null, the second-best fit included only Pod (ΔAIC =1.98), and the third included body class and Pod (ΔAIC =2.46). Because we were interested in the effect of body class, we reported the results of the third best fit model. J Pod was in the reference position.

	Estimate	**Std. Error**	**Z value**	**P value**
Intercept	-0.349	1.966	-0.178	0.859
Body class	1.929	1.404	1.375	0.169
Pod (L)	-3.935	2.505	-1.570	0.116

Table 4.6: The estimated power to detect varying correlations ranging from 0.1 to 0.9 of anisakid infection status with body condition. We ran a power analysis for our sample size (N_{fecals} = 19) and for a scenario in which all of the whales in the population were sampled with the same replication (N_{fecals} = 100). Power estimates were calculated with the powerSim() function in the simr package in R.

Correlation coefficient	**Power (N_{fecals} = 19)**	**Power (N_{fecals} = 100)**
0.1	4.4%	17.0%
0.3	17.2%	85.2%
0.5	42.8%	100%
0.7	72.8%	100%
0.9	89.8%	100%

CH. 4 FIGURES

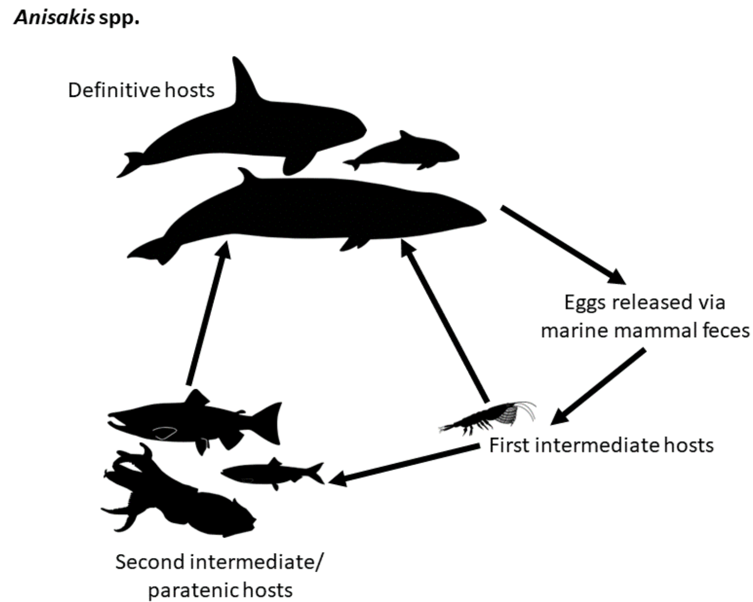


Figure 4.1: Life cycle of *Anisakis* spp. *Anisakis* spp. infect cetaceans as their definitive hosts, and after their eggs develop in the water column, larvae are consumed by an invertebrate which serves as their first intermediate host. The larva is transmitted to paratenic hosts when consumed by a fish or cephalopod - hosts in which the larvae does not develop, but which can help the parasite get to the definitive host. All images obtained from PhyloPic. Vector images of cetaceans courtesy of Chris Huh under the Creative Commons Attribution-ShareAlike 3.0 Unported license.

