RESEARCH ARTICLE

Abundance of an economically important nematode parasite increased in Puget Sound between 1930 and 2016: Evidence from museum specimens confirms historical data

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Abstract

1. Does disturbance increase or decrease parasite transmission among wildlife hosts? Ecologists cannot answer this controversial question, in part because few historical datasets rigorously document parasite abundance. Without such a baseline, it is difficult to determine whether contemporary ecosystems are experiencing elevated parasite burdens.

2. Here, we investigate change over time in the abundance of a parasite that affects the economic value of a fish species. *Clavinema mariae* is a nematode parasite of benthic fishes that is common in English sole (*Parophrys vetulus*) of Puget Sound, WA. We obtained historical records of its abundance from the literature and from unpublished government agency data, and resampled the same locations using the same methods in 2017. We also used a new approach by estimating the *C. mariae* burden for museum specimens of English sole collected between 1930 and 2016.

3. Both the historical data and museum specimen data suggested increases over time in *C. mariae* abundance, with robust agreement between the two approaches.

4. In addition to documenting a previously unrecognised ~eightfold increase in the burden of an economically important parasite, our work demonstrates—for the first time—that parasitological examination of liquid-preserved museum specimens can produce reliable data on long-term trends in parasite abundance, at a much greater temporal resolution than is possible to obtain from historical records.

5. *Synthesis and applications*. Defining a baseline state of infection is vital for natural resource management and policy, which must respond to the threat of disease; without such a baseline, managers attempting to maintain or recover the health of ecosystems under their stewardship are shooting in the dark. The method we present here—using museum specimens to reconstruct detailed chronologies of parasite abundance change—offers a solution. Our approach would allow managers to accurately characterise past disease states, informing the development of appropriate disease management targets. Given the broad representation and

1 | INTRODUCTION

Parasites are a natural part of ecosystems, and some even suggest that a healthy ecosystem is one that is rich in parasites (Hudson, Dobson, & Lafferty, 2006); but how much parasitism is too much parasitism? This question is pressing, because parasites can compromise the profitability and sustainability of fisheries (Lafferty et al., 2015) and can obstruct conservation efforts (Deem, Karesh, & Weisman, 2001). Management must strive to minimise the impacts of parasitism on hosts of economic or conservation value, while recognising that some amount of parasitism might be inherent and unavoidable in a healthy, functioning ecosystem. Identifying this balance point is critical to establishing a realistic baseline for infection management, and requires data on parasite abundance in a "healthy" ecosystem. For a given ecosystem, the best information on "healthy" infection levels would come from the same ecosystem at an earlier time point, prior to the recent years when human impacts altered that ecosystem.

Unfortunately, the abundance of parasites may be changing rapidly in the Anthropocene, and few historical data exist to document their populations prior to these changes. Pollution (Lafferty, 1997), eutrophication (McKenzie & Townsend, 2007), resource extraction (May & Dobson, 1987; Wood & Lafferty, 2015; Wood, Sandin, Zgliczynski, Guerra, & Micheli, 2014; Wood et al., 2015), climate change (Lafferty, 2009; Marcogliese, 2001), species invasions (Young, Parker, Gilbert, Sofia Guerra, & Nunn, 2017) and biodiversity loss (Wood, Lafferty, et al., 2014) are all expected to influence patterns of parasite transmission. Predictions for the outcome of these changes are complex; some kinds of environmental change—like species invasions—are predicted to decrease the abundance of parasites (Young et al., 2017), while others—like eutrophication—are predicted to increase parasite transmission (McKenzie & Townsend, 2007). Yet others—like chemical pollution (Lafferty, 1997) and resource extraction (Wood, Sandin, et al., 2014; Wood et al., 2015)—are predicted to have complex and context-specific effects on parasites.

Against this backdrop of rapid and dramatic change in parasitism, long-term data on parasite abundance are vital for developing realistic management baselines; unfortunately, these data are exceedingly rare. Although historical ecology is rich in examples of high-quality data on the density, body size and species composition of free-living species, it is uncommon to encounter a reliable source of parasitological information from the past. Ship's logs (e.g. Reeves & Cosens, 2003), fisheries landing records (e.g. Rosenberg et al., 2005), historical records (e.g. Jackson et al., 2001; Lotze et al., 2006), naturalists' accounts (e.g. Sagarin, Gilly, Baxter, Burnett, & Christensen, 2008; Willis, Ruhfel, Primack, Miller-Rushing, & Davis, 2008), living persons' memories (e.g. Lozano-Montes, Pitcher, & Haggan, 2008; Saenz-Arroyo, Roberts, Torre, & Cariño-Olvera, 2005) and photographs (e.g. McClenachan, 2009) rarely preserve information on parasites.

The solution to this problem has been hiding in plain sight: museum specimens are a reliable resource for reconstructing historical parasite assemblages and building baselines for infection in a "healthy" ecosystem. Many natural history collections contain vertebrates that were fixed in formalin and stored in ethanol, preserving parasite tissue as well as host tissue (e.g. Black, 1983; Hartigan, Phalen, & Šlapeta, 2010; Johnson, Lunde, Zelmer, & Werner, 2003). It is therefore possible to find, identify and count parasites in specimens collected decades or even centuries ago. The value of natural history collections for understanding long-term change in the biosphere is well-recognised (Johnson et al., 2011), but this resource is under-utilised for disease research (DiEuliis, Johnson, Morse, & Schindel, 2016). Past studies have used museum specimens to establish the historical presence of a parasite (e.g. Black, 1983; Frederick, McGehee, & Spalding, 1996; Hartigan et al., 2010; Hewson et al., 2014), but none—to our knowledge—have used museum specimens to reconstruct trajectories of change in the abundance of parasites. In part, this is because using museum collections to reconstruct parasite abundances of the past presents some problems. First, specimens selected for collection, preservation and storage may not accurately represent the original population from which they were selected if—for example—the collector selects specimens by size or appearance (e.g. rejecting or preferentially including "wormy" fish). In addition, specimen degradation due to fixation, storage protocols and the passage of time may make parasites less detectable in museum specimens than they might have been prior to fixation, leading to underestimates of historical parasite burden or artefactual relationships between specimen age and parasite burden. Given that natural history collections contain millions of specimens of many species across broad spectra of geography and time, it is worthwhile to ask: can these specimens provide a reliable reflection of the parasite assemblages that existed at the time of their collection?

