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Long-term change in the parasite burden of shore crabs (*Hemigrapsus oregonensis* and *Hemigrapsus nudus*) on the northwestern Pacific coast of North America

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The abundances of free-living species have changed dramatically in recent decades, but little is known about change in the abundance of parasitic species. We investigated whether populations of several parasites have shifted over time in two shore crab hosts, *Hemigrapsus oregonensis* and *Hemigrapsus nudus*, by comparing the prevalence and abundance of three parasite taxa in a historical dataset (1969–1970) to contemporary parasite abundance (2018–2020) for hosts collected from 11 intertidal sites located from Oregon, USA, to British Columbia, Canada. Our data suggest that the abundance of the parasitic isopod *Portunion conformis* has varied around a stable mean for the past 50 years. No change over time was observed for larval acanthocephalans. However, larval microphallid trematodes increased in prevalence over time among *H. oregonensis* hosts, from a mean of 8.4–61.8% between the historical and contemporary time points. The substantial increase in the prevalence of larval microphallid trematodes could be owing to increased abundances of their bird final hosts, increased production of parasite infective stages by snail intermediate hosts or both. Our study highlights the variability among parasite species in their temporal trajectories of change.

1. Introduction

In the current era of global change, the abundances of numerous taxa are reassorting. Geographically widespread declines have been reported among insects (e.g. [1]), amphibians (e.g. [2]), mammals (e.g. [3]) and birds (e.g. [4]). Meanwhile, other taxa may be on the rise, including plants that benefit from anthropogenic nutrient enrichment [5], invasive species [6], synanthropic species (e.g. [7]) and species recovering from historical impacts that have since been mitigated (e.g. [8]). Historical ecology research is identifying the 'winners' and 'losers' in this changing world, at least among free-living species, by reconstructing long-term trajectories of abundance. For parasitic species, we have few data to indicate the direction of temporal change, even though these changes are likely both (i) to occur and (ii) to be ecologically consequential.

Although parasites are small and mostly unseen, they can have a large impact on the communities and ecosystems in which they are embedded, influencing the abundance and diversity of other organisms and redirecting energy flow through entire ecosystems [9]. However, despite their ecological importance, we know little about how parasite populations have changed over

time in response to human-induced environmental change [10,11]. Long-term datasets could help to reveal trajectories of parasite abundance change in response to human impacts on ecosystems, but these datasets are exceedingly rare [10,11].

Among the few long-term datasets of parasite abundance that do exist, a diversity of temporal trajectories are observed. For example, Byers *et al*. [12] tracked the infection prevalence over 11 years of several trematode species in the intertidal snail *Littorina littorea*, finding stasis in parasite prevalence (an average change of less than 1%) over the period of the dataset. On the other hand, Cort *et al*. [13] examined the freshwater snail *Stagnicola emarginata angulata* from one location on Douglas Lake, Michigan, in 1936 and 1956—a 20-year time span—and found an overall reduction in the diversity of trematodes and in the prevalence of most trematode species over time. This study was followed up in 1994, almost 60 years after the initial time point, by Keas & Blankenspoor [14], who showed that the declines in diversity and prevalence originally detected by Cort *et al*. [13] continued in the ensuing decades. Other studies have found increasing parasite abundance through time; for example, Howard *et al*. [11] performed parasitological dissections of liquid-preserved fish specimens and found a previously undetected eightfold increase in the abundance of an economically important nematode parasite (*Clavinema mariae*) in English sole (*Parophrys vetulus*) between 1930 and 2016. These few long-term datasets suggest that—as we often observe for free-living species—there are likely to be 'winners' and 'losers' among parasites in a changing world.

We had access to a unique historical dataset on parasite abundance in two species of grapsoid crabs, *Hemigrapsus oregonensis* and *Hemigrapsus nudus*, sampled during the Pacific Transect Expedition (PacTrEx) in May 1969 and February 1970 [15]. This historical dataset was collected by A.M.K., who performed parasitological dissections of *H. oregonensis* and *H. nudus* collected at various intertidal sites from Baja California, Mexico, to British Columbia, Canada. The PacTrEx dataset focused on *Portunion conformis* [15], a castrating parasitic isopod. However, A.M.K. also tracked other parasite species, including larval cystacanths of acanthocephalans and larval metacercariae of trematodes in the family Microphallidae.