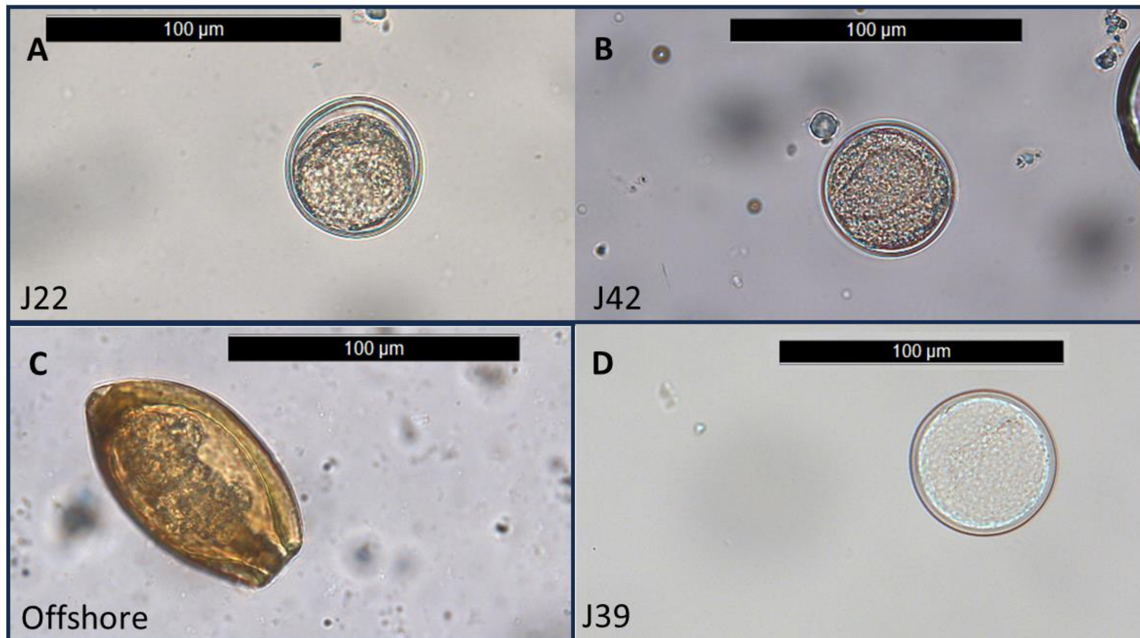


Figure 4.2: (A) Anisakis, as identified through the Dailey diagnostic key (Dailey et al. 1980), has a gap between the interior and the outer shell. (B) Contracaecum had no gap between the outer wall and the interior. (C) Likely Odhneriella spp., found in the offshore killer whale sample. (D) Possibly Balantidium spp., which has previously been found in fin whales (Hermosilla et al. 2016). Eggs in (A), (B) and (C) were identified and photographed from a fecal floatation, egg in (D) was identified and photographed from a fecal sedimentation.

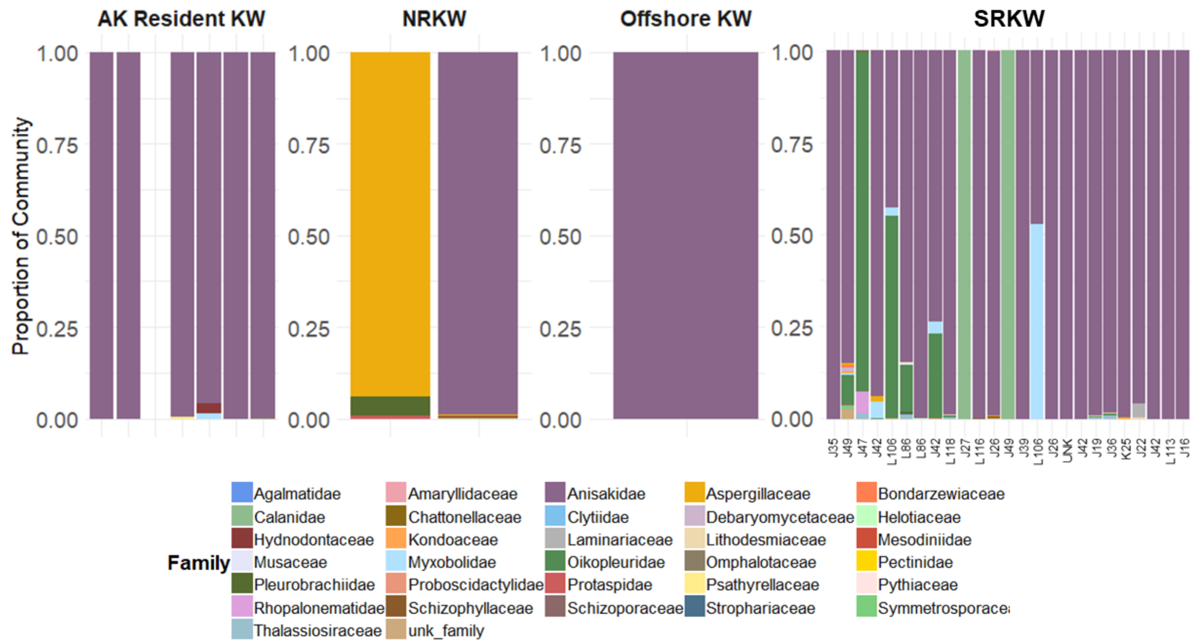


Figure 4.3: The relative abundance of sequences by family detected in each sample varied by population. Each bar represents an individual sample. Anisakidae was the most commonly detected sequence in our molecular analysis. Unk_family indicates a sequence that we could not identify to the family level.

