Parasites of the past: Tracking change in marine parasite abundance over time

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Abstract

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In recent years, disease ecologists have perceived a dramatic increase in infectious-disease-related mass mortality events among marine organisms. But does this increase reflect an actual rise in rates of infectious disease, or is it merely the artefact of improved detection and monitoring capacity? To help answer this question, I took two approaches: (1) I used meta-analysis to assess change in the abundance of two economically and ecologically important parasites over the past several decades and (2) I performed a manipulative experiment to test whether it might be possible to use liquid-preserved fish held in natural history collections to resurrect reliable information on parasites of the past. In Chapter 1, I harnessed the statistical power of the many published empirical studies that have measured the abundance of *Anisakis* spp. and *Pseudoterranova* spp. nematodes to reconstruct fifty years of change in the abundance

of these economically and ecologically important parasites, finding a 208-fold increase in *Anisakis* spp. and no change in *Pseudoterranova* spp. With this method, we can reconstruct change in disease for the past half-century, but we are constrained by the limited availability of electronic records for studies published prior to ~1970. To get back further in time, in Chapter 2, I performed an experiment to assess whether preservation biases parasite detectability in liquid-preserved fish held in natural history collections, finding only a minimal influence of museum preservation protocols on the ability to detect parasites. Because preservation has little influence on parasite detectability, parasitological dissection of such natural history specimens could provide a reliable estimate of the parasites that were present in a host at the time of its death. Thus, my work extends our ability to assess change in parasite abundance over time, arming disease ecologists with the tools needed to assess whether rates of infectious disease are indeed on the rise.

It's a wormy world: Meta-analysis reveals long-term change in the global abundance of parasitic anisakid nematodes in fishes and invertebrates

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Running head: Global rise of anisakids

Abstract

The Anthropocene has brought substantial change to ocean ecosystems, but whether this age will bring more or less marine disease is unknown. In recent years, the accelerating tempo of epizootic and zoonotic disease events has made it seem as if disease is on the rise. Is this apparent increase in disease due to increased observation and sampling effort, or to an actual rise in the abundance of parasites and pathogens? We examined the literature to track long-term change in the abundance of two parasitic nematode genera with zoonotic potential: *Anisakis* spp. and *Pseudoterranova* spp. These anisakid nematodes cause the disease anisakiasis and are transmitted to humans in undercooked and raw marine seafood. A total of 123 papers published between 1967 and 2017 met our criteria for inclusion, from which we extracted 755 host-parasite-location-year combinations. Of these, 69.7% concerned *Anisakis* spp. and 30.3% focused on *Pseudoterranova* spp. Meta-regression revealed a global increase in *Anisakis* spp. abundance (average number of worms / fish) over a 53-year period from 1962 to 2015 and no significant change in *Pseudoterranova* spp. abundance over a 37-year period from 1978 to 2015. Standardizing changes to the period of 1978 to 2015, so that results are comparable between genera, we detected a significant 208-fold increase in *Anisakis* spp. abundance and no change in abundance of *Pseudoterranova* spp. The global increase in *Anisakis* spp. abundance may have implications for human health, marine mammal health, and fisheries profitability.

Key words: Anisakiasis, anisakidosis, herring worm, cod worm, parasite burden, whales, dolphins, seals, fish, zoonoses

Introduction

Nematode parasites in the family Anisakidae are ubiquitous in marine fishes and can threaten human health when directly consumed (Deardorff et al 1991). The disease that they cause, anisakiasis, is considered an emerging zoonosis (Bao et al. 2017). Humans can contract anisakiasis from the consumption of raw, pickled, smoked, undercooked, or improperly frozen marine seafood that contains an anisakid nematode (Deardorff et al. 1991). Consumers often discover their infection status when they find live worms in their phlegm, mucus, vomit, or feces (Acha and Szyfres 2003; Figure 1a). In serious cases, symptoms include acute abdominal pain, nausea, vomiting, and diarrhea, which can persist for months (Bouree et al. 1995, Acha and Szyfres 2003). Misdiagnosis is common, as symptoms resemble those of many gastrointestinal ailments (Acha and Szyfres 2003, Shibata et al. 2014, Shrestha et al. 2014, Khan and Williams 2016). There are few epidemiological studies of anisakiasis in the United States; since it is not reportable at the county, state, or federal levels, its occurrence is not systematically tracked by local or state health departments or by the US Centers for Disease Control and Prevention (CDC). However, existing evidence suggests that anisakiasis prevalence may be significantly under-estimated (Schantz 1989, Nawa et al. 2005, Painter et al. 2013, Bao et al. 2017) and exposure may be substantial among individuals who consume raw fish (Garcia-Palacios et al. 1996, Uga et al. 1996, Bao et al. 2017). It is possible that the rising incidence of anisakiasis may be due to more accurate detection and diagnosis of anisakiasis (Lohi et al. 2007), increased consumption of raw or undercooked fish (Nawa et al. 2005), or an increased abundance of the parasites in the commercially fished hosts. We sought to assess whether the risk of anisakiasis to human consumers has increased over time due to increasing abundance of parasites in wild seafood.

To track change in parasite abundance over time when primary data are scarce, researchers can use meta-analysis of data from existing literature (Rudel 2008) to pool vast amounts of data. Meta-analysis demonstrated that the abundance of parasites in various host taxa had changed between 1970 and 2001 (Ward and Lafferty 2004; Wood et al. 2010); disease increased over time in turtles, corals, mammals, urchins, and mollusks (Ward and Lafferty 2004), while there was no significant change in the disease burden of decapods, sharks, and fish (Ward and Lafferty 2004; Wood et al. 2010). While Ward and Lafferty (2004; Wood et al. 2010) observed no overall change in rates of disease for fish, this average could mask increases or decreases for individual parasite species. For example, Howard et al. (2019) examined museum specimens of English sole (*Parophrys vetulus*) for the nematode parasite *Clavinema mariae* and found an eightfold increase in the parasite's abundance between 1930 and 2016. Therefore, a more narrow taxonomic perspective may be needed investigate change in the abundance of parasite species, especially for ecologically and economically important parasites.

Anisakid worms (Figure 1a) are globally distributed marine mammal parasites, reaching their adult life stage in the intestines of cetaceans and pinnipeds (Anderson 1992, McClelland 2002). Invertebrates (krill and copepods) serve as the first intermediate hosts, fish and cephalopods as the second intermediate and paratenic hosts, and marine mammals as the definitive hosts (Figure 1b; Mattiucci and Nascetti 2006). Anisakiasis occurs when humans consume raw or undercooked fish or invertebrates containing *Anisakis* spp. and *Pseudoterranova* spp. These two genera have similar life cycles and morphology, with the major distinction that *Anisakis* spp. infect cetaceans as their definitive hosts while *Pseudoterranova* spp. infect pinnipeds.

While anisakids have zoonotic potential, they can also cause disease in their intermediate and definitive hosts. In Atlantic salmon (*Salmo salar*), red vent syndrome – which causes fish vents to become red, swollen, hemorrhaging, and prolased – is attributed to *Anisakis simplex* infection (Beck et al 2008, Noguera et al 2009). While affected fish were in otherwise good condition, red vent syndrome can facilitate opportunistic secondary infection by pathogenic bacteria (Beck et al 2008). In their definitive hosts, anisakids are commonly found in necropsied marine mammals, some of which are listed are endangered (Gibson et al. 1998, Dailey and Stroud 1978). The presence of anisakids in marine mammals can cause gastric obstruction, ulceration, and, in some cases, death (Young and Lowe 1969, Geraci and St. Aubin 1987, Spraker et al. 2003). Anisakids are therefore a threat to both human and wildlife health.

Anisakids can also influence the profitability of fisheries. In 1987, German news broadcasters displayed a close-up shot of living anisakids nematodes emerging from a filet of fish, driving an immediate 80% drop in seafood sales and loss of fishery-based jobs in Germany (Karl 2008). This "nematode crisis" spurred industry and regulatory agencies to develop and implement more stringent controls to reduce the number of anisakids in seafood products (Karl 2008). It has been estimated that 'cod worm' (*Pseudoterranova* sp.) costs Canadian Atlantic fisheries at least $30 million annually (Malouf 1986), with some estimates upward of $50 million (Aryee and Poehlman 1991) to $80 million annually (Bonnell and Thota 1994). For Pacific cod (*Gadus macrocephalus*), it is estimated that monitoring and detection of anisakids constitutes up to 50% of the production cost (Choudhury and Bublitz 1992). In Spain, a majority of consumers are willing to pay more for fish that is guaranteed to be free of anisakids (Bao et al 2018).

Given the significant environmental change that global ocean ecosystems have experienced in the past century (Jackson et al. 2001; Lotze et al. 2006), there are several reasons

to predict that anisakid nematode abundance may have changed over this time period. As complex life-cycle parasites, anisakids can respond to changes in the abundance of any intermediate or definitive host (copepod, fish, or marine mammal; Arneberg et al. 1998). We do not know which host is the most important determinant of anisakid abundance (i.e., the worm's life cycle bottleneck; sensu Lafferty 2012), but we do know that several anisakid hosts have changed in abundance over the past half-century. Since the Marine Mammal Protection Act was adopted in 1972 (Magera et al. 2013), many marine mammal species have increased in abundance. This could lead to an increase in anisakid transmission, if definitive mammalian hosts are the life-cycle bottleneck (Lafferty 2012). On the other hand, as industrial fishing pressure has increased, fish host density may be on the decline (Christensen et al 2014), which could reduce transmission and abundance of the parasite. If fish hosts are not a transmission bottleneck (sensu Lafferty 2012) and fishing happens at a spatial scale commensurate with the scale of parasite transmission (Wood et al. 2013, Kuris and Lafferty 1992), a reduction in fish density could actually result in an increase in the per-fish burden of anisakid parasites, as worms become more concentrated in the remaining fish hosts. Long-term climate change could also influence the abundance of anisakid nematodes. Increases in temperature are thought to increase host susceptibility to disease and to increase pathogenicity and virulence of pathogens and parasites (Harvell et al 2002, Burge et al 2014). This could lead to increases in infection of all hosts, as these species lose the ability to fight off infection. Increased temperature is also known to decrease the hatching ability and survival of *Anisakis* spp. larvae (Hojgaard 1995, Hojgaard 1999). Thus, warming temperatures could also lead to decreased infection in fish and invertebrates as the life cycle is disrupted due to low survival of eggs and larvae. As land cover, agriculture, and logging practices change and expand, nutrients can be released into coastal

ecosystems (Cuo et al. 2009). These increases in nutrient export to coastal ecosystems can then fuel phytoplankton blooms that in turn fuel increases in abundances of copepods and other filter-feeding crustaceans, such as euphausiids (Trainer et al., 2003), and thus potentially increase abundances of anisakid nematodes if the crustacean first intermediate hosts are the life-cycle bottleneck.

We sought to assess change in global anisakid nematode abundance over the past ~50 years (since the 1970s) using meta-analytic techniques. Although no study before ours has tested for temporal change in anisakid abundance at a global scale, thousands of studies have empirically quantified the abundance of anisakids in fish and invertebrate hosts at specific times and locations across the world. We assimilated these studies into our meta-analysis, harnessing the statistical power of many studies conducted in many regions across many host species. We specifically focused on fish and invertebrate intermediate hosts, as they have been systematically assessed for parasites by many prior studies and link to human anisakiasis risk, impacts of anisakids on marine mammal health, and implications of anisakids for fisheries profitability.