We investigated change over time in abundance of the parasites of these two species of shore crab by replicating the collection and dissection methods used by A.M.K., so that our final dataset included measurements from 1969, 1970, 2018, 2019 and 2020. Given the substantial anthropogenic impacts experienced by Pacific northwest coastal ecosystems in the past five decades, we expected to observe change in the abundance of the three parasite species we tracked.

2. Methods

(a) Contemporary collection

We strove to reproduce the methods, sites, collection dates and levels of replication of the PacTrEx as closely as possible [15], with some important exceptions noted below. To facilitate this process, the leader of the PacTrEx study (A.M.K.) trained the data collectors of the present study (J.Q., D.G. and A.G.) in all crab collection and dissection protocols and was available for consultation as questions arose. *Hemigrapsus nudus* and *H. oregonensis* were collected from three sites in Oregon, six sites in Washington and two sites in British Columbia (electronic supplementary material, table S1; figure 1). All historical and contemporary crab collections were conducted by hand during low tide at each sampling site. Given that parasite burden can vary substantially over small spatial scales, we sought to ensure that the exact same locations were sampled within each sampling site. To that end, A.M.K. sampled several sites alongside J.Q. and D.G., using the detailed field notes from his 1969–1970 sampling expeditions to ensure that the exact same location was sampled. For contemporary sampling trips that A.M.K. was not available to join, the team used Google Earth to query A.M.K. about the exact location of sampling. The contemporary crabs were kept alive and transported to the laboratory, where they were euthanized by freezing. Crabs were stored in a freezer for at least 48 h before dissection.

There was one important difference between the methods of the historical PacTrEx study and the contemporary study. In the historical study, A.M.K. dissected all of the crabs collected immediately following euthanasia, without freezing, while in the contemporary study, J.Q., D.G. and A.G. froze crabs and dissected them after freezing. To test whether this difference in methods would influence parasite counts, we performed an experiment (see the electronic supplementary material, text S1).

(b) Crab dissection

We performed dissections of all sampled crabs in order to identify and count their parasites and matched our protocols as closely as possible to those used in the PacTrEx study. Dissections were performed identically for both *H. oregonensis* and *H. nudus*. Crabs were retrieved from the freezer and left to thaw in room-temperature sea water. The carapace width was measured in millimetres by placing calipers between the second and third carapace spikes. Sex was also recorded for each crab.

Parasitological dissections were performed by lifting the carapace of the crab, cutting the digestive tract at the juncture of the thorax and the abdomen, and carefully examining the tissues of the crab for *P. conformis*. The remaining digestive tissue of the crab was removed and searched for other parasites, such as acanthocephalans, which were counted. After the digestive tissue was removed and searched, the body cavity of the crab was searched for metacercariae. We used the same categorical system to quantify metacercariae that was used in the PacTrEx study: '0' for no metacercariae, '+' for 1–5, '++' for 6–25 and '+++' for more than 25.

(c) Statistical analysis

In the historical dataset, data were collected for *P. conformis*, larval cystacanths of acanthocephalans and larval metacercaria from trematodes in the family Microphallidae. Our response variables included the abundance (number of individual parasites in a host, including both infected and uninfected hosts; *sensu* [16]) of *P. conformis* and acanthocephalan cystacanths and the categorical abundance category of metacercariae (see above). Statistical models were customized to accommodate the ordinal nature of the metacercariae data (see below). We were concerned that differences in the host sex ratio between the historical and contemporary collections might influence our conclusions. Given that historical sampling focused primarily on females (n females in historical sample = 1982, n males = 10), we limited our analysis to female crabs. To test for effects of freezing on parasite detectability, we used generalized linear fixed-effects models. Details are provided in the electronic supplementary material, text S1.