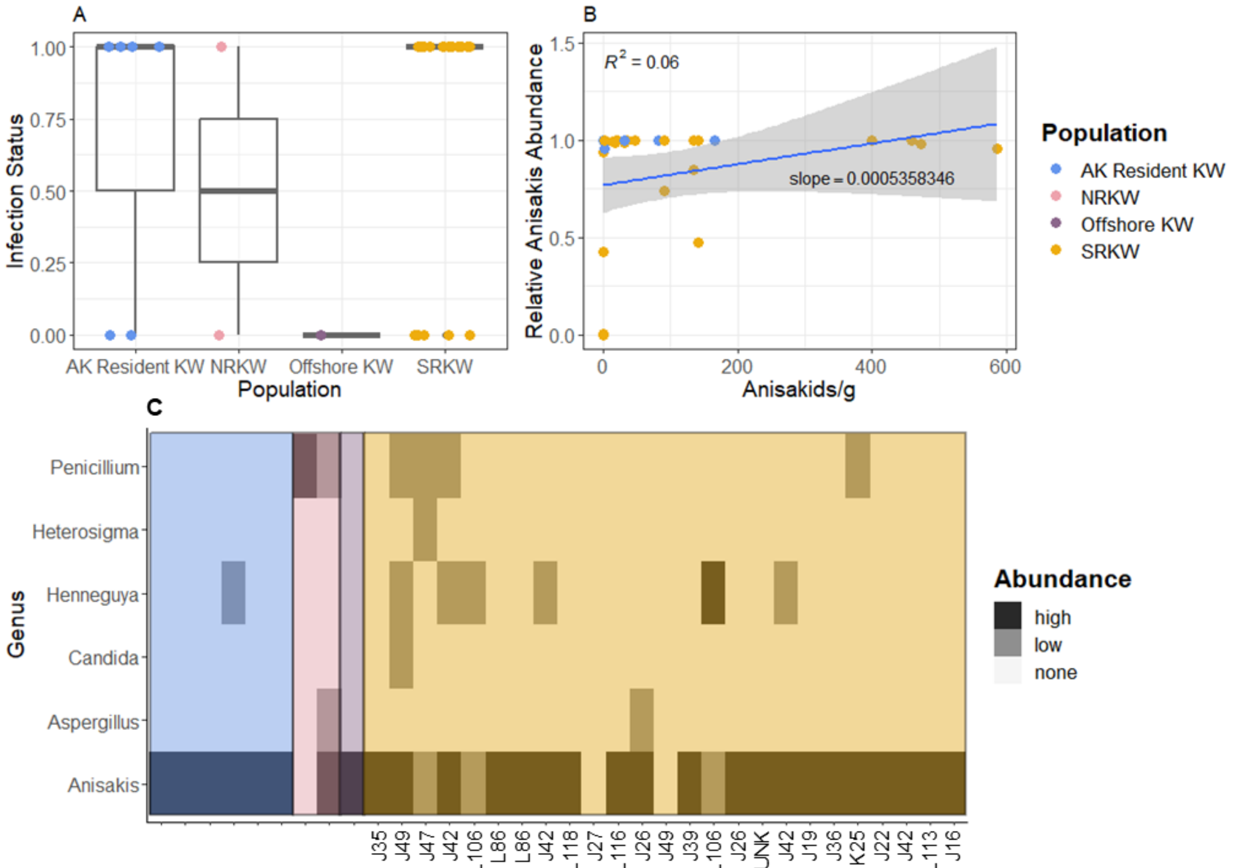


Figure 4.4: A) The infection status of each population, where 1 indicates that there were anisakid eggs present in the sample (infected), and 0 indicates that anisakid eggs were absent (uninfected). Color indicates the population from which the sample was collected. B) The weak relationship between egg counts of anisakids per gram, and Anisakis relative abundance in sequence read counts, determined through eDNA analysis. C) A heatmap of the relative abundance of each potentially pathogenic genus detected across all samples. High relative abundance indicates read counts for a given genus make up 50% or more of the total sample, while low relative abundance indicates the read counts made up less than 50% of the samples.