Methods

Data collection

To compile papers estimating anisakid abundance, we conducted a search in ISI Web of Science. We used the search string: *TS = anisak* or "herring worm" or "herringworm" or "herring-worm" or pseudoterranova or whaleworm or "whale worm" or phocanema or "whale-worm"*, on October 10th, 2017, which yielded 2,284 papers. We then used a systematic screening process to eliminate non-relevant papers (Figure 2a). We first screened titles for suitability, excluding papers that quantified anisakids in humans, birds, or marine mammals and titles that clearly

indicated that the paper concerned a different suite of parasite species. After title screening, we retained 1,336 papers for the next stages of the screening process. We screened abstracts to further focus the dataset, excluding publications that examined anisakids in humans, birds, or marine mammals, that performed experimental manipulation of parasites or hosts, or that were reviews. This process resulted in 576 retained papers which were read in full to determine their eligibility for inclusion in the study. To qualify for final inclusion, papers were required to contain information on host species identity, parasite species identity, location of host collection (either as a named place or latitude and longitude), collection year or year range, size of the host, how the hosts were examined for anisakids, and either prevalence and intensity of infection with an error estimate or abundance with an estimate of error. Ultimately, we extracted data from 123 papers, resulting in 755 data points (i.e., unique estimates of parasite abundance for a host species at a particular location at a particular time). Of these 755 data points, 69.7% described *Anisakis* spp. nematodes (Figure 2b) and 30.3% described *Pseudoterranova* spp. nematodes (Figure 2c). For each data point, we extracted information on host species identity, collection location, year of collection, portion of the host examined (i.e., all, viscera, musculature), host examination method (i.e., standard visual assessment, candling, UV light, acid digestion), parasite genus (*Anisakis* spp. or *Pseudoterranova* spp.), parasite prevalence (proportion of hosts infected), parasite intensity (average number of parasites per infected host), error associated with parasite intensity (i.e., standard deviation, range, standard error, or confidence intervals), parasite abundance (average number of parasites per host for all hosts), and error associated with abundance (i.e., standard deviation, range, standard error, or confidence intervals).

Data standardization

We standardized data prior to analysis. Length was reported in various ways (i.e., standard length, total length, fork length). We used the standard linear conversion equation,

$$Length_{standard} = a + b*Length_{reported}$$

to convert reported length measurements to standard length. We obtained the *a* and *b* parameters for each fish species from FishBase (Froese and Pauly 2019), using the average *a* and *b* if multiple values of *a* and *b* were provided by FishBase.

In our study, parasites were quantified by their abundance (i.e., number of parasites per host, including uninfected hosts; Bush et al. 1997). When parasite abundance was not reported, but parasite intensity and prevalence were, we calculated parasite abundance by multiplying intensity and prevalence and propagating the error through in quadrature (the square root of the sum of squares). If the range of intensity or abundance was provided but the standard deviation was not, we estimated standard deviation by optimizing the negative binomial distribution for the dispersion parameter. We assumed that the maximum abundance or intensity was the 95th quantile of the negative binomial distribution, since parasites often follow a negative binomial distribution (Shaw et al 1998). With this assumption, we then used the supplied mean intensity or abundance as the mean of the negative binomial distribution with the dispersion parameter unknown. We then used an optimization algorithm to estimate the dispersion parameter that best fit the supplied mean and 95th percentile. Then, with the given mean and estimated dispersion parameter, we were able to calculate the error of the mean given that the variance of a negative binomial distribution is the sum of the mean and the squared mean divided by the dispersion parameter. We also converted other forms of error reported in the studies (i.e., standard error,

confidence intervals) back to standard deviation. To convert from standard error to standard deviation, we multiplied the standard error by the square root of the sample size. To convert from a confidence interval, we took the difference from the upper bound of the confidence interval and the mean and then divided the difference by the appropriate z-score and multiplied by the square root of the sample size. For location information, we grouped data points based on major FAO fishing region (FAO) using ESRI ArcGIS (ESRI 2011), to match our data to FAO major fishing region.

Data analysis

To assess change over time in anisakid abundance, we developed two parallel meta-analytic regression models for the two genera of anisakid nematodes contained in our dataset (*Anisakis* and *Psuedoterranova*). For each model, we fourth-root transformed the abundance and standard deviation of the abundance to meet normality assumptions of the model. In our data, we had observations of anisakid abundance whose associated error was zero. This arose if only a single host was examined, if reported anisakid abundance was zero, or if anisakid abundance was greater than zero, but all fish sampled had the same anisakid burden. Since meta-regression uses the inverse of variance to weight each observation, we corrected the variance to account for observed zero error. We chose to add 1 to every variance to prevent over-weighting (i.e., adding a small value like 0.000001 to every zero-error observation will over-weight these observations). Adding a very small variance correction would give many orders of magnitude more weight to observations with zero variance compared to any observation that had greater than 1 variance. By adding 1 to variance, we prevent this issue and observations with zero error get full weight and all other observations receive progressively less weight. We included year of collection and

host length as moderators in the meta-regression model. We also included host species, portion of the host examined nested in host species, FAO major fishing area (to account for geographic clustering of points), method for detecting parasites, and paper ID as random effects using the *rma.mv* function of the package *metafor* (Viechtbauer 2010).

We were also interested in testing which fish species drove the patterns observed in the above meta-regression, so we sequentially excluded each host species and reran the model. We extracted an updated estimate of change over time and compared the estimate of the effect of time to the model with all host species. This allowed us to determine whether a single host species was driving the patterns we observed.

Results

We obtained 755 unique host-parasite-location-year combinations (data points) across 53 years and four oceans. A total of 56,778 fish were examined across all studies, resulting in enumeration of 446,615 anisakid nematodes. In our meta-regression models, we detected an increase through time (estimate = 0.0178, SE = 0.0069 , z = 2.5784, n =526 , p = 0.0099) in abundance of *Anisakis* spp. (Figure 3) and no change (estimate = –0.0019, SE = 0.0093, z = –0.2062, n =229 , p = 0.8366) in abundance of *Pseudoterranova* spp. (Figure 4).

For *Anisakis spp.,* we detected a 208-fold increase in abundance across the standardized time period of 1978 to 2015. Change in abundance over this time frame is measurable for both anisakid genera; thus, constraining our effect estimates to this period allows for fair comparison of results between anisakid genera. In terms of length-corrected modeled abundance, we observed an increase from an average of 0.0031 (95% CI: .000156, 0.202) *Anisakis* spp. worms per fish to 0.64 (95% CI: 0.083, 2.46) *Anisakis* spp. worms per fish over the entire time period

for which we have data of 1962–2015. We also tested for the effects of excluding the temporally outlying record from 1962 to assess its leverage in our model. When excluding the record from 1962, the effect of time is 0.0193 with a standard error of 0.0071; when the record from 1962 is included, the effect of time is 0.0178 with a standard error of 0.0069. When examining the results of sequentially removing single host species from our model, we found that there was no loss of significance of the effect of time when any one host was excluded, and the effect of time was always positive (Supplementary Figure S2). Despite not changing the overall temporal patterns, the host species that leveraged the largest absolute change to the effect size was Arctic cod (*Arctogadus glacialis*).

For *Pseudoterranova spp.,* we detected no change in abundance between 1978 and 2015. When examining the effects of individual host species on the temporal stability of *Pseudoterranova* spp. abundance, we found that excluding any host does not result in a significant effect of time either direction and there is always no significant change over time (Supplementary Figure S2).

Discussion

From our analysis of 123 manuscripts and 755 data points summarizing 56,778 fish and 446,615 anisakid nematodes, we conclude that there has been a long-term increase in the global abundance of *Anisakis* spp. nematodes and no long-term change in the global abundance of *Pseudoterranova* spp. nematodes. Our study is correlational and precludes conclusions regarding causation, but we do have several hypotheses for drivers that might explain the global increase in *Anisakis* spp.

Our analysis indicates that the abundance of *Anisakis* spp. has increased dramatically in recent decades. On average, we estimated that anisakid abundance increased from about 1 worm per every 100 hosts at the beginning of our time series to 2.08 worm per every 1 host at its end. This pattern is common across all host species, as no single host species exerted enough influence on the model to change the relationship between *Anisakis* spp. abundance and time. The host that had the largest influence on driving the temporal increase in *Anisakis* abundance was Arctic cod (*Arctogadus glacialis*), but this influence was not so great that it drove the temporal pattern observed across all hosts; when Arctic cod was excluded from the anlaysis, the temporal increase in *Anisakis* spp. abundance remained robust.

In contrast, *Pseudoterranova* spp. has not experienced increases in abundance over time. Because it is ecologically similar to *Anisakis* spp. and subject to many of the same long-term environmental changes (e.g., increases in definitive host abundance, climate change), we expected similar patterns between the two genera. Since we found no significant change in *Pseudoterranova* spp., this could indicate that either this genus is largely unaffected by the changes to ocean ecosystems over the past few decades, or that the factors determining abundance are responding to global ocean change antagonistically, such that any gain in abundance (e.g., from increases in pinniped populations), is counteracted by a negative effect on abundance (e.g., decreases in larval parasite survival with increasing temperatures). Furthermore, excluding different host species did not alter the estimates of the change over time. This could indicate that there are not host-specific patterns in relation to host abundance changes (discussed further below).

Anisakids are marine mammal parasites, and over the course of our study period marine mammals have increased in abundance in response to formal legal protections. Since 1972,

marine mammals have been protected in the US under the Marine Mammal Protection Act and many countries adhere to the moratorium on commercial whaling imposed by the International Whaling Commission in 1982. This protection has allowed for the recovery of many marine mammals populations (Magera et al. 2013). An increase in abundance of mammals may explain the increase in *Anisakis* abundance, although if marine mammals were the most important driver of this long-term increase, we would have expected a stronger increase in *Pseudoterranova* in response to the robust increase in pinnipeds compared to cetaceans, which host *Anisakis* spp. (Magera et al. 2013). Marine-mammal-driven increases in anisakids have been observed at small spatial scales; for example, fish collected near seal haul-out sites have been found to harbor a greater number of anisakid worms than fish collected near non-haul-out sites (Jensen and Idås 1992). As many marine mammal populations recover their former abundance, the increased number of definitive hosts may support larger parasite populations. While this may be perceived as a recent increase in abundance, our data cover only the period of time since the enforcement of the Marine Mammal Protection Act in 1972, and thus the increase could be a return to historic population levels present before declines in marine mammal abundance due to human exploitation.

Fish and large pelagic invertebrates (e.g., squid and other cephalopods) are key intermediate and paratenic hosts for anisakids, and their abundance has changed in complex ways over the past half-century (Christensen et al 2014). Fishing pressure has altered the abundance and density of many fishes (Anderson et al. 2008). For example, Atlantic cod have experienced declines on the northeastern coast of North America (Lilly et al. 2008), cephalopods have experienced increases in abundance (Doubleday et al 2016), forage fish have experienced fluctuations and shifts in dominant species (Chavez et al 2003), and there has been mixed

responses of large fish and invertebrates (Christensen et al 2014, McClenachan 2009). If fish and squid represent a life-cycle bottleneck for *Anisakis* spp. (sensu Lafferty 2012), then we would expect declines in their abundance to reduce anisakid transmission. However, if they are not a life-cycle bottleneck, decreases in intermediate host abundance could lead to a concentration of parasites in the remaining hosts, thus resulting in an increase in parasite abundance (i.e., number or parasites per fish host).

Increasing temperature could also determine whether anisakid populations are temporally stability or changing over time. We hypothesized that increasing temperature could have mixed effects due to changes in host performance (Burge et al 2014) or declines in egg hatching and larval survival (Hojgaard 1995). In the case of *Anisakis* spp., we found increases over time, which could be due to a decline in the ability of fish and invertebrate hosts to immunologically or behaviorally resist infection when temperatures increase beyond fish or invertebrate host optima or due to decreases in the maturation time and increasing growth rates of *Anisakis* spp. nematodes. Increasing temperatures often lead to faster growth and shorter generation times in aquatic parasites (Marcogliese 2001). Increases in nutrient loads to coastal ecosystems can fuel blooms of phytoplankton that in turn fuel filter-feeding crustacean populations (Trainer et al. 2003). If the crustacean hosts that harbor *Anisakis* spp. nematodes are more sensitive to these changes compared to the crustacean hosts that harbor *Pseudoterranova* spp. nematodes, increases in nutrients could facilitate increases in *Anisakis* spp. without driving change in *Pseudoterranova* spp.

Whatever the drivers of the global increase in anisakid abundance, such an increase could have substantial economic, ecological, and human health impacts on a global scale. First, increases in anisakid abundance could increase the risk of anisakiasis in people (Bao et al. 2017).