To approach this issue, we investigated temporal change in the abundance of an economically important parasite: *Clavinema*
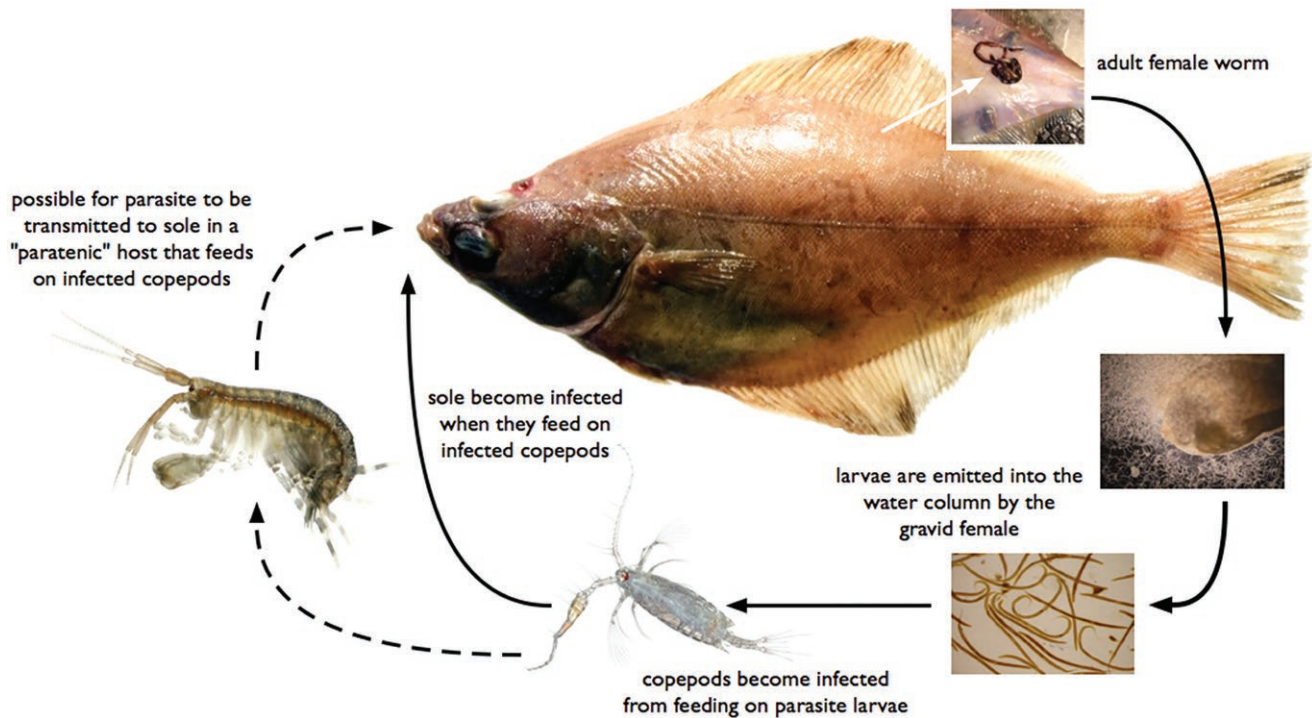
FIGURE 1 Complex life cycle of the nematode parasite *Clavinema mariae*. English sole image courtesy of Washington Department of Fish and Wildlife. Adult female worm courtesy of Ingrid Howard. Larval worm images reproduced from Sethi, Jithendran, and Dash (2013). Copepod courtesy of David Liittschwager/National Geographic Creative. Amphipid courtesy of Discover Life, www.discoverlife.org

mariae (Layman, 1930; superseding *Philometra americana* Kuitunen-Ekbaum, 1933 and *Philometra mariae* Layman, 1930; Arai & Smith, 2016), a nematode parasite of commercially important English sole (*Parophrys vetulus*) in Puget Sound, WA (Margolis & Arthur, 1979). *C. mariae* larvae develop in marine copepods, which the fish host then ingests (Figure 1). The parasite then moves into the tissues between the fin rays or subcutaneous tissues of its fish host, where it spends most of its adult life. When gravid, female worms form a lesion in the skin of its host and, through this breach, release larvae into the water, where they are ingested by intermediate host copepods (Holland, 1954).

Clavinema mariae is a large, easily visible parasite that renders fish unmarketable. Its ubiquity was responsible for the closure of the English sole fishery in a large swath of Puget Sound; the south Puget Sound (south and west of Point Defiance) English sole fishery was closed in 1948, in part because the *C. mariae* parasite was so abundant that filets from fish caught in the region could not be sold for human consumption (Holland, 1954, 1969). In 1989, the Washington State Legislature closed all of Puget Sound south of Admiralty Inlet to bottom trawling, in response to concerns about potential habitat destruction (Palsson, Hoeman, Bargman, & Day, 1996). In 1994, non-treaty bottom trawling was also banned in Admiralty Inlet, the eastern Strait of Juan de Fuca, and the San Juan archipelago (Palsson et al., 1996). All commercial groundfish gear was banned throughout Puget Sound in 2010 to protect Endangered Species Act-listed rockfish (WDFW, 2011). English sole was once among the most commercially important groundfish species in Puget Sound (Alverson,

Palmen, & Pasquale, 1956; Gooding, 1956; Holland, 1954; Quinnell, 1984; Smith, 1936), but by 2006 the species contributed <2% of the total mass of groundfish landed by Washington State non-treaty fisheries (TCW Economics, 2008).

Clavinema mariae is unique among parasites. Due to its economic importance, reliable, geographically explicit historical estimates of its abundance exist. This provided us with the opportunity to compare direct estimates of *C. mariae*'s abundance (historical data) with estimates derived from museum specimens—a "proof-of-concept" to test whether museum specimens bias estimates of parasite abundance or closely track the actual abundance of parasites in nature. We uncovered two valuable historical resources suitable to this "proof-of-concept" test: (a) geographically explicit estimates of *C. mariae* prevalence in peer-reviewed literature from the mid-1900s, and (b) raw data from two cruises conducted in 1949 and 1952 for the express purpose of estimating *C. mariae* abundance, commissioned by Washington State Department of Fisheries (now the Washington Department of Fish and Wildlife [WDFW]) and recovered from paper records in storage at WDFW headquarters (Data S1).

Here, we address two questions: (a) Has the abundance (i.e. proportion of individuals infected and the number of worms per infected individual) of *C. mariae* in English sole of Puget Sound changed since the 1930s? (b) Do museum specimens and historical records agree on the direction and magnitude of change in *C. mariae* abundance across time, and on the absolute abundance of the worm at different points in time? We hypothesised that the closure of fisheries

throughout Puget Sound might have increased English sole density, thereby facilitating enhanced transmission of *C. mariae*. Consistent with this hypothesis, our data indicate that *C. mariae* abundance has dramatically increased over time, and this increase is reflected in both historical data and data from museum specimens. Our study validates an approach for quantifying parasite assemblages of the past that could provide—for the first time—baselines with which to understand parasite transmission shifts in the Anthropocene.

2 | MATERIALS AND METHODS

To assess the *C. mariae* worm burden of English sole in Puget Sound over multiple decades, we took two approaches: estimating parasite burden in museum specimens of English sole, and comparing historical records of parasite burden with contemporary data collected by our team. We then compared the results of the two approaches, with the expectation that better correspondence of the two outcomes would increase certainty in the result. In addition to robustly testing for change over time in this economically important parasite, our two-pronged approach provided a rare opportunity to test the utility of fish museum specimens for assessing historical parasite burdens.