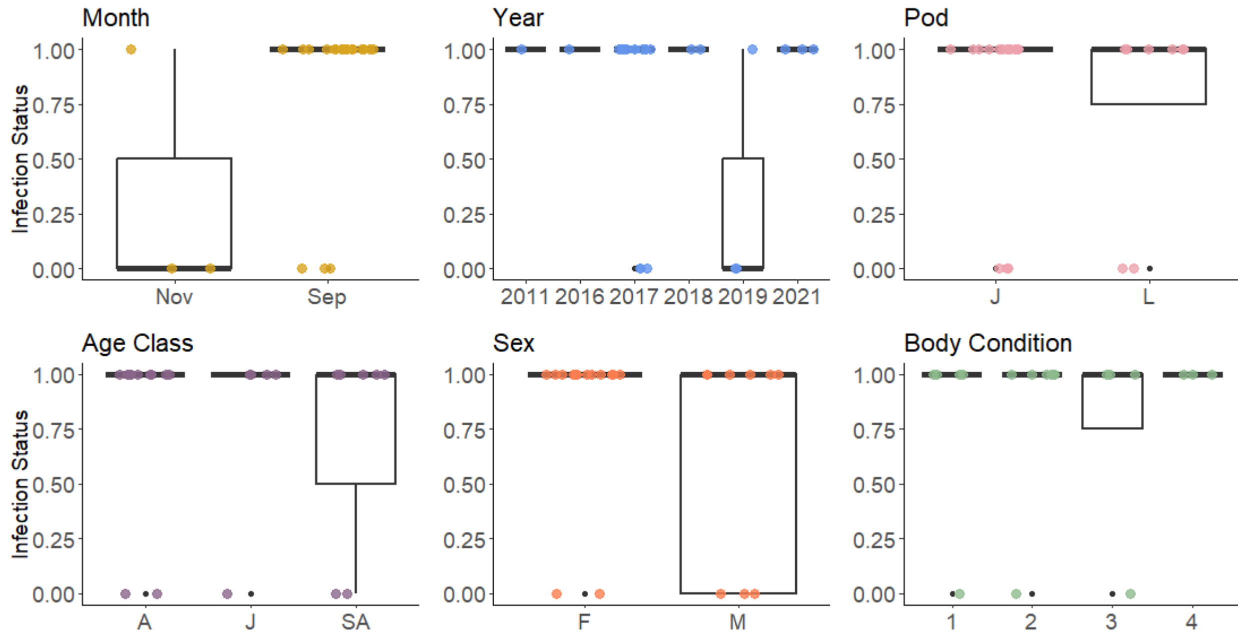


Figure 4.5: The relationship between anisakid infection status and temporal (year, month) and demographic (pod, age class, sex) factors, as well as body condition class. The only factor that had a marginally significant effect on parasite infection status was month. Pod indicates whether the sample was collected from a member of J or L pod (K was not well-represented), and age class indicates if the individual was a juvenile (J), subadult (SA), or adult (A).

APPENDIX CH. 4A:

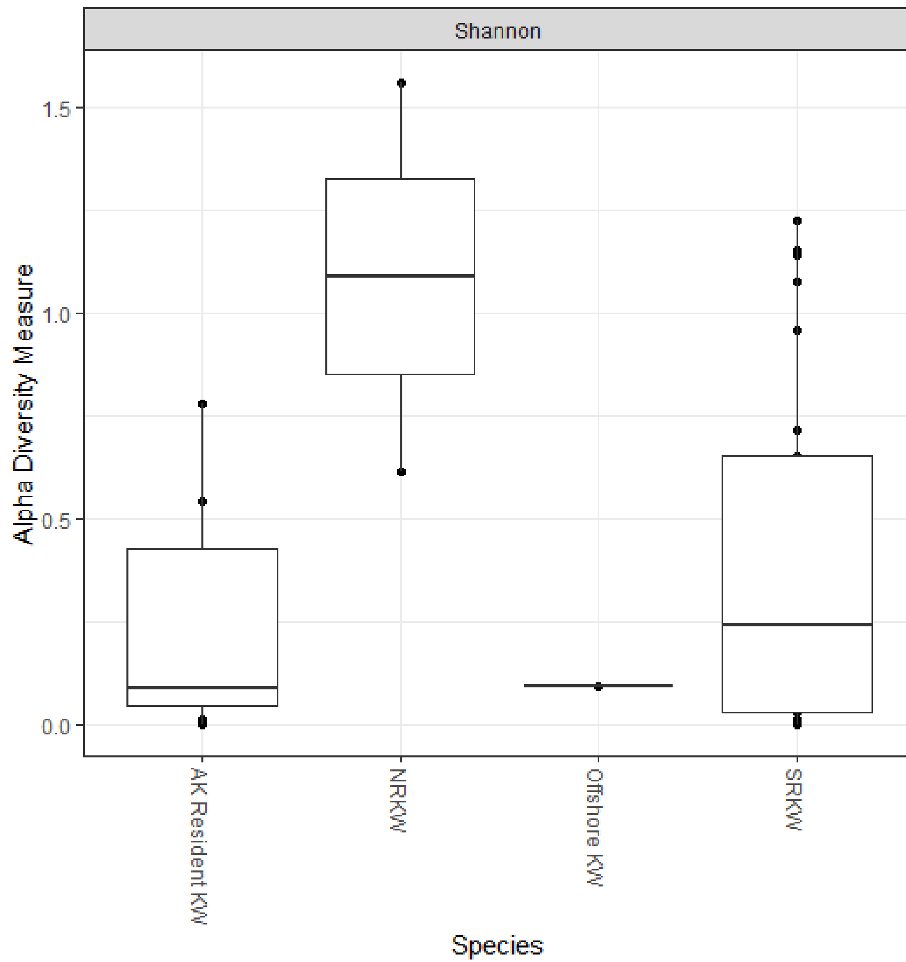
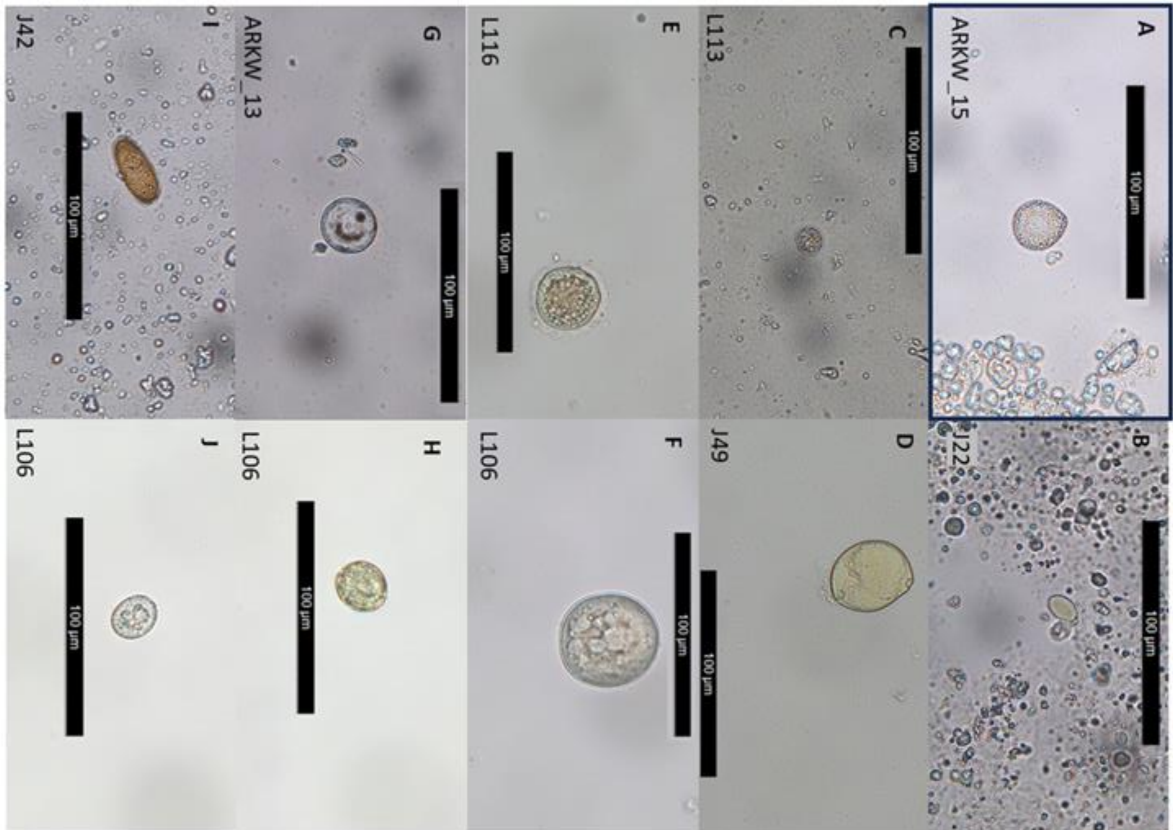