Increased anisakid abundance in commercially exploited fish and invertebrates increases the likelihood that the resulting seafood meal contains a worm. While the symptoms of anisakiasis are rarely life-threatening, increased incidence of anisakiasis could increase hospitalizations, representing a burden on public health (Bouree et al 1995). It could also alter public perceptions of seafood safety (Karl 2008), reducing the market value of a vast number of commercially fished species (Bao et al. 2018).

Increases in anisakid abundance can also affect marine mammal and fish health. In marine mammals, anisakids divert energy away from the host that would otherwise be used for survival, growth, and reproduction (Dailey and Stroud 1978). Some have suggested that intestinal parasites (including anisakids) may be hampering recovery and even causing mortality of endangered populations of killer whales (Krahn et al. 2002). Anisakid infections have been empirically linked to a mass mortality of sea otters in Cordova, Alaska, where necropsies revealed fatally heavy anisakid burdens (Ballachey et al 2002). Anisakids also affect their fish hosts; they can reduce swimming efficiency of fish when encysted in the musculature (Buchmann and Mehrdana 2016). Slower swimming speeds reduce survival by increasing the likelihood that the fish will be consumed a predator (Persson 1991). Anisakids may therefore be exerting an increasing burden on wildlife health.

Our study is limited in its temporal scope by the available literature. ISI Web of Science is a commonly used database of peer-reviewed publications, although the amount of literature accessible is heavily weighted to the past fifty years (Supplementary Figure S2). This limits meta-analyses to near history (~1960s to present), by which time the oceans had already been drastically altered by human activity (Lotze et al. 2006). This limitation prevents meta-analyses from characterizing ocean ecosystems as they were before they were impacted by fishing,

pollution, and climate change. This lack of an appropriate "baseline" should be acknowledged alongside the results presented here. While we did detect an increase in anisakid nematodes over the past 50 years, does this increase represent a rise in infection, or a recovery of anisakids to some pre-impact baseline? In other words, are anisakid abundances increasing in response to human impacts on the environment (e.g., fishing, pollution, climate change), or are they recovering alongside their exploited marine mammal hosts? Our data cannot discriminate among these possibilities, but parasitological dissection of natural history collections (Harmon et al. 2019, Howard et al. 2018), could reveal trajectories of anisakid changes over the past century or more.

Although disease ecologists suspect a long-term increase in the frequency and severity of these outbreaks, few sources of data exist to test this hypothesis. The meta-analysis we conducted yielded temporally resolved, long-term data on the abundance of two ecologically and economically important parasites, and revealed a long-term increase in the abundance of *Anisakis* spp. However, this is the story of only two parasite species and we encourage others to use historical ecology approaches (e.g., meta-analysis, parasitological dissection of museum specimens) to track change across a diversity of marine parasite species. Only then will we have the data to indicate whether contemporary oceans are facing a "rising tide" of marine disease.

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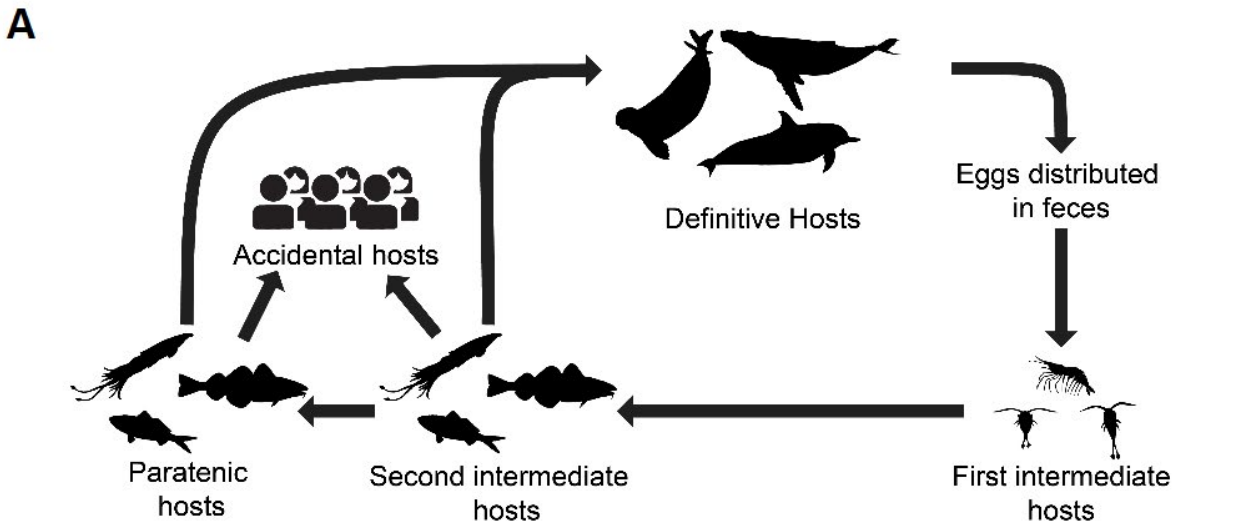
Figures



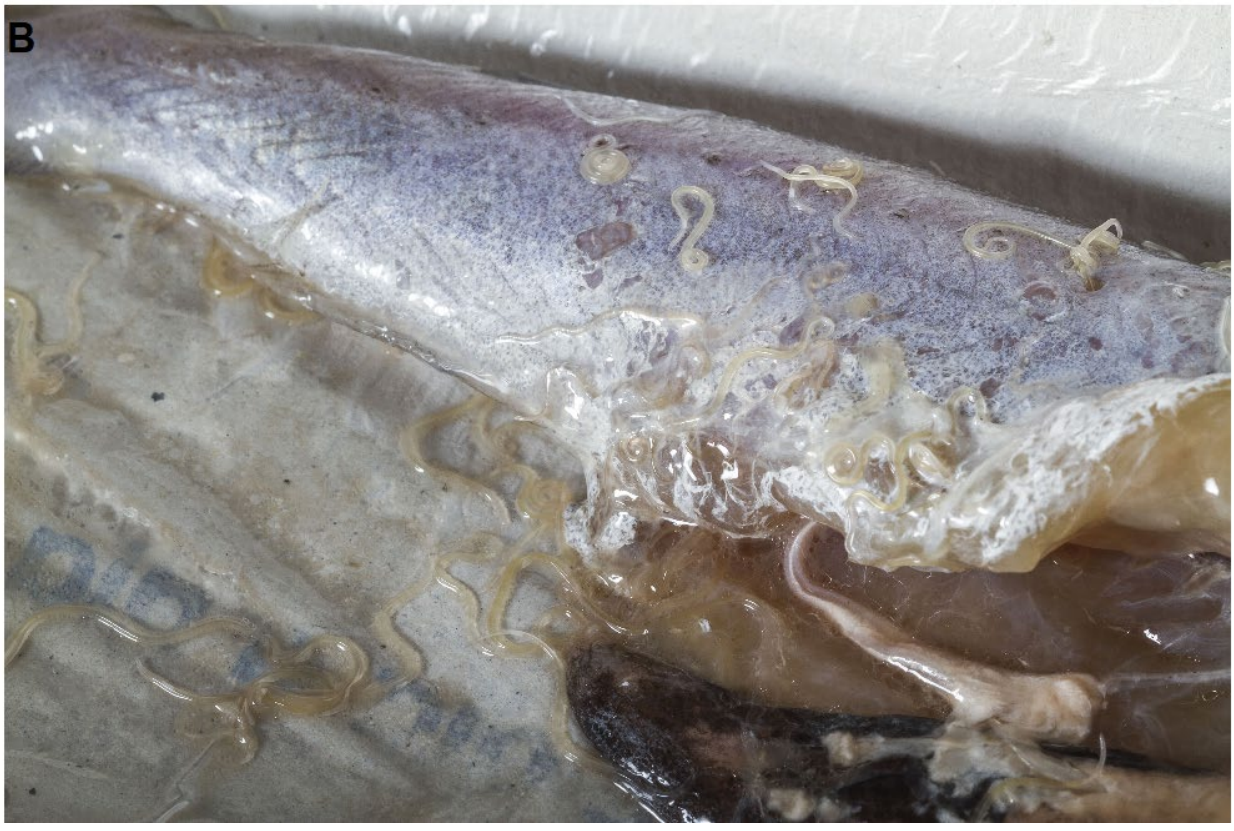


Figure 1. A: Generalized life cycle of an anisakid nematode. Anisakid nematodes begin their life as eggs deposited in the feces of marine mammals, which hatch into juveniles that are consumed by and infect planktonic invertebrates such as krill or copepods. Infected invertebrates are then consumed by fish or cephalopods which become infected with anisakids. Here, if infected fish or cephalopods are consumed by larger fish or cephalopods, the anisakids can re-encyst in these

paratenic hosts to work their way up the food web, eventually reaching their definitive hosts, marine mammals. Once a marine mammal consumes an infected fish or cephalopod, the anisakid matures in the stomach and begins to reproduce and release eggs with the mammal's feces. Attribution for host vector images Kim Kraeer, Lucy Van Essen-Fishman, Tracey Saxby, Joanna Woerner, Dieter Tracey, Jane Hawkey, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/). B: Anisakid nematodes in blue whiting (*Micromesistius poutassou*). Image by Gonzalo Jara and reproduced with permission via Shutterstock (stock photo ID 656259196).

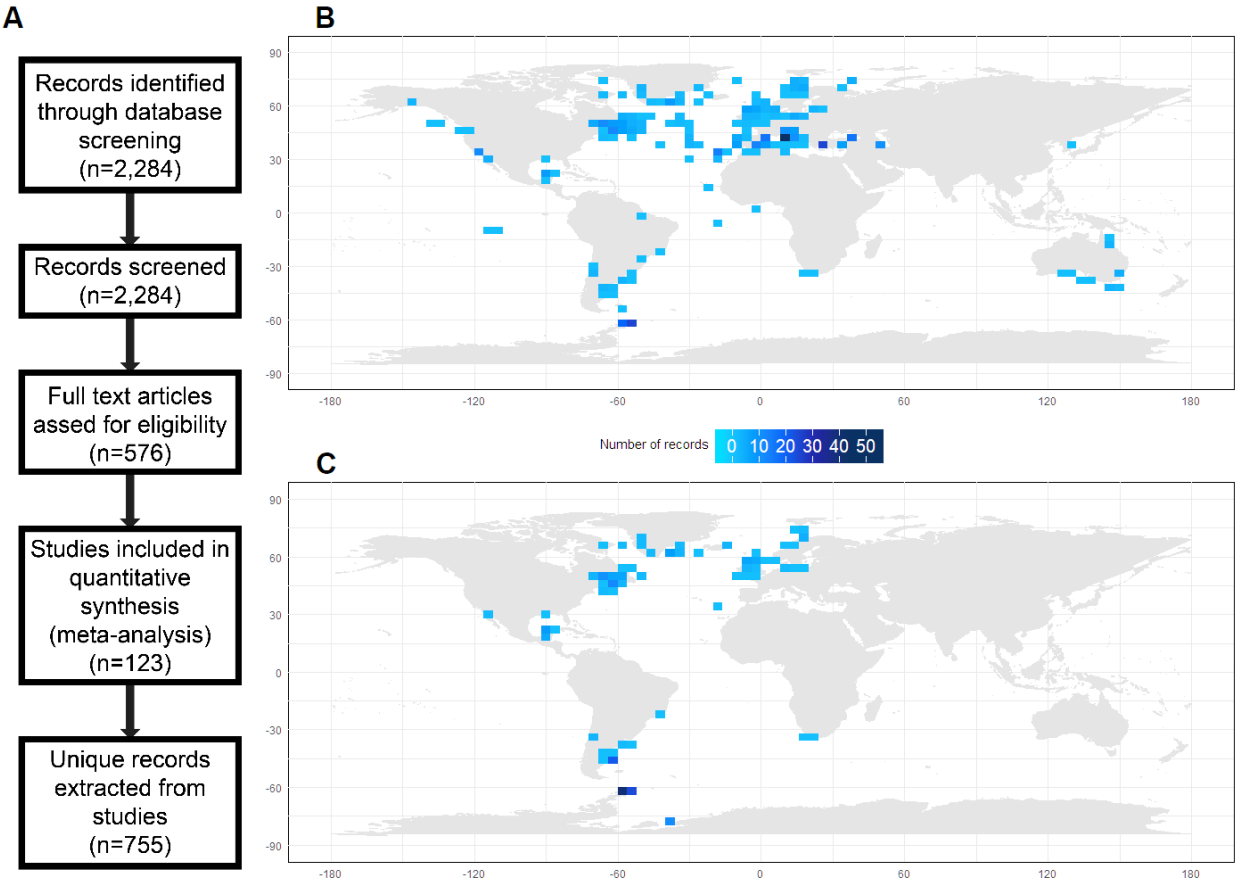


Figure 2. A: Flow diagram for our process of screening papers. This represent the screening process from the initial Web of Science search to the usable full-text article after examination of titles and abstracts. For all full-text articles, we assessed eligibility for inclusion and returned 123 articles. From these 123 articles, we were able to extract 755 unique records of anisakid abundance. B: Geographic density of unique estimates of *Anisakis* spp. abundance from our meta-analytic dataset. C: Geographic density of unique estimates of *Pseudoterranova* spp. abundance from our meta-analytic dataset.