Our primary aim was to assess whether parasite burden differed between historical (1969–1970) and contemporary (2018–2020) samples. We sought to avoid confounding variability

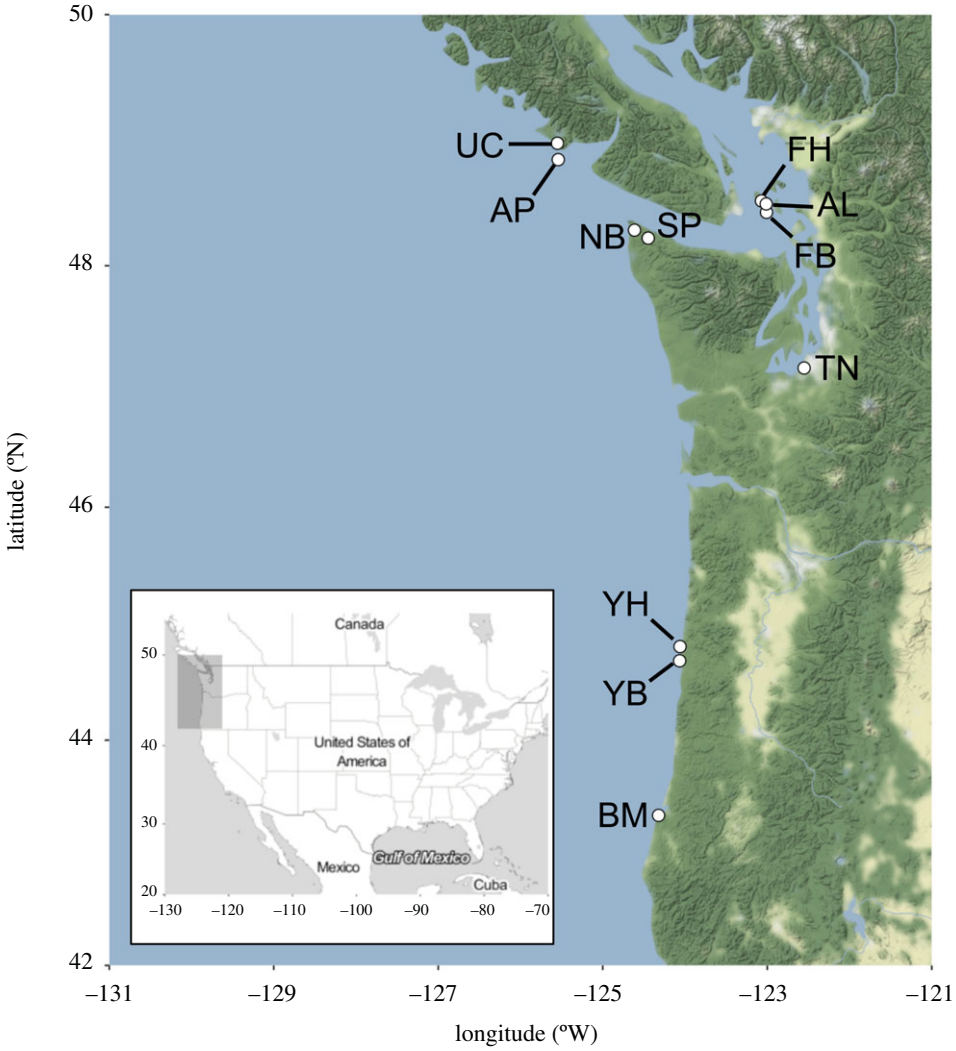

Figure 1. Map of all collection sites where the number of female crabs of at least one species (*H. oregonensis* and *H. nudus*) was sufficient to make comparisons of parasite burden between the historical and contemporary datasets (electronic supplementary material, table S1). Site locations are jittered slightly in the *y*- (i.e. latitudinal-) dimension to allow better visualization of nearby sites. Site names for each code are shown in the electronic supplementary material, table S1. (Online version in colour.)