Figure S4.1: The Shannon Diversity measures of all sequences, grouped by population. The mean diversity was greatest in NRKW, though the sample size for this population was 2. SRKW showed the most variability (n = 25).



	Total eggs found in the population's samples			
Parasite	**ARKW**	**NRKW**	**Offshore**	**SRKW**
Unknown A	2	0	0	0
Unknown B	0	0	0	2
Unknown C	7	0	0	13
Unknown D	0	0	0	1
Unknown E	0	0	0	2
Unknown F	0	0	0	1
Unknown G	1	0	0	0
Unknown H	0	0	0	1
Unknown I	0	0	0	1
Unknown J	0	0	0	1

Figure S4.2: Unidentifiable parasites were found in fecal sedimentation and floatation in small numbers.

APPENDIX CH. 4B

Fecal floatation and sedimentation identification guide for Northeast Pacific killer whales

Nematodes

Anisakis sp.

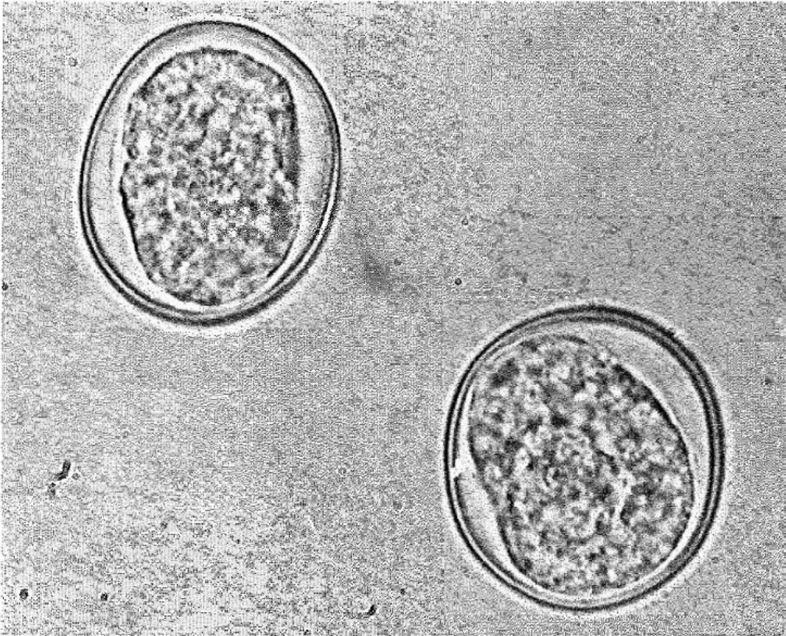


Figure 8.
***Anisakis* sp. ova**

- Photo from Dailey 2001.