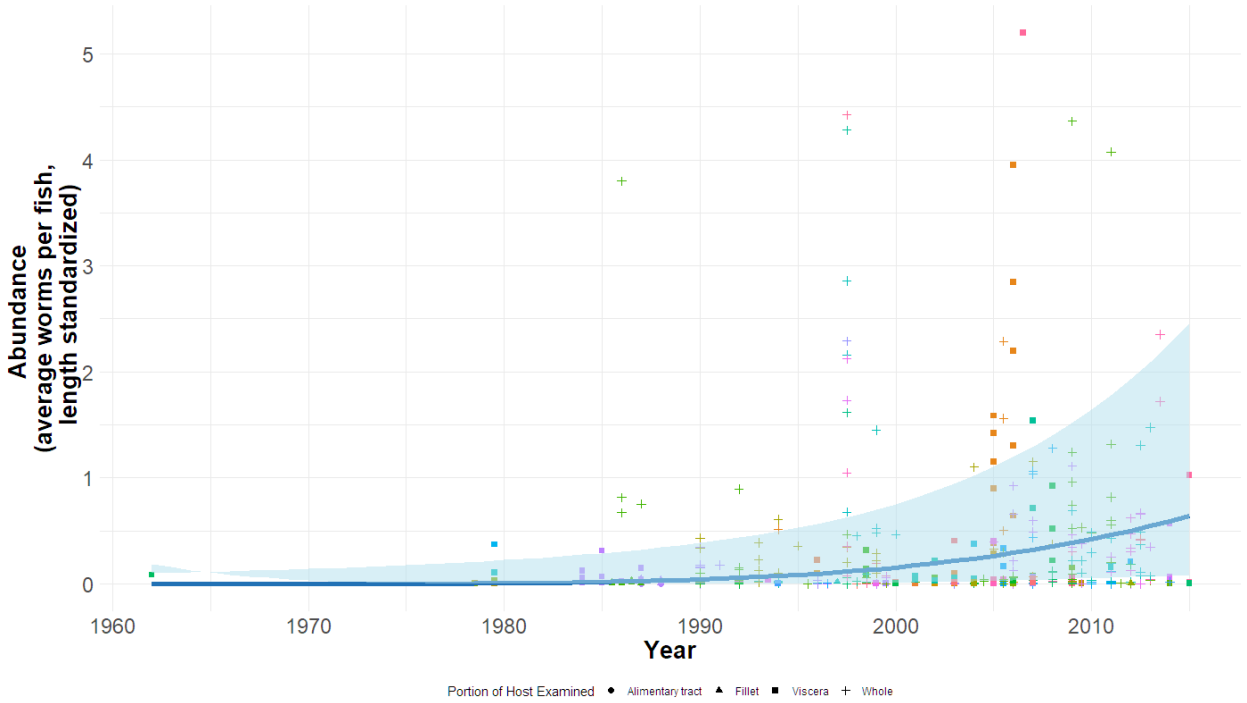


Figure 3. Change over time in *Anisakis* spp. abundance with 95% confidence interval. Points represent extracted, length-corrected mean abundances. Shapes indicates which portion of the hosts were examined and color indicates host species (n = 185).

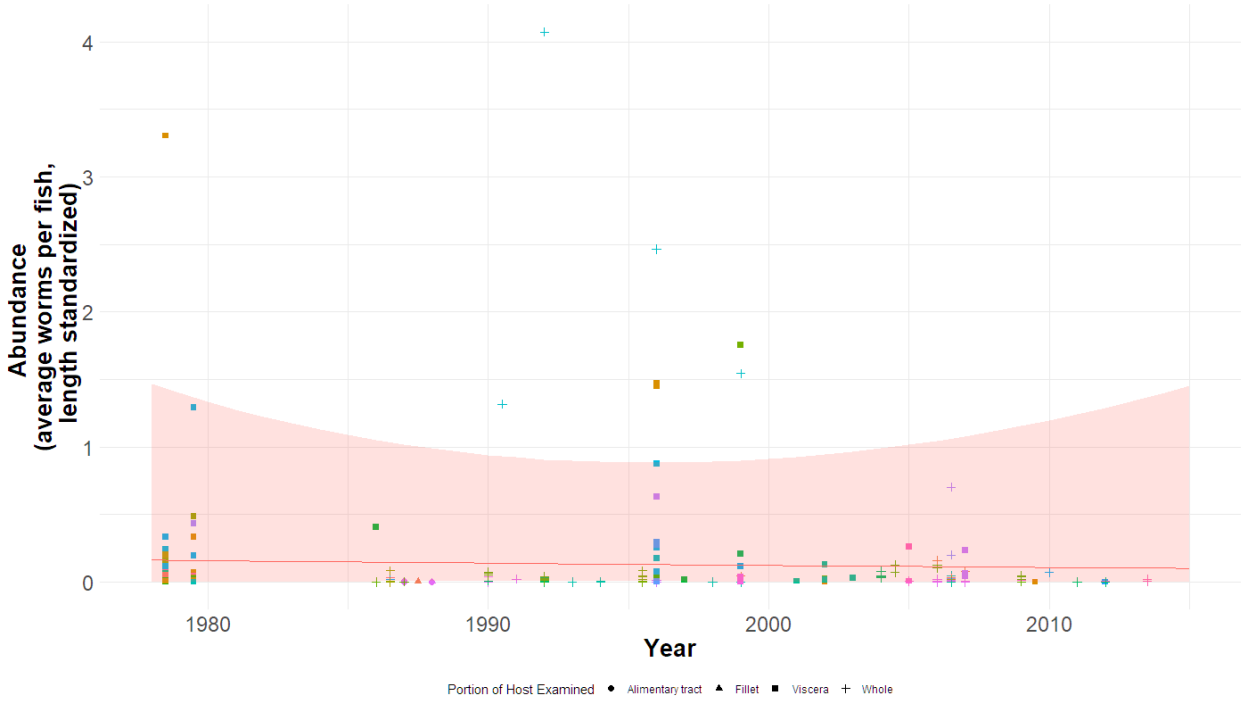
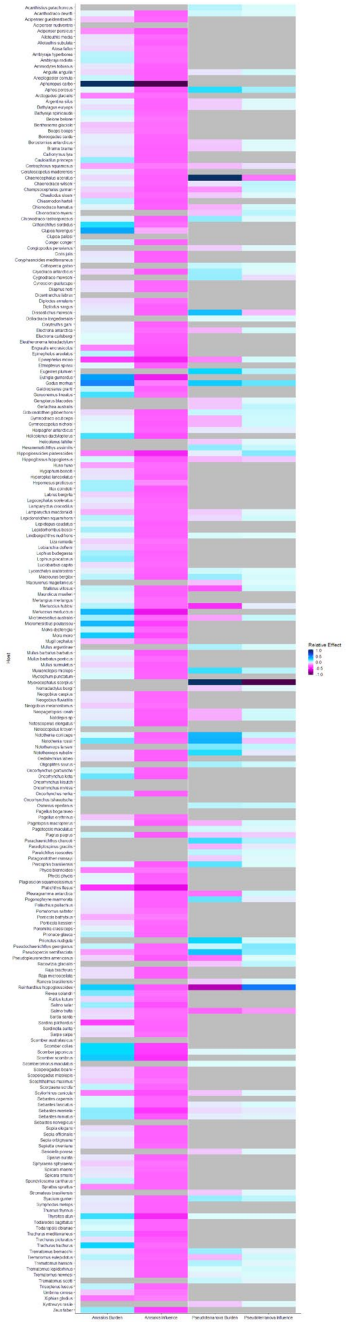
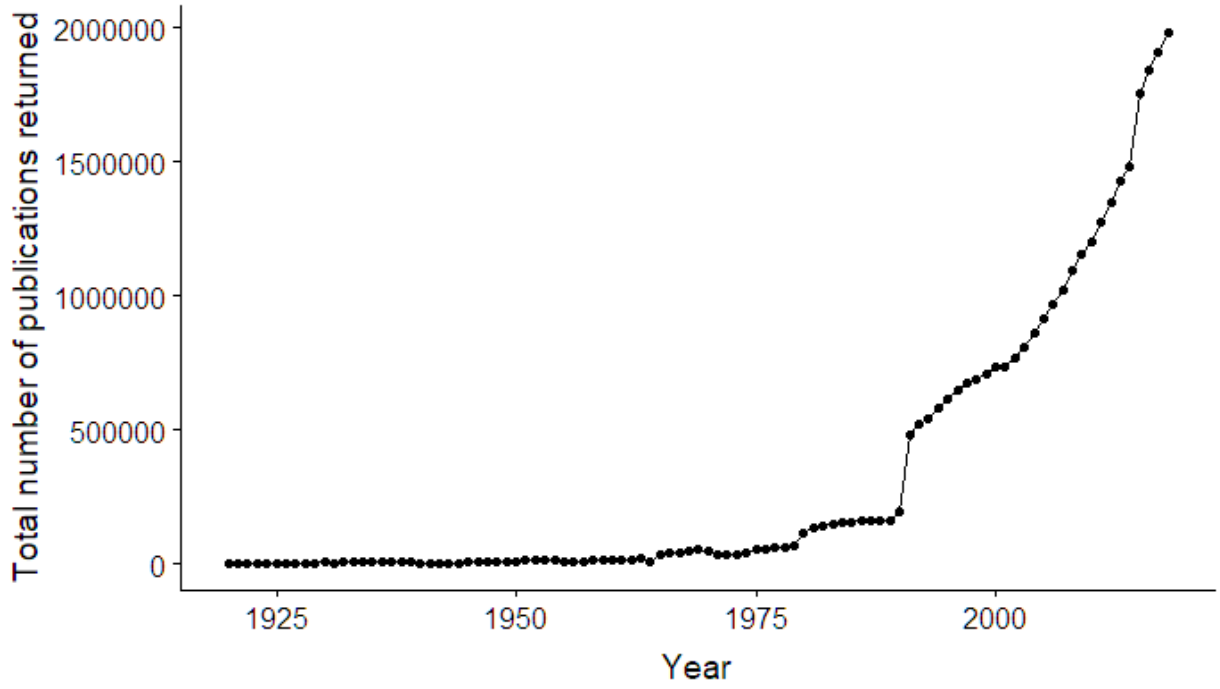


Figure 4. Change over time in *Pseudoterranova* spp. abundance with 95% confidence interval for all hosts. Shapes indicates which portion of the hosts were examined and color indicates host species (n = 93).



Supplementary Figure S1. Host species names, random effects estimates, and influence on the temporal patterns for both *Anisakis* spp. and *Pseudoterranova* spp. Burden represents the extracted random effects estimates from our models and provides an indication of which host species have higher or lower anisakid abundance compared to average. Influence represents the relative effect of excluding the host species on the estimate of temporal change. Positive values indicate that excluding the host decreased the estimate of the temporal effect, showing that the effect of time is greater when the host is included, while negative values indicate that when the host is excluded, the estimate of the effect over time was increased, and thus the host dampens

the effect over time when included in the model. Gray boxes represent hosts that were not contained in our data for that anisakid genus.