2.1 | Parasitological examination of museum specimens

We reconstructed a timeline of *C. mariae* parasite abundance by counting worms in museum specimens of English sole (Data S2). All specimens were obtained from the University of Washington's Fish Collection at the Burke Museum, and were originally collected, preserved and accessioned between 1930 and 2016 from locations across Puget Sound, WA (Figure 2). To minimise sampling bias, we assessed the parasite burden of every English sole specimen held by the UW Fish Collection >150 mm in total length (few fish <150 mm have been documented with *C. mariae*; Holland, 1954). For each of the 306 available fish, we performed a thorough external physical examination and counted the number of *C. mariae* present. The presence of the parasite is indicated by lumps or protruding worms in the flesh and fins (Figure 3); where we found a bump or lesion that was not obviously caused by *C. mariae*, the area was cut with a scalpel and explored to find worms beneath the skin's surface. We recorded the total length of each fish in millimetres and all information from the tag identifying the specimen, including catalogue number, sampling location and collection date. For tags where information was missing, we used the online UW Fish Collection database to obtain additional information (http://www.burkemuseum.
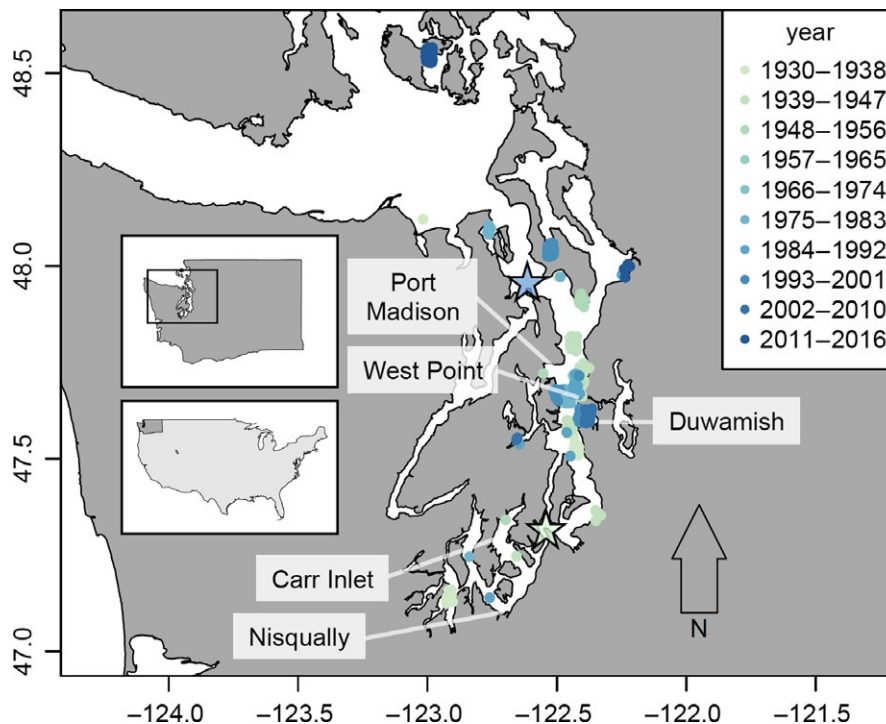

FIGURE 2   Map of collection locations for all 306 preserved English sole (*Parophrys vetulus*) museum specimens assessed for *Clavinema mariae* infection. These specimens represent all of the English sole originally collected from within Puget Sound (and >150 mm) that are held in the UW Fish Collection. Greener colours indicate collection locations of older specimens; bluer colours indicate more recent specimens (see colour legend). Locations are jittered by a factor of 100 to display the number of specimens and collection dates for each specimen at locations that yielded multiple specimens. The English sole fishery south of Point Defiance (green star) was closed in 1948; south of Admiralty Inlet in 1989 (blue star); and in Admiralty Inlet, the eastern Straight of Juan de Fuca, and the San Juan archipelago in 1994. In 2010, all commercial groundfish gear was banned throughout Puget Sound. Coastline map from NOAA's Global Self-consistent, Hierarchical, High-resolution Geography Database (https://www.ngdc.noaa.gov/mgg/shorelines/gshhs.html)
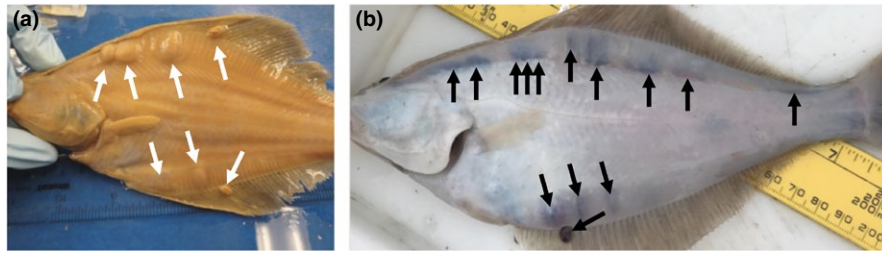
FIGURE 3 (a) Preserved English sole (TL = 250 mm) from Elliot Bay, Puget Sound (catalogue number UW 110312, from the University of Washington Fish Collection). Specimen was collected and preserved in 1992 and examined on 18 October 2016. Seven worms are visible on non-eyed side. (b) Freshly collected English sole (TL = 230 mm) from Elliot Bay, Puget Sound, examined on 2 May 2017. Fourteen worms are visible on non-eyed side

org/research-and-collections/ichthyology). For specimens with missing or incomplete location information (e.g. only collection site name recorded on tag), we estimated longitude and latitudes of the collection sites using Google Earth.

To test for change over time in the burden of *Clavinema mariae* in the museum specimens we examined, we used a generalised linear mixed model with Poisson error, implemented using the *glmmadmb()* function of the GLMMADMB package in R (Fournier et al., 2012; Skaug, Fournier, Nielsen, Magnusson, & Bolker, 2016). The model had the following form:

$$\text{Number of Clavinema mariae} \sim \text{year of collection}$$
$$+ \text{fish total length (mm)}$$

We tested models with error structures set as Poisson, negative binomial without zero inflation, and negative binomial with zero inflation; from among these models, we discarded those where residual plots revealed violations of model assumptions and, after this process, were left with the Poisson model. We tested whether the body size of museum specimens changed over time using a simple linear model with Gaussian error; we recognised that this change over time could reflect either (a) change in the true average body size of the population or (b) change in the size-bias of the collection process, but sought to characterise any temporal changes in body size regardless of their cause. We tested for spatial autocorrelation using Moran's I, implemented using the *Moran.I()* function of the fields library in R, and by examining a spatial variogram, implemented in the gstat library in R.

2.2 | Comparison of historical versus contemporary data

To develop an assessment of temporal change in *C. mariae* abundance that was independent of the museum specimen approach discussed above, we compared contemporary parasite burdens with historical reports of parasite burden. We identified two sources of historical data. The first was the peer-reviewed literature (Holland, 1954; Miller et al., 1977; Williams, 1950), which yielded eight estimates of prevalence (proportion of hosts infected with *C. mariae*) from five sites around Puget Sound (i.e. some sites were sampled at multiple time points in the past) in 1949,

1950–1951, 1951, 1975 and 1976. All estimates were obtained by examining English sole captured by otter trawl. Information about the collection depths for two historical data sources (Holland, 1954; Williams, 1950) were unavailable, and prevalence for the final data source (Miller et al., 1977) reflects pooling across trawls at 5, 25, 45, 70 and 95 m depth. While useful, these data merely estimate the proportion of hosts infected—not the number of worms carried by each fish, nor the distribution of worms among fish as a function of host body size, which is often an important predictor of parasite burden (Hudson & Dobson, 1995; Pacala & Dobson, 1988; Poulin & Morand, 2000). The second data source provided these valuable details. We obtained a set of raw data sheets (individual host records, documenting total length and number of parasites per host individual collected) from two cruises conducted in 1949 and 1952 by the vessel *Panther*, for the express purpose of estimating *C. mariae* abundance at two locations (Carr Inlet and Nisqually Reach; Data S3). This cruise was commissioned by Washington State Department of Fisheries and the raw data sheets were recovered from paper records in storage at WDFW headquarters (Data S1). All English sole were captured by otter trawl, but information on collection depth was unavailable. In some cases, worms were too numerous to count and were noted as "many"; to avoid dropping these values (and therefore biasing estimates of parasite burden downward), we noted the burden of *C. mariae* as $n + 1$, where n = the highest number of worms counted for that site on that date. This substitution was necessary for only 21 fish in our historical dataset of 1002 fish.