Contracaecum sp.

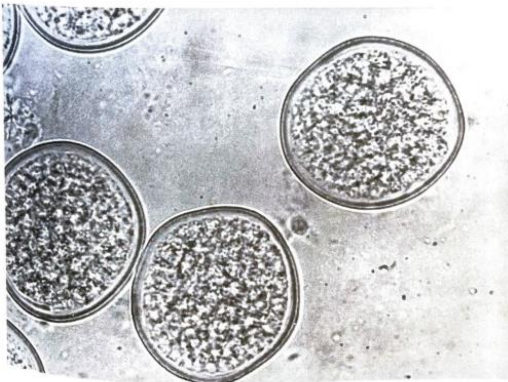


FIGURE 6 *Contracaecum* egg (original magnification ×400).

- Photo from Dailey 2001.

Contracaecum multipapillatum
- Egg has three layers, including a smooth or slightly rough outer layer.
- 53 × 43 µm,
- After the larvae had developed inside, egg size increased to 66 × 55 µm. (Valles-Vega et al. 2017)

Trematodes

Braunina cordiformis

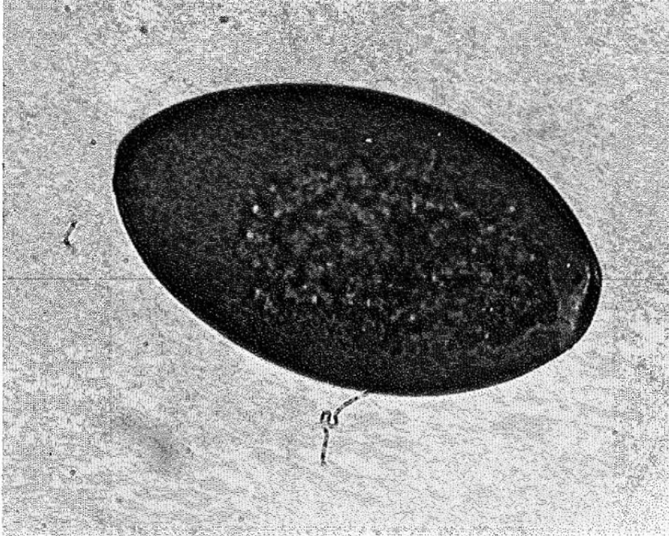


Figure 3.
Braunina cordiformis ovum

- Photo from Dailey et al. 1980

Campula sp.

- Eggs are triangular or sub-triangular in cross-section (Gibson 2005)

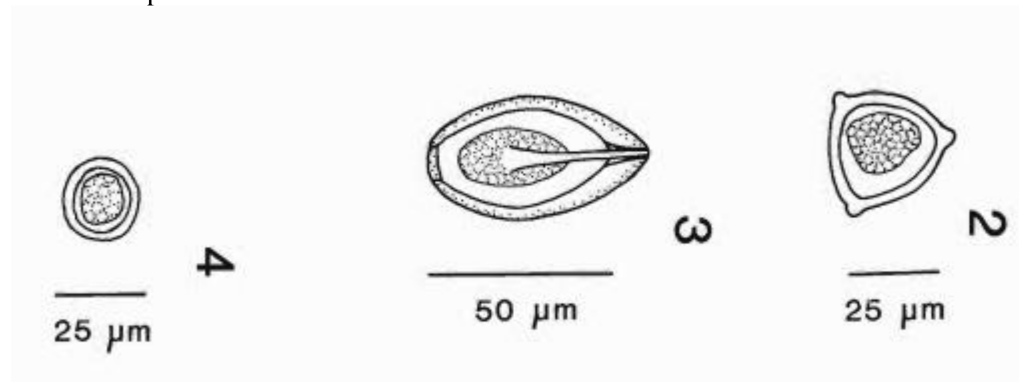
Campula palliata

- Eggs oval, with a noticeable cap at one pole and mammiform appendage at other pole (Delyamure 1955)
- Length of eggs 0.063-0.66mm, width 0.036-0.047 mm (Delyamure 1955)

Fasicola skrjabini

- Eggs oval, 0.120-0.127 mm long, 0.06-0.67 mm wide (Delyamure 1955)

Hadwenis sp.