Supplementary Figure S2. Publications returned for the search string 'a' in ISI Web of Science over time. The jump in 1991 is potentially attributable to the invention and spread of the internet.

Liquid preservation causes minimal reduction of parasite detectability in fish specimens: A new approach for reconstructing parasite communities of the past

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Running head: Preserved fish for parasites

Key words: Historical ecology, marine fish parasites, natural history, parasite abundance, museum collections,

Abstract

1. Long-term datasets are a prerequisite for evaluating temporal patterns in wildlife disease burdens. Unfortunately, historical data on parasite abundance are extremely rare. But for more than a century, natural history collections have been accumulating liquid-preserved specimens, which should contain most of the parasites infecting the host at the time of preservation. By performing parasitological dissection of these specimens, it is possible to reconstruct long-term change in parasite abundance. Before this unique data source can be exploited, we must identify the biases that are introduced by the preservation process. Here, we experimentally address whether the preservation process alters the detectability of metazoan parasites in liquid-preserved fish specimens.

2. We randomly assigned fish of three species (Alaska pollock *Gadus chalcogrammus,* eulachon *Thaleichthys pacificus,* and English sole *Parophrys vetulus*) to two treatments. In the first treatment, fish were frozen and we performed parasitological dissection after they were thawed. In the second treatment, fish were frozen and, after thawing, we preserved according to standard natural history collection procedures. After the liquid-preservation process was complete, we performed parasitological dissection on those specimens. We compared parasite abundance and diversity between the two treatments.

3. Across 298 fish and 59 host–parasite pairs, we found few differences between treatments. Although our experiment had sufficient statistical power to detect moderate differences between treatments, we found that 24 of 27 host–parasite pairs were equally abundant in the two

treatments. Of these, one pair was significantly more abundant in the preservation treatment than in the control treatment, and two pairs were significantly less abundant in the preservation treatment than in the control treatment.

4. Our data suggest that the liquid preservation process does not have a substantial effect on the detectability of metazoan parasites. Natural history collections contain a wealth of hidden information on parasite diversity and abundance. With this technique, ecologists can reconstruct novel, long-term datasets on parasite diversity and abundance over the past century.

Introduction

Contemporary observations suggest that rates of wildlife disease have recently increased in frequency and magnitude (Harvell et al. 1999; Harvell et al. 2002). Past decades have seen outbreaks of infectious disease among marine organisms resulting in die-offs of endangered black abalone in California's Channel Islands (Moore et al. 2000), sea urchins in the Caribbean (Lessios et al. 1984), and pilchards in Australia (Whittington et al. 1997). But one important question about these disease outbreaks remains unanswered: how unusual are they? Although ecologists have access to many long-term datasets, parasites tend not to be included among these historical data (Harmon et al. 2019). Because we lack long-term datasets on parasite abundance, we cannot evaluate whether recent increases in infections represent departures from historical patterns, or business as usual (Harmon et al. 2019).

One approach to reconstructing timelines of parasite abundance is to use meta-analysis (e.g., Ward & Lafferty, 2004; Wood, Lafferty, & Micheli, 2010, Fiorenza et al. in prep). However, this approach has its limitations. Ward and Lafferty (2004) used the rate at which disease was reported in the literature to quantify temporal trends in disease, finding increases in disease for turtles, corals, marine mammals, urchins, mollusks, and fish between 1970 to 2001. However, a later re-analysis found that the reported positive trend for fish was driven by an artifact, and found instead that rates of fish disease had not changed over time (Wood, Lafferty, & Micheli 2010), demonstrating that conclusions drawn by meta-analysis can be susceptible to literature bias and to improper inclusion or exclusion of studies. In another meta-analysis of the literature, Fiorenza et al. (in prep) demonstrate a global increase in *Anisakis* spp. nematode abundance across the globe from 1962 to 2015. Despite its power for resolving decadal-scale change in parasite abundance, meta-analysis is constrained to the time period represented in

searchable online databases, typically the 1960s forward (Fiorenza et al in prep, Supplemental Figure S2). By the 1960s, the ocean had already undergone substantial anthropogenic change, including warming, increasing fishing pressure, species invasions, and reductions in pH (Stachowitz et al. 2002, Takahashi et al. 2009, Last et al. 2010). However, there is a way to quantify parasites across longer time scales.

Natural history collections contain specimens that can be used to extract information about historical parasite diversity and abundance over the past century or more (Figure 1) (Harmon, Littlewood, & Wood, 2019). Howard et al (2019) examined blood worm *Clavinema mariae* abundance in English sole *Parophrys vetulus* over 84 years by counting the *C. mariae* present in liquid-preserved fish specimens from the University of Washington Ichthyology Collection (Howard, Davis, Lippert, Quinn, & Wood, 2018). Others have used natural history collections to assess whether a parasite of an invasive cane toad in Australia was present in native species before the introduction of the cane toad in 1935 (Hartigan, Phalen, & Šlapeta, 2010) and to determine whether amphibian limb abnormalities were caused by trematode metacercaria in the past, as they are in contemporary amphibians (Johnson, Lunde, Zelmer, & Werner, 2003). However, in these cases of using natural history collections to examine parasites, they were targeting particular parasites and not reconstructing parasite communities.

Although there is substantial promise in the use of natural history collections as sources of information on parasites of the past, this approach remains to be validated (Swetnam, Allen, & Betancourt 1999). Validation of new historical ecology approaches can be accomplished by comparison of the novel data source with established sources of information when and where they overlap, use of multiple lines of evidence, or mathematical models that test whether the results given by the novel approach are biologically and ecologically possible (McClenachan &

Cooper, 2008; Mcclenachan, Ferretti, & Baum, 2012; McClenachan, Cooper, McKenzie, & Drew, 2015). Howard et al. (2019) provided qualitative validation for the prevalence of *C. mariae* using a historical dataset contemporaneous with specimens from the natural history collections. They found that results from the two approaches (i.e., parasitological data from natural history collections versus historical datasets) both demonstrated increases in parasite abundance over time. But Howard et al. (2019) were unable to perform more formal validation (e.g., matching natural history collection samples to historical data points and comparing the absolute abundance of parasites in the two) due to lack of natural history specimens from the appropriate times and location. Natural history collections can fill a large gap in the information available about historical rates of parasitism (Figure 1), but we must first determine whether the parasitological information stored in these collections is accurate.

Here, we address this research gap with an experimental study testing how the liquid preservation process used by natural history museums affects the detectability of metazoan parasites in preserved specimens. More broadly, we assessed whether parasitological dissection of natural history specimens might be used to reconstruct long-term changes in parasite communities. Many natural history collections use formalin fixation followed by long-term storage in 70% ethanol, which could affect parasite detectability and lead to loss of ectoparasites (Kvach et al 2018). The liquid-preservation process dehydrates the specimen and alters the coloration and physical properties of the host's tissues (Waller and Eschmeyer 1965, Gaston et al 2013), changes that could make finding parasites in the tissues more difficult; color differences between parasite and host tissue could be eliminated, and the fixation process might bind parasites more firmly to the host tissues in which they are embedded.

Kvach et al (2018) examined the use of different field preservation techniques (live, frozen, ethanol storage, formalin preservation) on the detectabilities of parasites in two freshwater fish hosts (*Perca fluviatilis* and *Rhodeus amerus*). They found an overall decrease in the abundance and diversity of parasites from fish due to preservation. The authors detected 27 parasite taxa, including protozoa, myxozoa, monogenea, cestode, digenea, acanthocephala, nematoda, copepoda, and branchiura. Across the 27 parasite taxa, there were declines in abundance of 2 taxa after freezing, 7 taxa after formalin preservation, and 9 taxa after ethanol preservation, when compared to freshly sacrificed fish. Our study differs from Kvach et al. (2018) in that we address the impact on parasite detectability of the preservation protocol used by natural history collections, which involves fixation in 10% buffered formalin followed by storage in 70% ethanol. This will allow for the identification of preservation-driven biases in natural history collections and will address whether natural history collections are potential sources of information on historical parasite communities.

To understand how the preservation process affects the detectability of metazoan parasites, we recreated the preservation process for a variety of fish species and compared parasite abundances in experimentally preserved fish to control fish. We hypothesized that there would be a loss of parasite detectability due to the preservation process. Specifically, we expected that mobile ectoparasites would be dislodged and lost through the preservation process as fish are moved and handled, but that permanently anchored ectoparasites would persist through the preservation process. We expected that endoparasites would generally be preserved with the host tissues, with the degree of degradation in detectability depending on the taxonomic group to which the parasite belongs. For example, nematodes, with their hard cuticles, would be likely to persist through the preservation process. Adult trematodes, adult cestodes, and adult

acanthocephalans might be harder to identify after fixation, due to degradation of structures used for taxonomic diagnosis and loss of translucency. Trematode metacercariae and encysted cestodes might be harder to detect after fixation, as metacercaria and cysts are often embedded in host tissues and fixation could make host tissues harder to squash and pass light through host tissues to observe cysts.

We tested these hypotheses by performing parasitological dissection of fish specimens in two treatments: one in which we experimentally re-created the liquid preservation process used by natural history collections, and a control. For any parasite taxon where there was no significant difference between the two treatments, natural history collections might represent an accurate and useful source of information about parasite abundance in the past.

Methods

What constitutes a long-term dataset in the disease ecology literature?

First, we were interested in understanding the extent to which long-term datasets are available in the literature, with the aim of identifying temporal gaps that could be filled by parasitological dissection of fish specimens held in natural history collections. To determine the average length of a long-term dataset on the abundance of fish parasites, we performed a meta-analysis. We searched ISI Web of Science for articles on long-term studies of fish parasites in the natural environment. We used the search string *TS = ((long-term OR long term) AND parasite AND fish)* on May 17th 2019, which returned 262 potential articles. We then screened the titles and abstracts of these articles for relevance to our question. To be included, each study: (1) had to contain annual or nearly annual (i.e., >50% of temporal period had observations) observations of

parasite abundance or prevalence in fish (i.e., a study comparing two observations of parasite abundance 40 years apart is not eligible), (2) was not experimental, (3) did not use natural history collections, and (4) included the phrase “long term” in the title or abstract. This winnowing yielded 23 articles. From each of these articles, we extracted the length of the study in years to calculate summary statistics.

Study species

We used three species of marine fish for our experiment. Two species, Alaska pollock *Gadus chalcogrammus* and eulachon *Thaleichthys pacificus*, were provided to us by the UWFC. These fish were collected by National Oceanic and Atmospheric Administration research cruises in Alaska and stored frozen by the UWFC. On 11 and 12 May 2018, we collected English sole *Parophrys vetulus* by bottom trawl in Port Madison, WA, in conjunction with the Fisheries Ecology course offered by the School of Aquatic and Fisheries Sciences at the University of Washington. These three species of fish represent a variety of trophic strategies (suspension feeder, benthic predator, and pelagic predator, respectively), life history (i.e., short lived [eulachon] and long lived [English sole and Alaska pollock]), habitats (benthic [English sole] and pelagic [Alaska pollock and eulachon]), as well as body plans (i.e., flat fish [English sole] and fusiform [Alaska pollock and eulachon]). Fish traits might influence the parasites that the fish can be infected with or the effects of preservative on fish tissues, and thus having a diversity of fish traits produces more robust conclusions regarding the effects of preservation on parasite detectability. In total, we dissected 99 English sole, 109 Alaska pollock, and 90 eulachon.