To compare these two historical baselines against contemporary conditions, we then reconstructed sampling at each of the sites for which we had historical data (Data S3). This was accomplished: (a) on a research bottom trawl conducted by the Toxics-Focused Biological Observing System unit (TBiOS) and the Marine Fish Science (MFS) unit of the Washington Department of Fish and Wildlife in April and May of 2017 at Nisqually Reach, Carr Inlet, Duwamish Waterway, and West Point, and (b) on an annual research bottom trawl conducted by a University of Washington (UW) undergraduate fish ecology class (led by Thomas P Quinn) in May of 2017 at Port Madison. English sole were captured by otter trawls in depths between 56 and 104 m for WDFW MFS trawls (at Carr Inlet), between 7 and 137 m for WDFW TBiOS trawls (at

Nisqually Reach, Duwamish Waterway, and West Point), and at 10, 25, 50 and 70 m for UW trawls (at Port Madison). All English sole >150 mm in total length caught at each station were examined for *C. mariae* infection.

For historical data from published literature, we tested whether prevalence changed over time with a general linear model that included sampling location as a random effect (to account for multiple observations at some sampling locations). For historical data from the *Panther* cruise, we tested whether the relationship between worm burden and body size varied between historical and contemporary datasets, hypothesising that worm burden would be higher and would rise with body size more rapidly at contemporary than historical time points. We used a generalised linear model with a random effect of site, implemented using the *glmmadmb()* function in the GLMMADMB package in R (Fournier et al., 2012; Skaug et al., 2016). We tested models with error structures set as Poisson, negative binomial without zero inflation and negative binomial with zero inflation; from among these models, we discarded those where residual plots revealed violations of model assumptions and, from among the remaining models, chose the one with the lowest AIC (negative binomial without zero inflation).

2.3 | Comparison of estimates of parasite abundance change between museum specimens and historical data

One way to test whether the parasite burden of museum specimens reflects the parasite burden of the original population is to compare values obtained from parasitological examination of museum specimens to those obtained from historical data. This is often impossible, because it is very rare to find historical data that rigorously document parasite abundance. We had historical data from several sites that matched the geographic and temporal

sampling profile of museum specimens in our dataset. We did not have a large number of museum specimens of English sole from Carr Inlet or Nisqually Reach (where we had ample raw data on *C. mariae* abundance in fish from 1949 and 1952, collected by the *Panther* research cruise). However, we were able to compare prevalence calculated from a limited number of museum specimens with published literature in Golden Gardens in 1950–1951, West Point in 1975–1978 and Carr Inlet in 1942–1951. We assessed the relationship between prevalence as estimated in the literature versus prevalence as estimated by our examination of museum specimens using a simple linear model with Gaussian error, of the form:

$$\text{prevalence in museum specimens} \sim \text{prevalence in historical data}$$

3 | RESULTS

3.1 | Parasitological examination of museum specimens

The abundance of *C. mariae* worms in museum specimens of English sole increased with year of specimen collection (estimate ± *SE* = 0.0440 worms/year ± 0.0081, *z* = 5.44, *df* = 303, *p* < 0.0001; Figure 4; Data S2), such that each fish had, on average, 0.11 worms in the first 30 years of the dataset (1930–1960) and 0.86 worms in the last 30 years of the dataset (1986–2016; Figure 4)—a nearly eightfold increase in burden. The average body size of the English sole included increased over time (estimate ± *SE* = 0.5850 mm/year ± 0.1040, *z* = 5.61, *df* = 303, *p* < 0.0001), and body size had a positive effect on worm burden (estimate ± *SE* = 0.0103 worms/mm ± 0.0021, *z* = 4.90, *df* = 303, *p* < 0.0001), but this was controlled for in our models. Moran's I was not significant (observed = −0.0014, expected = −0.0033, *p* = 0.3872) and a spatial variogram showed no relationship between distance and semivariance, indicating low spatial autocorrelation in the dataset.
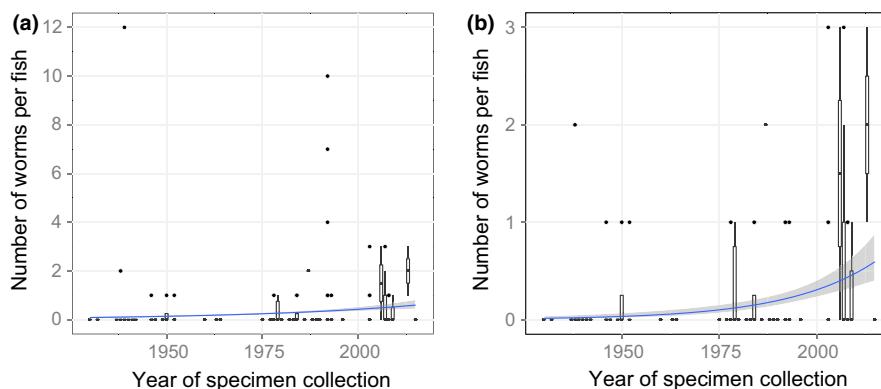

FIGURE 4 Abundance of *Clavinema mariae* in specimens of English sole from the UW Fish Collection, as a function of year of their collection. Boxplots indicate 25% quantile (lower hinge), median (centre line), 75% quantile (upper hinge), and outliers (dots). (a) Full range of data and (b) *y*-axis truncated to better illustrate the temporal relationship. Shaded area indicates confidence interval around Poisson regression line, demonstrating an increase in parasite burden over time
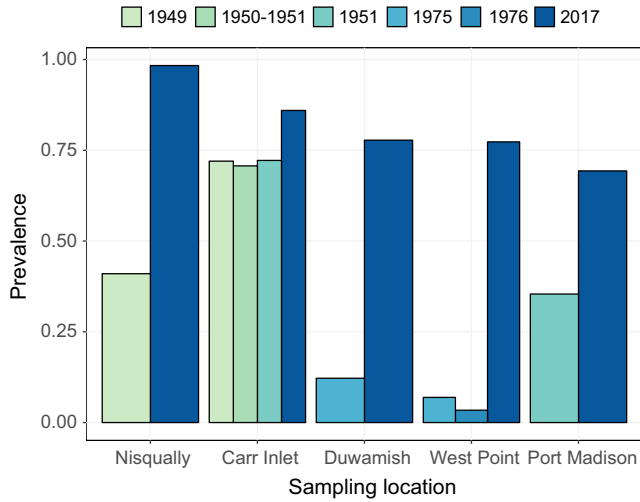
FIGURE 5 Historical (1949–1976) and contemporary (2017) prevalence of *Clavinema mariae* in English sole at five sites, ordered from southernmost to northernmost (see Figure 1): Nisqually Reach, Carr Inlet, Duwamish, West Point, and Port Madison. Historical data from (Holland, 1954; Miller et al., 1977; Williams, 1950). Values labelled "1950–1951" are averaged valued from cruises conducted across that time span (Holland, 1954). Contemporary data are from WDFW and UW research cruises conducted in 2017

3.2 | Comparison of historical versus contemporary data

We compared historical data derived from published literature sources to contemporary data collected during 2017 WDFW and UW research cruises. Historical prevalence of *C. mariae* was, on average, 48.7% lower than contemporary prevalence (z = 5.06, df = 6, p < 0.0001; Figure 5).