- Egg illustrations from Raga et al. 1994.
- 2. Cross-section at abopercular pole
- 3. Lateral view
- 4. Cross-section at opercular pole

Odhneriella sp.

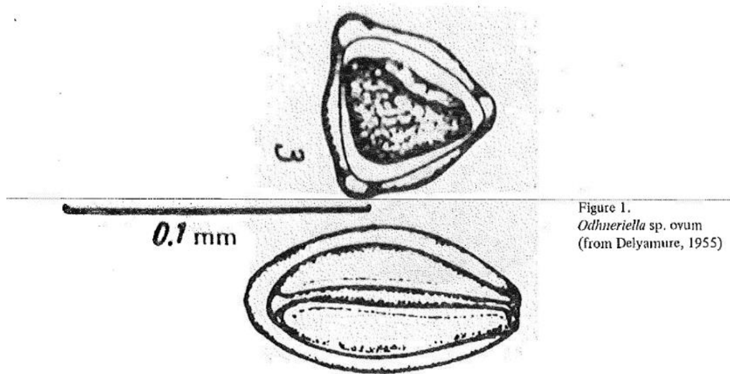


Figure 1.
Odhneriella sp. ovum
(from Delyamure, 1955)

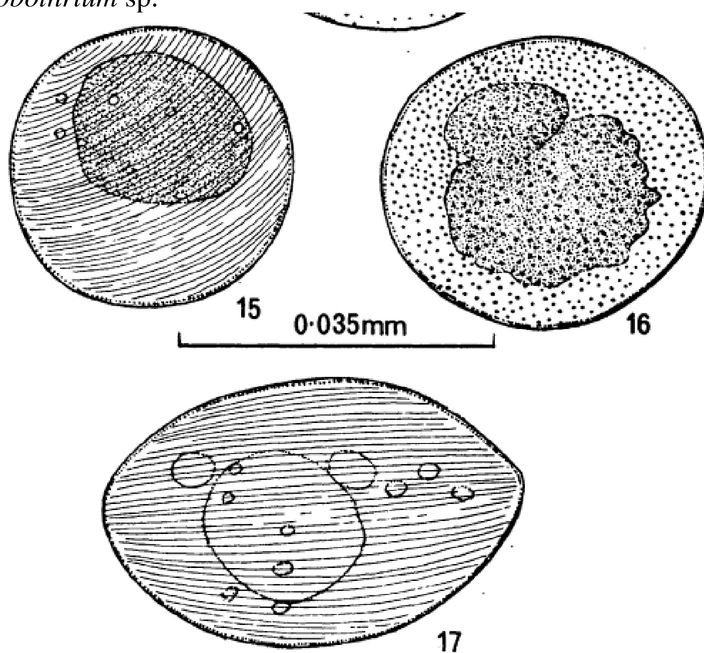
- Photo from Dailey et al. 1980.
- Eggs oval
- Truncate at opercular pole
- Triangular in cross section
- 75-98 x 44-55 µm (Balbuena et al. 1989)
- Thick light brown shell (Delyamure 1955)

Oschmarinella albamarina

- Eggs triangular in cross section (Demaree et al. 1997)
- Eggs range in size by species:
  - *O. macrochis* 80–98 x 45–55 µm
  - *O. sobolevi* 110–120 x 62–66 µm
  - *O. laevicaecum* 63–81 x 42–48 µm
  - *O. mascomai* 80–84 x 40–42 µm

Cestodes

Phyllobothrium sp.



- Image of *Phyllobothrium britannicum* from Williams 1968
- *P. radioductum*
    - Egg is thin-shelled
    - No operculum
    - Nearly spherical
    - Diameter ~ 35 µm (Williams 1968)
- *P. brittanicum*
    - Egg contains structures resembling oil globules (Williams 1968)

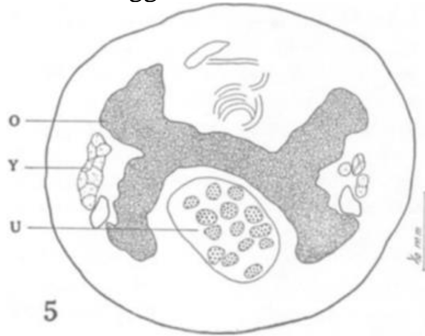


PLATE LXII

- *Phyllobothrium magnum* egg, from Hart 1936.
- *P. magnum*
    - Eggs spindle-shaped, 0.08 mm – 0.04 mm (Hart 1936)

Pyramicocephalus phocarum

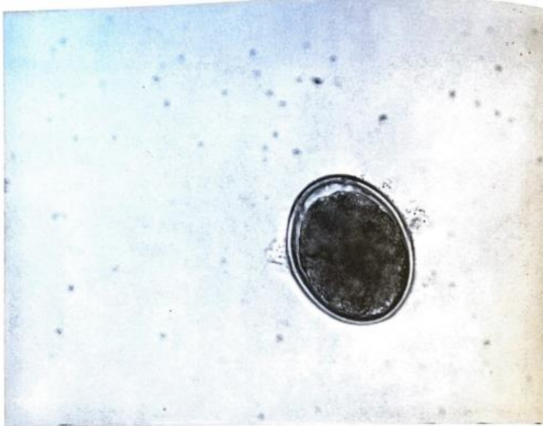


FIGURE 7 Tapeworm egg (*Diphyllobothrium* sp.) (original magnification ×400).