Experimental test of the effect of preservation on parasite detectability

To assess whether and how the preservation process affects the detectability of parasites, we took an experimental approach. We obtained approximately 100 individuals of three fish species (English Sole *Parophrys vetulus*, Alaska pollock *Gadus chalcogrammus*, and eulachon *Thaleichthys pacificus*; see *Study species,* above, for collection details). For each species, individuals were randomly assigned to one of two treatments using a stratified design. Prior to randomly assigning fish to a treatment, fish were visually paired according to length within each species of fish. Then, an individual from each pair was randomly assigned to the preservation treatment by a coin toss. This stratified random design equalized host size, a potential driver of parasite abundance, between treatments, and ensured that the mean, median, and range of sizes were similar between the two treatments. In the first treatment, frozen fish were thawed and dissected, and their parasites identified. In the second treatment, frozen fish were thawed and preserved according to methods used at the University of Washington's Fish Collection (UWFC, see *Preservation protocol,* below). After preservation was complete, fish were dissected, and their parasites identified. To avoid confounding the effects of preservation and dissection method, we used a consistent dissection protocol across both treatments. This could result in lower overall detectability (across both treatments) for parasites found in muscle, since candling was used to avoid excessive destruction of the musculature of specimens (Levsen, Lunestad, & Berland, 2005). Methods for all other tissues follow standard parasitological techniques (see *Dissection methods*, below). We compared parasite abundance between the two treatments to assess the effects of preservation on detectability for each parasite taxon detected.

Power analysis

We sought to ensure that we had sufficient statistical power to detect even small differences between treatments, so that if no effect of treatment was found, we could confidently conclude that there were no differences between the treatments (i.e., so that we could rule out the possibility that the experiment was insufficiently powered to detect differences between treatments). To determine the minimum sufficient sample size, we conducted a power analysis using simulated data. In the simulation, we generated data based on a negative binomial distribution – a distribution that has previously been suggested as an accurate representation of most parasite populations (Shaw, Grenfel, & Dobson 1998) – for the two treatments. We varied the sample size per treatment and the difference between means (and therefore variance, since variance is based on the mean in a negative binomial distribution) to determine the level of power needed to detect differences between treatments. We assumed that a small effect size would = 0.2, a moderate effect size would = 0.5, and a large effects size would = 0.8 (Cohen 1969). Using the simulated data, we then created and ran generalized linear models using a negative binomial error distribution. The first model included the effect of treatment while the second model was a null model. After running the two models, we compared them using Akaike information criterion corrected for small sample sizes (AICc) and recorded whether the treatment model was the better model (greater than 2 AICc units lower than the null model) for each simulation. We repeated the simulation 1,000 times for each sample size and difference between means and used the proportion of times the treatment model was supported by AIC to calculate power for each combination of sample size, mean, and difference between means.

Preservation protocol

We followed the preservation protocol used by the UWFC, by first placing completely thawed fish in 10% buffered formalin solution until the fish is fixed (absolute amount of time is variable due to variation in fish body size). A fish was considered to be fixed when its tissues had become firm but retain some pliability. Over-fixation and decalcification are possible if fish are left in formalin beyond the time required to fix all the tissues. To prevent this from happening, fish were monitored during the preservation process at least every three days. Once the fish were fully fixed, they underwent two consecutive freshwater rinses, each lasting 24 hours. After this, the fish were placed directly in 70% ethanol, where they remained for at least three days prior to dissection. Across the entire preservation process, care was taken to avoid favoring preserving parasites that could be dislodged during the preservation process, to mimic actual preservation conditions in collections.

Dissection methods

For each specimen, we made a ventral incision from the anus to the gill isthmus. We then removed all viscera for examination. The viscera were separated by organ and individually squashed between two glass plates and examined for parasites. Gills from the right or blind side of the fish were removed, placed in a vial with artificial seawater or 70% ethanol, depending on treatment, shaken to free parasites from the gills, and examined under a dissecting microscope. External surfaces and the buccal cavity of the fish were also examined for ectoparasites. The specimen was then spread open along its ventral incision and placed over a strong light to examine the flesh and skin for parasites. All examinations were conducted under a dissecting microscope to best capture the entire parasite burden and not simply the large-bodied parasite

taxa. This method was used for both treatments of fish to avoid confounding the effects of how parasite dissections were performed with the treatments.

Comparing host body size between treatments

To ensure that there were no systematic differences between treatments in fish length, we used linear mixed-effects models (LMMs) to compare standard lengths, using experimental treatment as a fixed effect and location of collection as a random effect for each of the three species.

Comparing abundance of each parasite taxon between treatments

To test for differences in mean abundance of each parasite taxon between treatments, we used generalized linear mixed-effects models (GLMMs) for each host–parasite pair with an overall prevalence greater than five percent. In each model, we included standard length of the host and treatment as fixed effects. Collection tow was included as a random effect for English sole only. Models used a negative binomial error structure with a log-link and were implemented in glmmADMB (Fournier et al., 2012; Skaug, Fournier, Bolker, Magnusson, & Nielsen, 2016). The resulting p-values were Bonferroni-corrected to control for false positives.

Comparing empirical distributions of each parasite taxon between treatments

We then tested for differences in distributions of length-corrected parasite burdens between the treatments using Kolmogorov-Smirnov two-sample tests for each host–parasite pair. This non-

parametric test allows for the comparison of the empirical cumulative distribution functions and tests for the greatest difference between the cumulative distribution functions.

Comparing abundance of each parasite group between treatments

We were not only interested in whether there were significant differences between treatments in the abundance of individual parasite taxa; we also wanted to test whether there were tendencies toward over- or under-representation in the preservation treatment across all parasite taxa, across all parasite taxa within higher-order taxonomic groupings of parasites, and across all parasite taxa within life stage (i.e., adult versus larva). That is, we sought to pool replication across parasite taxa to rigorously test whether there were consistent detectability differences between the preservation and control treatments across groups of parasites that share characteristics in common. We used a meta-regression approach to assess the responses of parasites to treatment across parasite taxa. For effect size estimates, we used regression coefficients for the effect of treatment on abundance of each parasite in each host, extracted from the GLMMs described above. All analyses were implemented in *metafor* (Viechtbauer, 2010; after Wood, Sandin, Zgliczynski, Guerra, & Micheli, 2014). We first calculated a cumulative effect size across all host–parasite combinations, using a fixed-effects model weighted by the inverse of the variance for each effect size. We tested our hypotheses about how different parasitic taxa and life stages would respond to preservation with several meta-analytic fixed-effects general linear models, by including the moderators; parasite taxonomic grouping (Acanthocephala, Cestoda, Nematoda, Hirundea, and Trematoda), life stage (larval and adult), and the interaction of the two.

Comparing detectability of parasites across fish organs

Not only were we interested in how fixation influenced parasite detectability across parasite taxa and life stages; we were also interested how this differed across host tissues. To test this, we used a meta-regression approach where we extracted the effect size and error estimates from our individual parasite taxon GLMMs outlined above. In our meta-regression model, we correlated the effect sizes to the organs where parasites were found (i.e., body cavity, buccal cavity, heart, gills, stomach, intestine, pyloric ceca, fins, muscle, liver, kidney, gonads, and eyes), based on a presence/absence basis. This allowed us to determine whether the influence of fixation on parasite detectability was modulated by which host tissue in which a parasite was found.

Results

What constitutes a long-term dataset in the disease ecology literature?

We found that published fish parasites studies that are considered "long-term" have an average length of 12.5 ± 9.5 years, with a minimum of three years, a median of 10 years, and a maximum of 41 years.

Experimental test of the effect of preservation on parasite detectability

We dissected 298 fish of three species and found 15,246 parasites. These parasites occurred in 59 host–parasite pairs (21 parasite morphotypes in English sole, 24 in pollock, and 14 in eulachon). Of these 59 host–parasite pairs, 27 pairs had prevalence greater than 5% and were therefore

included in analyses. These 27 pairs included adult and larval trematodes, adult and larval cestodes, adult acanthocephalans, adult and larval nematodes, and adult leeches.

Power analysis

We found that a sample size of 50 fish per treatment (100 fish total) would give us the power to detect an effect size of 0.5 at least 95% of the time, which is equivalent to a difference in abundance of 1.25 parasites per host between treatments (Figure 2).

Comparing host body size between treatments

There were no significant differences between treatments in host standard length (English sole: $t = –0.751$, df = 92, $p = 0.455$; eulachon: $t = –0.911$, df = 77, $p = 0.366$; Alaska pollock: $t = –1.306$, df = 104, $p = 0.194$).

Comparing abundance of each parasite taxon between treatments

Across the 27 host–parasite pairs, we found that 24 of the pairs showed no statistically significant difference in abundance between treatments. We also detected three host–parasite pairs that were significantly different in mean abundance between treatments after Bonferroni correction. Of these, one parasite taxon was more abundant in the preservation treatment compared to the control treatment (*Cucullanus* sp.) and two were less abundant in the preservation treatment compared to the control treatment (*Pseudoterranova* sp. and Hemiuridean sp. trematode; Figure 3).

Comparing empirical distributions of each parasite taxon between treatments

Of the 59 host–parasite pairs, we detected no differences in empirical cumulative distributions of length-corrected abundance for 57 pairs. The two pairs that did differ in their empirical distributions were *Cucullanus* sp. in English sole and *Pseudoterranova* sp. in eulachon. *Cucullanus* sp. showed a greater number of high-abundance hosts in the preservation treatment, which matches the increased mean abundance revealed in the generalized linear mixed model. *Pseudoterranova* sp. also matched the results of the generalized linear mixed model with a greater number of higher abundance hosts in the frozen treatment compared to the preservation treatment.

Comparing abundance of each parasite group between treatments

Using the GLMM model estimates (from *Comparing abundance of each parasite taxon between treatments*, above), we ran a set of meta-regression models to determine whether there were consistent effects of preservation on detectability across all parasites, among parasite life stages (larval or adult), and among taxonomic group (Trematoda, Cestoda, Acanthocephala, Hirudinea, Nematoda). We found that there were no consistent differences in detectability between treatments across all parasitic taxa, nor across life stage or taxonomic group. We did find that adult Cestoda tended to have enhanced detectability in preserved fish (Figure 4).

Comparing detectability of parasites across fish organs

Using the GLMM model estimates (from *Comparing abundance of each parasite taxon between treatments*, above), we ran a set of meta-regression models to determine whether there were consistent effects of preservation on detectability among different host tissues. We found no consistent differences in detectability between treatments in the body, fins, gills, stomach, intestine, pyloric cecum, muscle, eyes, kidney, liver, gonads, and buccal cavity. We did find that parasite detectability was lower in the preservation treatment relative to the control for parasites detected in the heart (Figure 5).

Discussion

Natural history collections may contain a wealth of information about historical parasite populations, but before we can use these data, they require rigorous validation. We tested whether the liquid-preservation process alters the detectability of parasites and assessed whether estimates of parasite abundance from natural history collections would be biased by parasite life history stage or taxon. Overall, we found that there were few differences in parasite abundance and distribution between the preservation and control treatments.

Across the 26 host–parasite pairs, we only found significant differences between preserved and control treatments in three pairs. Among these, the direction of the effect was mixed, with some parasites displaying elevated abundances in the preservation treatment relative to the control and some the reverse. We found that adult hemiuridean trematodes had decreased detectability in the preservation treatment compared to the control treatment. This could arise because during dissection, we noticed that preservation can bind intestinal contents, complicating the detection of adult trematodes in the intestinal lumen. Compared to the intestinal contents of

frozen fish, contents from preserved fish often had to be broken apart manually with forceps instead of agitation. Larval *Pseudoterranova* sp. nematodes were also less abundant in the preservation treatment than in the control treatment. Here, the decline in detectability may arise because preservation reduces the color difference between parasite and host tissues, making it more difficult to detect these parasites by transmitted light. For adult *Cucullanus* sp. nematodes, we found that detection was enhanced in the preservation treatment relative to the control treatment, which might arise because preservation can stiffen nematodes, making them easier to distinguish from intestinal contents. No single cestode taxon differed significantly in abundance between treatments; however, when we used meta-regression to pool replication over all parasite taxa, we found that preservation increased detectability among adult cestodes, probably because increased replication allowed the non-significant positive effect of preservation on tetraphyllideans (Figure 2) to become significant (Figure 3). While there is potential for adult cestodes to have better detectability after preservation, further information would be needed as we only detected a single adult cestode taxon across our three species of fish. With our sample sizes, we were only able to reliably detect significant differences that had an effect size greater than 0.5. Therefore, our analysis is limited in its ability to detect small differences between treatments.