 We also compared data on parasite burden of English sole from the 1949–1952 *Panther* cruise with data from a 2017 WDFW research trawl. The fish collected in 1949–1952 (average total length ± SE = 309.6 ± 6.4 mm) were larger than those collected in 2017 (average total length ± SE = 244.8 ± 4.2 mm; z = 15.5,

df = 1007, p < 0.0001). Controlling for body size, fish from 2017 had higher worm burdens than those from 1949–1952, and those burdens increased with body size, whereas there was no relationship between body size and worm burden in 1949–1952 (body size*[years 1949–1952 vs. 2017] estimate = −0.01959 ± 0.00281, z = −6.96, p < 0.0001; Figure 6a). This was true even after we excluded those fish from 1949–1952 that were larger than the largest fish from 2017 (body size*[years 1949–1952 vs. 2017] estimate = −0.01799 ± 0.00323, z = −5.58, p < 0.0001; Figure 6b).

3.3 | Comparison of estimates of historical parasite abundance between museum specimens and historical data

There was a strong, positive relationship between estimates of prevalence based on the literature and estimates of prevalence based on our examination of museum specimens (slope ± SE = 0.7195 ± 0.0123, z = 58.72, df = 2, p < 0.0001), although this relationship was based only on the three points where we could match geography and time between historical data and museum specimen data, and some of the museum specimen values associated with those points had extremely low replication. The historical data corresponded to the museum specimen data as follows: Golden Gardens in 1950–1951 by historical data (Holland, 1954) = 0.6% (8/1323), by museum specimens = 0% (0/2); West Point in 1975 by historical data (Miller et al., 1977) = 6.5% (83/1282), by museum specimens = 3.2% (1/31); Carr Inlet in 1942–1951 by historical data (Holland, 1954) = 70.7% (2921/4132), by museum specimens = 50.0% (1/2). Estimates of prevalence in museum specimens therefore corresponded well with historical data, although they slightly underestimated prevalence, especially for higher prevalence values.

4 | DISCUSSION

The two orthogonal datasets presented here both strongly suggest that the abundance of *C. mariae* in English sole hosts of Puget Sound
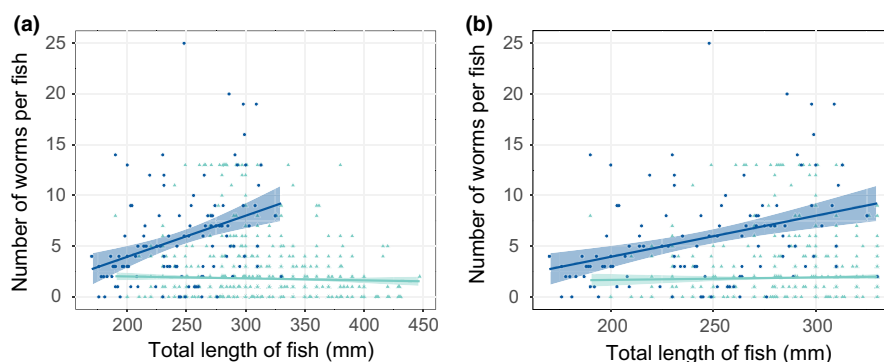

FIGURE 6 Number of *Clavinema mariae* in specimens of English sole from a WDFW research cruise conducted in 2017 (blue dots) and a research cruise commissioned by WDFW in 1949–1952 (green triangles), as a function of the total length of the fish in mm. (a) Full dataset, (b) only those fish equal to or smaller than the largest fish collected in 2017 (330 mm). Shaded area indicates confidence interval around regression line, demonstrating an increase in parasite burden with increasing fish size in 2017 and no such relationship in 1949–1952

increased between the mid-1900s and the present day. Museum specimens of English sole collected from 1930 to 2016 were more heavily burdened by *C. mariae* in later years (Figure 4). For those historical reports (1949–1976) of *C. mariae* prevalence that we were able to reconstruct with sampling in the present day (2017), contemporary sampling consistently yielded higher estimates of *C. mariae* prevalence (Figure 5) and burden (Figure 6). Furthermore, where we were able to link historical reports to the same time and place as museum specimens, the two methods yielded consistent estimates of prevalence. Together, these findings suggest that examination of museum specimens may be a useful method for reconstructing change in the abundance of parasites that—unlike *C. mariae*—lack documentation in the historical record.

Examination of the *C. mariae* burden of museum specimens allowed us to develop a highly resolved timeline of infection that was impossible even with the high-quality historical data to which we had access (Figure 4). That timeline clearly showed an increase in *C. mariae* burden between the earliest collections in 1930 and the most recent, in 2016. However, this pattern might not necessarily reflect a true underlying increase in *C. mariae* abundance. For example, it might arise if earlier generations of scientific collectors discriminated against infected fish (e.g. because they judged those specimens unattractive or non-representative of the larger population). It also might arise if preservation and storage render *C. mariae* lesions more difficult to detect with the passage of time—if, for example, texture changes that accompany ageing of fixed specimens make it harder to distinguish healthy from diseased host tissues. To test whether the increase in *C. mariae* abundance among English sole museum specimens was real or an artefact, we turned to the historical record.

Comparison of historical data to contemporary data also revealed an increase in *C. mariae* prevalence over time. For literature records of *C. mariae*, prevalence was consistently higher at contemporary time points compared to their matched historical counterparts (Figure 5); these data also suggested a decrease in *C. mariae* prevalence with increasing latitude in contemporary Puget Sound (blue bars in Figure 5). For raw data on *C. mariae* burden, fish collected in 2017 carried significantly higher burdens than fish collected in 1949–1951, even though they were, on average, smaller than the fish from the historical collections (Figure 6). In fact, while there was a strong relationship between body size and *C. mariae* burden for contemporary fish (a common pattern across many parasites; Pacala & Dobson, 1988; Hudson & Dobson, 1995; Poulin & Morand, 2000), there was no such relationship for historical fish. This pattern could be explained by several processes. First, infection pressure might have been significantly lower in the past, such that infection was a stochastic process unrelated to fish age or size. Second, it might be the case that adult fish are eating or incidentally ingesting more copepods today than they did in the past, reflecting a temporal change in ontogenetic diet shifts. Third, *C. mariae* might have been more virulent in the past (Read, 1994), causing mortality of infected juveniles and thereby selecting for adults resistant to infection (sensu Hawlena, Abramsky, & Krasnov, 2006). Finally, fish immune systems might have been more capable of clearing infections in

the past—if, for example, pollutants erode fish immunocompetence (Segner, Wenger, Möller, Köllner, & Casanova-Nakayama, 2012)—thereby effectively decoupling fish age/size from infection burden (see Appendix M in Wood, Sandin, et al., 2014). Our data cannot discriminate among these explanations for the divergence in body size–parasite burden relationship between the historical and contemporary data.