- Image from Dailey 2001
- Description (Hillard 1960):
    - Elongate eggs
    - Narrowly obovoid, greatest diameter near operculate end
    - Shell ~ 2.5 µm thick
    - Amber in color
    - Scrobiculate, with pits ~ 0.4 µm in diameter
    - Average diameter of operculum 19.5 µm
    - 38–43 x 58–68 µm
    - Sutures strongly incised
    - Apical knob much reduced or lacking
    - Coracidium situated centrally
        - Coracidia within shell averages 35 x 38 µm

Trigonocotyle spasskyi

- Eggs are 0.071 mm in diameter (Delyamure 1955)

Acanthocephalans

Corynosoma sp.

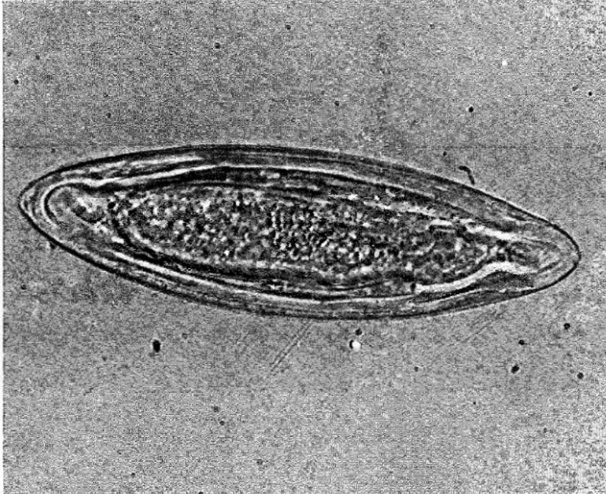
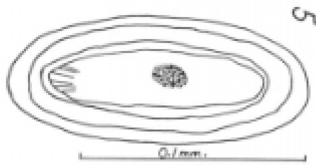


Figure 6.
Corynosoma sp. ovum

- Image from Dailey et al. 1980



- Image of *C. macrosomum* egg from Neiland 1962

Bolbosoma sp.

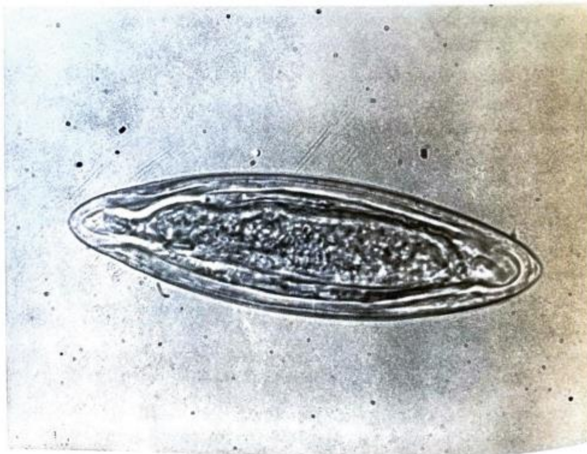


FIGURE 8 Acanthocephalan (*Bolbosoma* sp.) egg (original magnification ×400).

- Image from Dailey 2001

- Image of *Bolbosoma balaenae* from Santoro et al. 2021

Protozoa

Balantidium coli

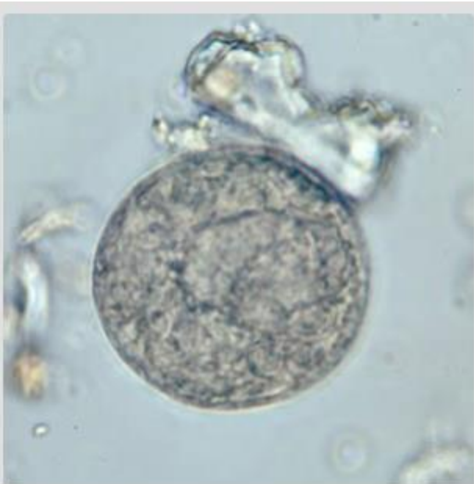


Figure A: *B. coli* cyst in a wet mount, unstained.

- Image from CDC website

APPENDIX CH. 4B REFERENCES

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