Although we found few significant differences in mean parasite abundance between treatments, we questioned whether there still might be differences in parasite metrics that wouldn't reveal themselves in generalized linear mixed effects models. Consistent differences between the distributions of parasite abundance in the preservation versus control treatment could arise from differences in mean, variation, skew, or kurtosis, and some of these may not be detectable with a generalized linear mixed effect model. To test this, we ran Kolmogorov-

Smirnov (KS) two-sample tests for each host–parasite pair. This allowed us to determine whether there are significant differences in the empirical cumulative distribution function between the two treatments, thus elucidating differences that could be due to differences in variance, skew, presence of zeros, and modality. This test showed only two significantly different host–parasite pairs among 26 total pairs, confirming that most parasite taxa were detected in similar numbers between preservation and control treatments. The taxa where significant differences were found (*Cucullanus* sp. and *Pseudoterranova* sp.) overlap with the taxa that showed significant differences in abundance between treatments (Figure 2).

While there were few significant differences between treatments across parasite group and life stage, we were interested to know if there were potential differences in detectability across different host organs. We found that, in general, there were few systematic biases in detectability across host tissues, with the one exception being the heart. Parasites from the heart had decreased detectability after preservation. However, we were only able to model a single parasite from the heart, *Pseudoterranova* spp., and thus would caution that further examinations of other fish species that can potentially have parasites in the heart to conclusively claim that parasite detectability is decreased in the heart after fixation.

Our study is taxonomically limited by the fact that there are certain parasitic taxa that we did not detect or did not detect in sufficient abundance, including crustaceans and monogenean trematodes. We also did not examine for the presence of non-metazoan taxa, such as protistan, viral, or bacterial parasites. In a study comparing several preservation techniques (live, frozen, formaldehyde, and ethanol), it was found that monogenean abundance was reduced with various methods of preservation (Kvach, Ondračková, Janáč, & Jurajda, 2018). Preservation-associated declines in abundance for monogeneans are expected because monogeneans do not have a hard

cuticle, as nematodes or encysted parasites do, and this would make them more subject to dehydration during the preservation process. For crustaceans, we would not expect a decline in abundance, since crustaceans have a hard exoskeleton and would be less subject to dehydration and destruction from the preservation process. However, many crustaceans are mobile ectoparasites and could potentially become dislodged during specimen preparation and transfer between preservation liquids. According Kvach et al (2018), there were no differences in abundance of crustaceans among any of the treatments, thus supporting the idea that crustacean abundance from natural history collections might accurately reflect actual abundance.

We used frozen fish as our experimental control because this is the most common state of fish at the time they are accessioned into the UW Ichthyology Collection (i.e., it is common for fish to be frozen at sea before fixation, preservation, and accessioning; K Maslenikov and L Tornabene, pers comm). The act of freezing a fish is known to reduce the detectability of many parasites, especially protozoa (Kvach et al., 2018). We chose to freeze our fish to accurately reflect the actual process of preservation as it occurs in a major ichthyology collection. Furthermore, while dissection of fresh, recently sacrificed fish is ideal (Kvach et al., 2018), the feasibility of this is not always possible due to the remoteness of field collection, time constraints, or lack of experienced parasitological dissectors at the location of collection. A useful extension of this work would be to compare fresh, frozen, and preserved fish to determine how this other component of the natural history collection preservation process could influence the detectability of parasites.

In our experiment, fish were dissected after three to 10 days of ethanol storage. In contrast, some specimens in natural history collections have been stored in ethanol for over one hundred years (Harmon et al. 2019). Long-term storage in ethanol could further alter the detectability of

parasites and result in a loss of information. To determine whether there is a loss of information due to long-term storage in ethanol, the procedure used here would need to be repeated, but with multiple time points of dissection over a long time span. Our group has initiated this experiment; we are holding experimentally preserved fish that we will dissect at multiple time points over the next decade to determine the long term effects of storage in ethanol on parasite detectability.

Although our study suggests that a fixed fish specimen accurately reflects the parasite burden of the fish at the time of its fixation, it does not address the potential for systematic bias in the choice of which fish to preserve, which could have a substantial influence on estimates of parasite abundance. Some collections may receive exhaustive fish samples from a specific location year after year; for example, the eulachon and pollock we dissected came from research trawls in which the entire contents of the catch is retained. In this case, the collections represent an unbiased sample of the natural populations and should accurately reflect natural populations. On the other hand, many collections are the result of more haphazard collections, or collections motivated by past research projects or curator interests. This can introduce bias as collectors and collections managers select which fish to be catalogued, which might either favor parasitism or disfavor parasitism (Harmon et al. 2019). For example, curators might choose not to include a fish that is visibly parasitized for aesthetic reasons, or might choose to include it as a curiosity. These biases can also change through time as personnel and research practices change. Addressing this potential source of bias requires discussions with collections managers, staff, and curators to understand the history of policies and practices in different natural history collections.

Conclusions

Holding tens of millions preserved fish, natural history collections represent a treasure trove of information (Harmon et al. 2019). Our results suggest that the liquid-preservation approach used by these collections has little effect on the detectability of parasites. This opens the door to use parasitological dissection of natural history specimens for reconstructing long-term parasite change over the past 100 years or more. Many previously intractable questions in disease ecology will be within our reach using this new approach. Most importantly, the long-term perspective provided by this approach will allow us to evaluate contemporary parasite burdens in their historical context.

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Author contributions

EAF, KPM, LT, MET and CLW conceived the ideas and designed methodology; EAF and KLL collected the data; EAF analysed the data; EAF and CLW led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility: Code will be available through GitHub and data will be accessioned on Dryad Digital Data Repository.

Figures

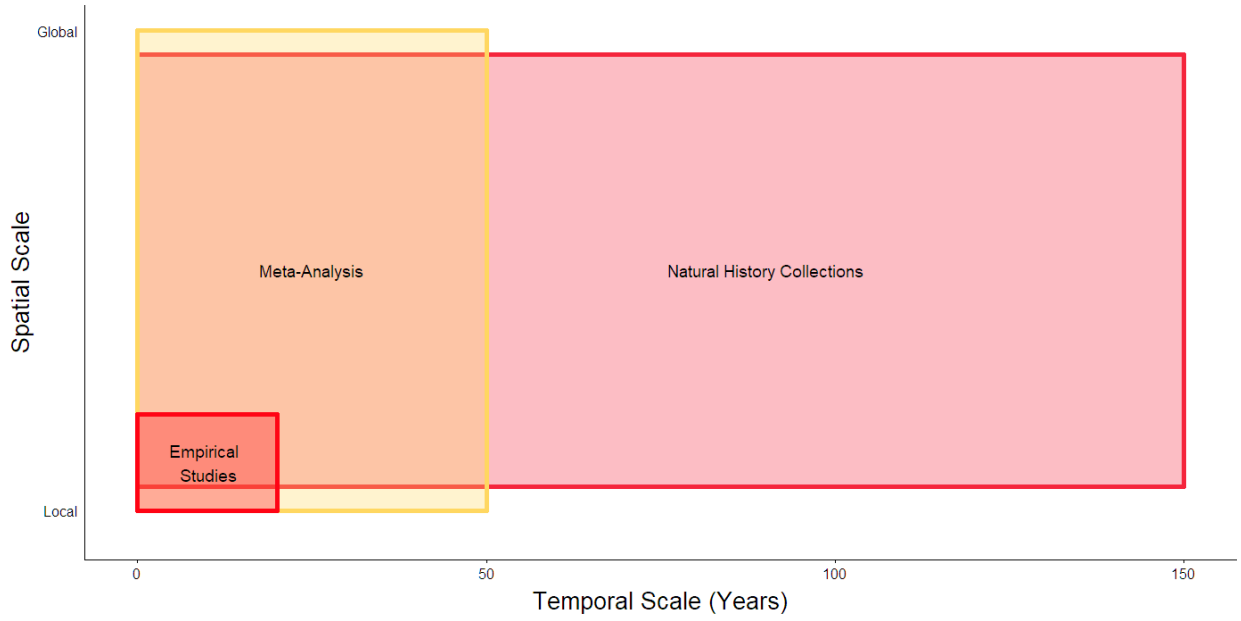

Figure 1. Conceptual diagram displaying the dimensions of time and space that can be characterized by three parasite ecology approaches. The first, empirical studies, involves the collection of parasite data in real time by research teams. This approach is often limited in spatial and temporal scale due to constraints of funding and personnel to cover large areas over long periods of time. Meta-analysis is another method in which researchers can summarize results across studies. Here, one is limited by the availability of accessible published research, which by many meta-analytic methods limits one to a period of approximately 50 years, though depending on the questions, can span local, well studied areas to global patterns. The third method is the use of natural history collections. Here, one is limited by what is present in natural history collections. Collections can date back 150 years and can be from one locality over many years to species of global distribution over many years. Natural history collections have great potential to fill information gaps in the temporal dimension.

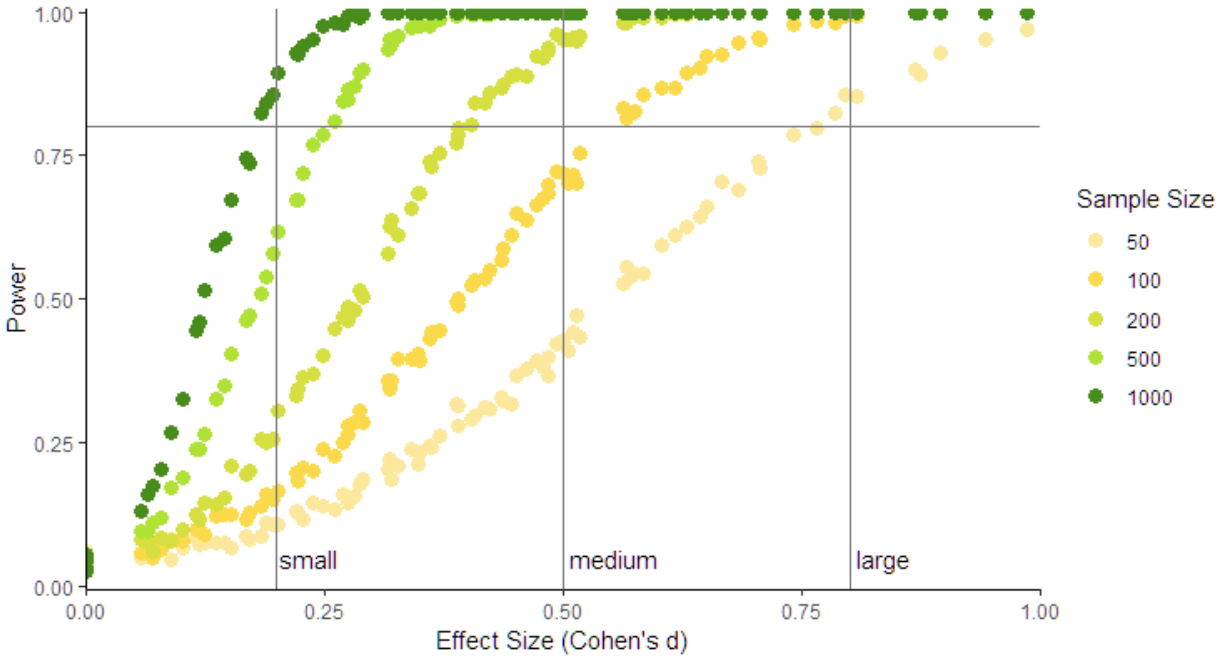

Figure 2. Estimates of effect size and power from simulated data to determine adequate sample size. Vertical black lines correspond to the different 'levels' of effect sizes based on Cohen 1969. The horizontal black line represents a power of 0.8. In order to achieve adequate power to detect small effect sizes, approximately 1,000 fish per species would be needed.