Given that these different datasets all show the same pattern of increasing infection over time, we can conclude that patterns of relative temporal change in *C. mariae* inferred from museum specimens agree with those that are directly observed. Do museum specimens also give an accurate absolute estimate of *C. mariae* abundance? To test this, we matched historical records to museum specimens collected from the same place and roughly (within one decade) the same time. This process yielded only three temporal–spatial matches between historical data and museum specimens, and the museum specimen data were sparse, with extremely low replication, so the results should be interpreted with substantial caution. However, these data suggest correspondence between prevalence calculated by the two methods, with underestimation of prevalence by the museum specimen method. This might be expected because preservation reduces colour and texture differences between infected and healthy tissue, leading to lower probabilities of detection (Figure 3).

Our orthogonal datasets have converged on the conclusion that Puget Sound English sole experienced an increase in *C. mariae* infection between the mid-1900s and the present day, but what drove this increase? We suggest several, non-mutually exclusive hypotheses. As English sole move over a scale of only hundreds of kilometres (Stewart, 2007) and exhibit extreme site-fidelity in Puget Sound (Day, 1976), their infection status is likely to reflect local conditions. In the past, increases in *C. mariae* prevalence have been attributed to locally high densities of fish. For example, in 1943, Washington State Department of Fisheries employee Fred Cleaver wrote a memorandum about unusually high proportions of *C. mariae*-infected flatfish in Holmes Harbor, WA, reporting that the fishermen "thought it to be due to concentrations of fish in the small area of the harbor" (Cleaver, 1943). Such an explanation is consistent with our data. English sole fisheries were closed south of Point Deception in 1948 (green star in Figure 2) and south of Admiralty Inlet in 1989 (blue star in Figure 2), leading—presumably—to an increase in English sole density. Such an increase in sole density could facilitate an increase in *C. mariae* transmission; May and Dobson (1987) argued on theoretical grounds that it may be possible to "fish out" or locally extirpate parasites by reducing fish host density below that level needed for parasite transmission, and Wood, Sandin, et al. (2014) and Wood et al. (2015) demonstrated that even artisanal, non-commercial fishing pressure can depress the abundance of parasites. However, many factors other than cessation of fishing pressure have changed in Puget Sound since the 1930s, and are as likely to contribute to the observed increase in *C. mariae* abundance as fishing cessation, particularly in the light of the life cycle of this parasite. As a complex life cycle parasite,

C. mariae requires a copepod first intermediate host to complete its life cycle (Figure 1). Copepods, which are primary consumers, tend to be sensitive to changes in the abundance of phytoplankton, which is itself sensitive to changes in nutrients and temperature. Puget Sound has undergone significant changes in nutrient and temperature regimes in the past 100 years. Development of the Puget Sound watershed—including removal of vegetative cover through logging and addition of impermeable surface cover through development—has led to increases in the amount of nutrient-laden runoff reaching the Sound (Cuo, Lettenmaier, Alberti, & Richey, 2009; Trainer et al., 2003). Simultaneously, water temperatures in Puget Sound have risen in recent decades as the result of global climate warming; at the Race Rocks lighthouse in the Strait of Juan de Fuca, surface waters have warmed 1.0°C since 1950 (Snover, Mote, Whitely Binder, Hamlet, & Mantua, 2005). These factors could combine to shift the composition of the zooplankton community, perhaps favoring copepod species suited to hosting *C. mariae*; indeed, a shift towards smaller bodied copepod species with more southerly ranges has already been observed along the Pacific coast of Canada (Chandler, King, & Perry, 2017). Nutrient-mediated increases in infection by complex life cycle parasites have been demonstrated in other systems (McKenzie & Townsend, 2007; Wood et al., 2015). Although we do not examine which of these hypotheses (fishing cessation, nutrient pollution, climate change) is more likely to explain the observed change in *C. mariae* abundance over time, the timeline of infection built by our museum specimen dataset is well-suited to the task. For example, we might ask whether annual-scale sea surface temperature anomalies correlate (perhaps with a time-lag) to elevated *C. mariae* abundance. Alternately, if nutrient pollution or copepod density varies among regions within Puget Sound, we might test whether change in *C. mariae* abundance over time varies among these regions. Finally, we could compare historical estimates of English sole density (or, inversely, fishing pressure) against historical estimates of *C. mariae* abundance. We might even be able to compete these factors against one another simultaneously, to assess which best explains variation in *C. mariae* burden.

Our success in using museum specimens to reconstruct long-term change in *C. mariae* prevalence and burden bodes well for the use of this technique with other parasite species. *C. mariae* infections are obvious upon gross external examination of a fish (Figure 3), making *C. mariae* infection likely to affect the collector's choice of whether to collect, preserve and retain a fish specimen. That we found strong correspondence between historical data and estimates of prevalence from museum specimens suggests that the effect of such bias is minimal. We conclude that the use of museum specimens for reconstructing timelines of parasite abundance holds significant promise, especially for the vast majority of parasites whose presence is invisible to the naked eye.

Defining a baseline state for infection is vital for marine resource management and policy, which must respond to the threat of disease; without such a baseline, managers attempting to maintain or recover the health of ecosystems under their stewardship are shooting in the dark. The method we present here—using museum specimens to reconstruct detailed chronologies of parasite abundance change—offers a solution. For a rare instance in which both historical records and museum specimens exist, we showed that museum specimens provide an unbiased estimate of change in parasite abundance over time, at a much finer temporal resolution than is possible to obtain from historical records. The method we outline could allow managers of marine, freshwater and terrestrial resources to reconstruct historical baselines of parasite abundance for their focal biological resource, in any ecosystem represented by liquid-preserved vertebrate or invertebrate host specimens. Natural history collections contain hundreds of millions of liquid-preserved specimens from diverse geographic regions, host taxa and time points (Harmon, Littlewood, & Wood, in press). In many cases, this approach will be the only avenue available for obtaining data on parasite abundance in a "healthy" ecosystem and thereby developing a realistic baseline for infection management. Such baselines are more vital than ever, as parasites increasingly influence natural resource use and conservation, and our approach of using museum specimens could provide a promising way forward.

AUTHORS' CONTRIBUTIONS

I.H., T.Q., and C.L.W. conceived ideas and designed methodology; I.H., E.D., G.L. and C.L.W. collected the data; I.H. and C.L.W. analysed the data; I.H. and C.L.W. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.sc09839 (Howard, Davis, Lippert, Quinn, & Wood, 2018).

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REFERENCES

Alverson, D. L., Palmen, A. T., & Pasquale, N. (1956). *Report on the 1955 trawl investigations*. Olympia, WA: State of Washington Department of Fisheries.

Arai, H. P., & Smith, J. W. (2016). Guide to the parasites of fishes of Canada Part V: Nematoda. *Zootaxa*, *4185*, 1–274. https://doi.org/10.11646/zootaxa.4185.1.1

Black, G. A. (1983). Taxonomy of a swimbladder nematode, *Cystidicola stigmatura* (Leidy), and evidence of its decline in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Science*, *40*, 643–647. https://doi.org/10.1139/f83-085

Chandler, P. C., King, S. A., & Perry, R. I. (2017). *State of the physical, biological and selected fishery resources of the Pacific Canadian marine ecosystems in 2016*. Canadian Technical Report of Fisheries and Aquatic Sciences 3225. Fisheries and Oceans Canada Institute of Ocean Sciences, Sidney, BC.

Cleaver, F. (1943). *Regarding wormy flatfish in Holmes Harbor, trawling in Birch Bay, and the use of gill nets to catch dogfish*. Seattle, WA: State of Washington Department of Fisheries.

Cuo, L., Lettenmaier, D. P., Alberti, M., & Richey, J. E. (2009). Effects of a century of land cover and climate change on the hydrology of the Puget Sound basin. *Hydrological Processes*, *23*, 907–933. https://doi.org/10.1002/hyp.7228

Day, D. E. (1976). Homing behavior and population stratification in central Puget Sound English sole (*Parophyrs vetulus*). *Journal of the Fisheries Research Board of Canada*, *33*, 278–282. https://doi.org/10.1139/f76-039

Deem, S. L., Karesh, W. B., & Weisman, W. (2001). Putting theory into practice: Wildlife health in conservation. *Conservation Biology*, *15*, 1224–1233. https://doi.org/10.1046/j.1523-1739.2001.00336.x

DiEuliis, D., Johnson, K. R., Morse, S. S., & Schindel, D. E. (2016). Specimen collections should have a much bigger role in infectious disease research and response. *Proceedings of the National Academy of Sciences of the USA*, *113*, 4–7. https://doi.org/10.1073/pnas.1522680112

Fournier, D. A., Skaug, H. J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M., ... Sibert, J. (2012). AD Model Builder: Using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software*, *27*, 233–249. https://doi.org/10.1080/10556788.2011.597854

Frederick, P. C., McGehee, S. M., & Spalding, M. G. (1996). Prevalence of *Eustrongylides ignotus* in mosquitofish (*Gambusia holbrooki*) in Florida: Historical and regional comparisons. *Journal of Wildlife Diseases*, *32*, 552–555. https://doi.org/10.7589/0090-3558-32.3.552

Gooding, D. (1956). *64th Annual Report: Washington Department of Fisheries, Year 1954*. Olympia, WA: State of Washington Department of Fisheries.

Harmon, A., Littlewood, D. T. J., & Wood, C. L. (in press). Parasites lost: Using natural history collections to track disease change across deep time. *Frontiers in Ecology and the Environment*.

Hartigan, A., Phalen, D. N., & Šlapeta, J. (2010). Museum material reveals a frog parasite emergence after the invasion of the cane toad in Australia. *Parasites and Vectors*, *3*, 50. https://doi.org/10.1186/1756-3305-3-50

Hawlena, H., Abramsky, Z., & Krasnov, B. R. (2006). Ectoparasites and age-dependent survival in a desert rodent. *Oecologia*, *148*, 30–39. https://doi.org/10.1007/s00442-005-0345-4

Hewson, I., Button, J. B., Gudenkauf, B. M., Miner, B., Newton, A. L., Gaydos, J. K., ... Fradkin, S. (2014). Densovirus associated with sea-star wasting disease and mass mortality. *Proceedings of the National Academy of Sciences of the USA*, *111*, 17278–17283. https://doi.org/10.1073/pnas.1416625111

Holland, G. A. (1954). *A preliminary study of the populations of English sole (Parophrys vetulus, Girard) in Carr Inlet and other localities in Puget Sound. University of Washington*. MS thesis, Department of Fisheries, University of Washington, Seattle, WA.

Holland, G. A. (1969). *Age, growth, and mortality of races of English sole (Parophrys vetulus) in Puget Sound*. Portland, OR: Pacific Marine Fisheries Commission.

Howard, I., Davis, E., Lippert, G., Quinn, T. P., & Wood, C. L. (2018). Data from: Abundance of an economically important nematode parasite increased in Puget Sound between 1930 and 2016: Evidence from museum specimens confirms historical data. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.sc09839

Hudson, P. J., & Dobson, A. P. (1995). Macroparasites: Observed patterns in naturally fluctuating animal populations. In B. T. Grenfell, & A. P. Dobson (Eds.), *Ecology of infectious disease in natural populations*. Cambridge, UK: Cambridge University Press.

Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution*, *21*, 381–385. https://doi.org/10.1016/j.tree.2006.04.007

Jackson, J. B., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., ... Hughes, T. P. (2001). Historical overfishing and the recent collapse of coastal ecosystems. *Science*, *293*, 629–637. https://doi.org/10.1126/science.1059199

Johnson, K. G., Brooks, S. J., Fenberg, P. B., Glover, A. G., James, K. E., Lister, A. M., ... Young, J. R. (2011). Climate change and biosphere response: Unlocking the collections vault. *BioScience*, *61*, 147–153. https://doi.org/10.1525/bio.2011.61.2.10

Johnson, P. T., Lunde, K. B., Zelmer, D. A., & Werner, J. K. (2003). Limb deformities as an emerging parasitic disease in amphibians: Evidence from museum specimens and resurvey data. *Conservation Biology*, *17*, 1724–1737. https://doi.org/10.1111/j.1523-1739.2003.00217.x

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., ... Saksida, S. M. (1997). Environmental parasitology: What can parasites tell us about human impacts on the environment? *Parasitology Today*, *13*, 251–255. https://doi.org/10.1016/S0169-4758(97)01072-7

Lafferty, K. D. (2009). The ecology of climate change and infectious diseases. *Ecology*, *90*, 888–900. https://doi.org/10.1890/08-0079.1

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., ... Saksida, S. M. (2015). Infectious diseases affect marine fisheries and aquaculture economics. *Annual Review of Marine Science*, *7*, 471–496. https://doi.org/10.1146/annurev-marine-010814-015646

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., & Jackson, J. B. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, *312*, 1806–1809. https://doi.org/10.1126/science.1128035

Lozano-Montes, H. M., Pitcher, T. J., & Haggan, N. (2008). Shifting environmental and cognitive baselines in the upper Gulf of California. *Frontiers in Ecology and the Environment*, *6*, 75–80. https://doi.org/10.1890/070056

Marcogliese, D. (2001). Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology*, *79*, 1331–1352. https://doi.org/10.1139/z01-067

Margolis, L., & Arthur, J. (1979). *Synopsis of the parasites of fishes of Canada*. Fisheries Research Board of Canada Bulletin 199, Fisheries and Oceans, Government of Canada, Ottawa, Canada.

May, R., & Dobson, A. (1987). The effects of parasites on fish populations – Theoretical aspects. *International Journal for Parasitology*, *17*, 363–370.