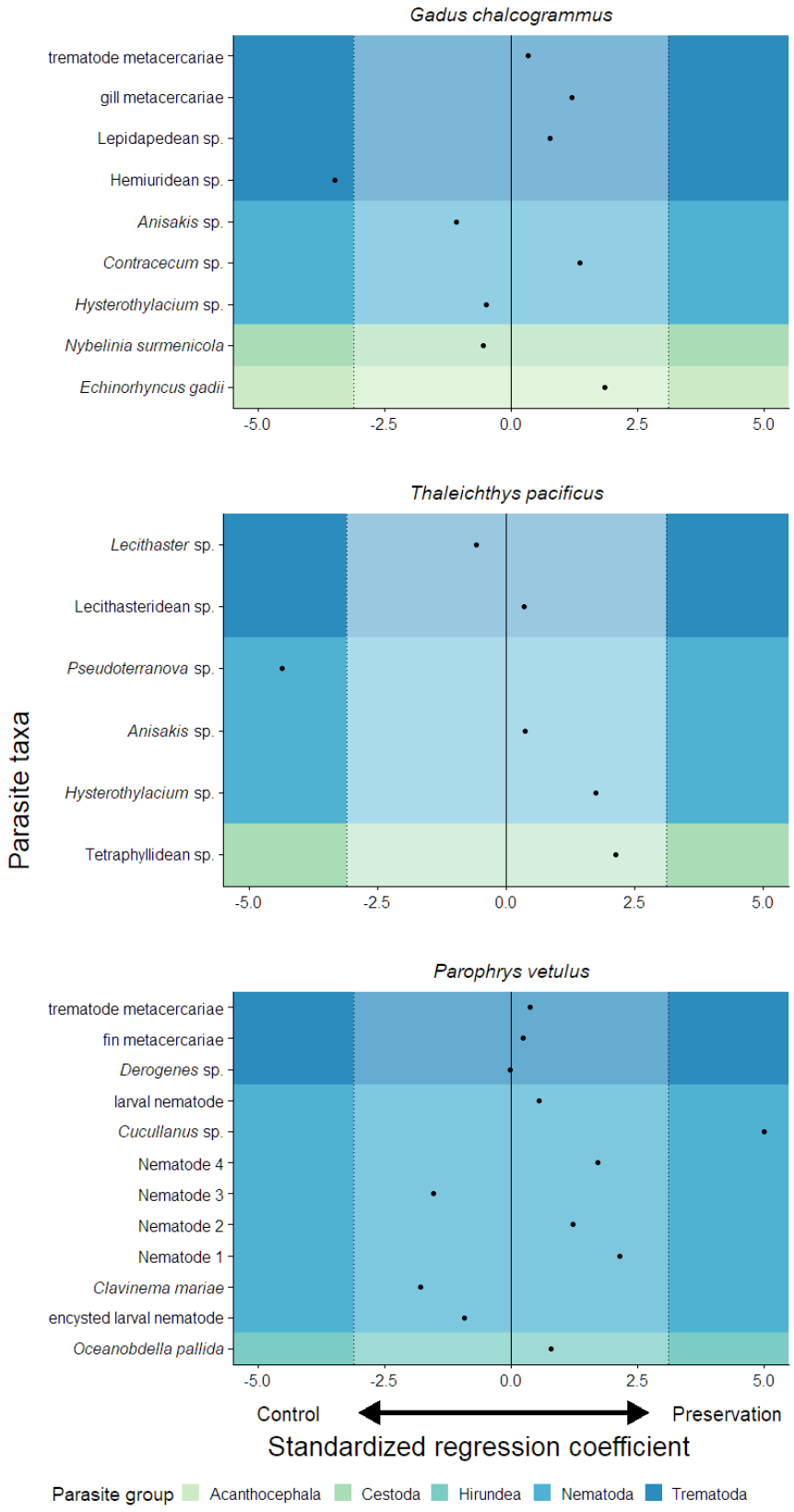
Gadus chalcogrammus
trematode metacercariae
gill metacercariae
Lepidapedean sp.
Hemiuridean sp.
Anisakis sp.
Contracecum sp.
Hysterothylacium sp.
Nybelinia surmenicola
Echinorhyncus gadii
-5.0
-2.5
0.0
2.5
5.0
Thaleichthys pacificus
Parasite taxa
Lecithaster sp.
Lecithasteridean sp.
Pseudoterranova sp.
Anisakis sp.
Hysterothylacium sp.
Tetraphyllidean sp.
-5.0
-2.5
0.0
2.5
5.0
Parophrys vetulus
trematode metacercariae
fin metacercariae
Derogenes sp.
larval nematode
Cucullanus sp.
Nematode 4
Nematode 3
Nematode 2
Nematode 1
Clavinema mariae
encysted larval nematode
Oceanobdella pallida
-5.0
-2.5
0.0
2.5
5.0
Control
Preservation
Standardized regression coefficient
Parasite group
Acanthocephala
Cestoda
Hirundea
Nematoda
Trematoda

Figure 3. Standardized regression coefficients (z-score) for the effect of preservation on the detectability of individual parasite taxa across the three studied hosts. Negative values indicate parasite taxa in which more individuals were detected in the control treatment than in the preservation treatment. Positive values indicate parasite taxa in which more individuals were detected in the preservation treatment than in the control treatment. Values greater than 3.1 indicate enhanced detection in the preservation treatment after Bonferroni corrections. Values less than –3.1 indicate decreased detection of the parasite taxa in the preservation treatment after Bonferroni corrections.

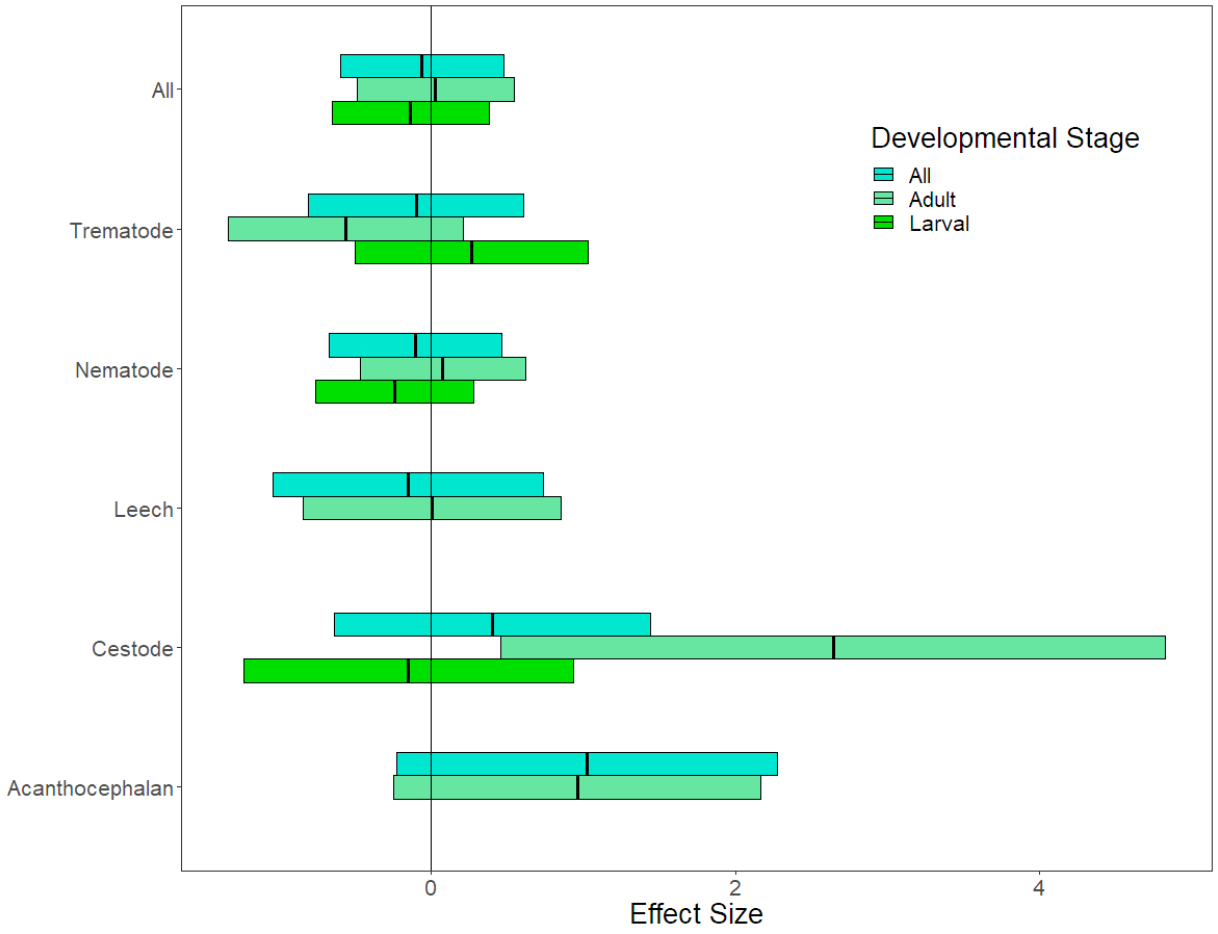

Figure 4. Meta-regression estimates for the effects of preservation on mean abundance of parasites. Estimates of the effect sizes come from meta-regression models testing the hypotheses of if there is an overall effect of preservation on detectability, if detectability is moderated by parasitic group, if it is moderated by parasite life stage, and if it is a combination of life stage and parasitic group. Positive values indicate higher abundance in preserved fish while negative values indicate higher abundance in control fish. Estimates are shown with 95% confidence intervals.

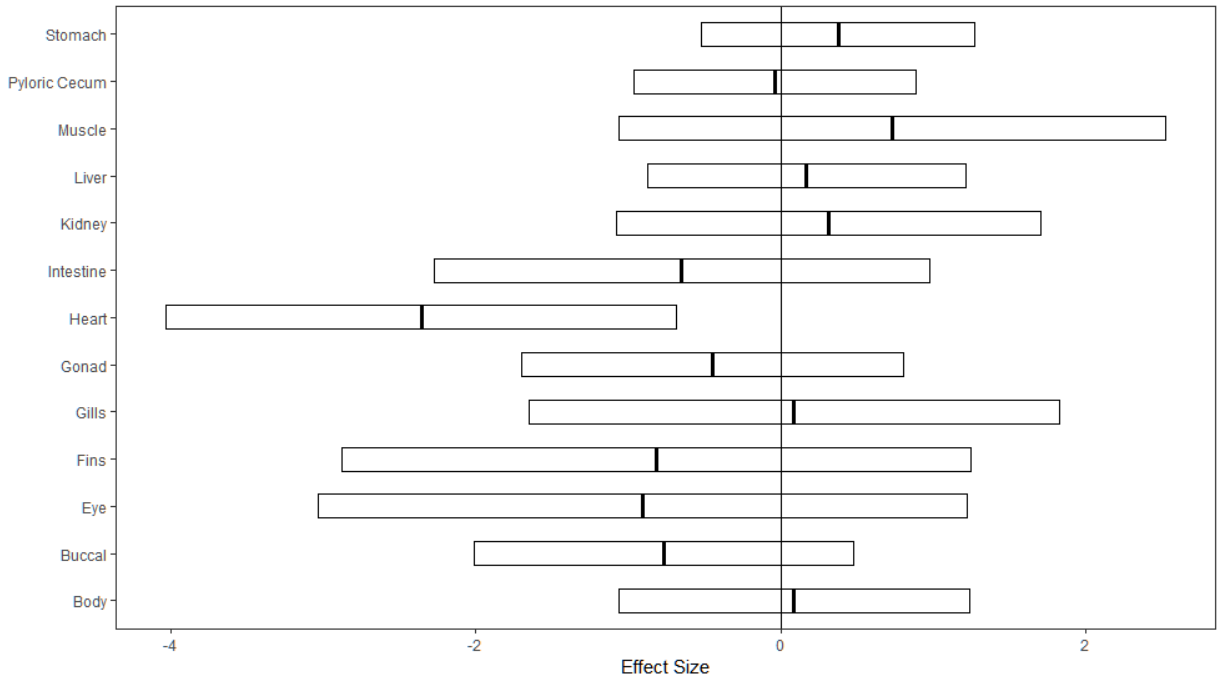

Figure 5. Meta-regression estimates of the effects of preservation on parasite detectability in different host organs. Positive values indicate higher abundance in the preserved treatment while negative values indicate higher abundance in the control treatment. Estimates are shown with 95% confidence intervals.

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