McClenachan, L. (2009). Documenting loss of large trophy fish from the Florida Keys with historical photographs. *Conservation Biology*, *23*, 636–643. https://doi.org/10.1111/j.1523-1739.2008.01152.x

McKenzie, V. J., & Townsend, A. R. (2007). Parasitic and infectious disease responses to changing global nutrient cycles. *EcoHealth*, *4*, 384–396. https://doi.org/10.1007/s10393-007-0131-3

Pacala, S. W., & Dobson, A. P. (1988). The relation between the number of parasites/host and host age: Population dynamic causes and

maximum likelihood estimation. *Parasitology*, 96, 197–210. https://doi.org/10.1017/S0031182000081762

Palsson, W. A., Hoeman, J. C., Bargman, G. G., & Day, D. E. (1996). *1995 status of Puget Sound bottomfish stocks*. Olympia, WA: Washington Department of Fish and Wildlife.

Miller, B. S., McCain, B. B., Wingert, R. C., Borton, S. F., Pierce, K. V., & Griggs, D. T. (1977). *Puget Sound interim studies: Ecological and disease studies of demersal fishes in Puget Sound near Metro-operated sewage treatment plants and in the Duwamish River*. Seattle, WA: Fisheries Research Institute, College of Fisheries, University of Washington.

Poulin, R., & Morand, S. (2000). The diversity of parasites. *Quarterly Review of Biology*, 75, 277–293. https://doi.org/10.1086/393500

Quinnell, S. (1984). *The trawl fisheries for English sole (*Parophrys vetulus*) of Washington State's inside waters: Fisheries trend*. Olympia, WA: State of Washington Department of Fisheries.

Read, A. F. (1994). The evolution of virulence. *Trends in Microbiology*, 73, 73–76. https://doi.org/10.1016/0966-842X(94)90537-1

Reeves, R. R., & Cosens, S. E. (2003). Historical population characteristics of bowhead whales (*Balaena mysticetus*) in Hudson Bay. *Arctic*, 56, 283–292.

Rosenberg, A. A., Bolster, W. J., Alexander, K. E., Leavenworth, W. B., Cooper, A. B., & McKenzie, M. G. (2005). The history of ocean resources: Modeling cod biomass using historical records. *Frontiers in Ecology and the Environment*, 3, 84–90. https://doi.org/10.2307/3868514

Saenz-Arroyo, A., Roberts, C. M., Torre, J., & Cariño-Olvera, M. (2005). Using fishers' anecdotes, naturalists' observations and grey literature to reassess marine species at risk: The case of the Gulf grouper in the Gulf of California, Mexico. *Fish and Fisheries*, 6, 121–133. https://doi.org/10.1111/j.1467-2979.2005.00185.x

Sagarin, R. D., Gilly, W. F., Baxter, C. H., Burnett, N., & Christensen, J. (2008). Remembering the Gulf: Changes to the marine communities of the Sea of Cortez since the Steinbeck and Ricketts expedition of 1940. *Frontiers in Ecology and the Environment*, 6, 372–379. https://doi.org/10.1890/070067

Segner, H., Wenger, M., Möller, A. M., Köllner, B., & Casanova-Nakayama, A. (2012). Immunotoxic effects of environmental toxicants in fish – How to assess them? *Environmental Science and Pollution Research*, 19, 2465–2476. https://doi.org/10.1007/s11356-012-0978-x

Sethi, S. N., Jithendran, K. P., & Dash, G. (2013). Occurrence of *Philometra lateolabracis* (Philometridae) in the subcutaneous caudal fin region of sciaenid fishes of Bay of Bengal Large Marine Ecosystem (BOBLME), Tamil Nadu (India). *Indian Journal of Geo-Marine Sciences*, 42, 606–610.

Skaug, H., Fournier, D., Nielsen, A., Magnusson, A., & Bolker, B. (2016). Generalized linear mixed models using 'AD Model Builder'. R package version 0.8.3.3. Retrieved from http://glmmadmb.r-forge.r-project.org

Smith, R. T. (1936). *Report on the Puget Sound Otter Trawl Investigations*. Olympia, WA: Department of Fisheries, State of Washington.

Snover, A. K., Mote, P. W., Whitely Binder, L. C., Hamlet, A. F., & Mantua, N. J. (2005). *Uncertain future: Climate change and its effects on Puget Sound*. A report for the Puget Sound Action Team by the Climate Impacts Group (Joint Institute for the Study of the Atmosphere and Oceans, University of Washington, Seattle). Puget Sound Action Team, Office of the Governor, State of Washington, Olympia, WA.

Stewart, I. J. (2007). Defining plausible migration rates based on historical tagging data: A Bayesian mark-recapture model applied to English sole (*Parophrys vetulus*). *Fishery Bulletin*, 105, 470–484.

TCW Economics. (2008). *Economic analysis of the non-treaty commercial and recreational fisheries in Washington State*. Olympia, WA: Prepared for Washington Department of Fish and Wildlife.

Trainer, V. L., Eberhart, B. T. L., Wekell, J. C., Adams, N. G., Hanson, L., Cox, F., & Dowell, J. (2003). Paralytic shellfish toxins in Puget Sound, Washington. *Journal of Shellfish Research*, 22, 213–222.

WDFW. (2011). *Final Puget Sound rockfish conservation plan: Policies, strategies, and actions, including preferred range of actions*. Olympia, WA: Washington Department of Fish and Wildlife.

Williams, R. (1950). *The bottom fishery and otter trawling*. Olympia, WA: Washington State Department of Fisheries.

Willis, C. G., Ruhfel, B., Primack, R. B., Miller-Rushing, A. J., & Davis, C. C. (2008). Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the USA*, 105, 17029–17033. https://doi.org/10.1073/pnas.0806446105

Wood, C. L., Baum, J. K., Reddy, S. M. W., Trebilco, R., Sandin, S. A., Zgliczynski, B. J., ... Micheli, F. (2015). Productivity and fishing pressure drive variability in fish parasite assemblages of the Line Islands, equatorial Pacific. *Ecology*, 96, 1383–1398. https://doi.org/10.1890/13-2154.1

Wood, C. L., & Lafferty, K. D. (2015). How have fisheries affected parasite communities? *Parasitology*, 142, 134–144. https://doi.org/10.1017/S003118201400002X

Wood, C. L., Lafferty, K. D., DeLeo, G., Young, H. S., Hudson, P. J., & Kuris, A. M. (2014). Does biodiversity protect humans against infectious disease? *Ecology*, 95, 817–832. https://doi.org/10.1890/13-1041.1

Wood, C. L., Sandin, S. A., Zgliczynski, B., Guerra, A. S., & Micheli, F. (2014). Fishing drives declines in fish parasite diversity and has variable effects on parasite abundance. *Ecology*, 95, 1929–1946. https://doi.org/10.1890/13-1270.1

Young, H. S., Parker, I. M., Gilbert, G. S., Sofia Guerra, A., & Nunn, C. L. (2017). Introduced species, disease ecology, and biodiversity–disease relationships. *Trends in Ecology and Evolution*, 32, 41–54. https://doi.org/10.1016/j.tree.2016.09.008

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