across space with variability across time; therefore, we included in our analysis only those sites that yielded at least $n = 10$ crabs at both the historical and contemporary time point (electronic supplementary material, table S1). For each parasite species encountered in both the historical and contemporary collections, we constructed a generalized linear mixed model with the fixed effects of time (historical versus contemporary), crab species (*H. nudus* versus *H. oregonensis*) and their interaction, as well as crab carapace width, with a random effect of site (to account for multiple observations at each site) and a random effect of year nested in time (i.e. historical versus contemporary; to account for the fact that samples collected within the same year are more likely to be similar than samples collected in different years). Both host species were included in one model because our scientific questions pertained to change in the parasites' overall abundance through time and were not specific to one host or the other. For *P. conformis* and acanthocephalans, we chose whether the response variable would be modelled as a Gaussian, Poisson or negative binomial distribution by selecting the model with the lowest Akaike's information criterion. Acanthocephalan and *P. conformis* models were fitted using the *glmer()* and *glmer.nb()* functions in the lme4 package in R [17]. For metacercariae (which were classified into abundance categories rather than counted), we used an ordinal fixed-effects linear regression with a probit-link function using the *clm()* function in the ordinal package in R [18]. Because we were interested in the interaction time*crab species in models that contained the crab species effect, we systematically switched each crab species into the 'baseline' or 'reference' position (i.e. so that n identical models were run, each with a different one of n crab species represented by the intercept) and recorded the coefficient for each crab species in table 1. We plotted predictions from each best-fit model using the *ggeffect()* function in the ggeffects package in R [19].

We made the choice to exclude some data owing to one data quality issue. Three data sheets from 1970 indicated the metacercariae ordinal category by data sheet page, and not by individual crab (i.e. the collector noted, 'few to many metacerc in most crabs' at the top of the page). If we had excluded only the three affected pages, we would have been disproportionately excluding those pages most likely to contain crabs infected with metacercariae, thereby biasing historical estimates of metacercarial abundance downwards. Therefore, for metacercariae only, data from 1970 were excluded and, in the statistical model for metacercariae, the random effect of year nested in time was dropped (because only 1 year (1969) remained in the 'historical' time category) and a random effect of year was introduced (see model formulation above).

We were also interested in testing whether the long-term changes we detected (i.e. historical versus contemporary) were larger or smaller in magnitude than the short-term changes we observed (e.g. 1969–1970). For each parasite species that changed

Table 1. Results of generalized linear mixed models comparing historical and contemporary estimates of parasite abundance for each parasite taxon.

	estimate	s.e.	*z*-value	*p*-value
(*a*) *Portunion conformis* (negative binomial; *n* crab species=2, *n* crabs=1790, *n* sites=11, *n* years=5)				
crab_species[*H. nudus*]	−0.29414	0.18432	−1.596	0.1110
time_point[historical/*H. oregonensis* in reference position]	0.30472	0.27137	1.123	0.261
time_point[historical/*H. nudus* in reference position]	−0.54703	0.27860	−1.963	0.0496
host_carapace_width_mm	0.09323	0.01201	7.763	<0.0001
crab_species[*H. nudus*]*time_point[historical]	−0.85175	0.18273	−4.661	<0.0001
(*b*) acanthocephalan cystacanths (Gaussian; *n* crab species=1, *n* crabs=1025, *n* sites=7, *n* years=5)				
time_point[historical]	−0.00038	0.01657	−0.023	0.9818
host_carapace_width_mm	−0.00067	0.00281	−0.235	0.8144
(*c*) microphallid trematode metacercariae (ordinal: *n* crab species=1, *n* crabs=696, *n* sites=7, *n* years=4)				
time_point[historical]	−1.41207	0.38959	−3.625	0.0003
host_carapace_width_mm	0.17430	0.02373	7.346	<0.0001

significantly in abundance between the historical and contemporary collections, we took the average abundance of the parasite at each site in each year (average prevalence for metacercariae, because its abundance was classified ordinally) and found the difference among every pair of years sampled for that site. Because this metric compares each site against itself through time, it avoids confounding variability across space with variability across time. We then compared the absolute value of the magnitude of the short-term differences at a given site (i.e. 1969–1970, 2018–2019, 2019–2020 and 2018–2020) against the absolute value of the magnitude of the long-term differences at the same site (i.e. 1969–2018, 1969–2019, 1969–2020, 1970–2018, 1970–2019 and 1970–2020) with a general linear mixed model containing the response variable parasite difference (i.e. difference in parasite abundance or prevalence between the 2 years examined), fixed effects of time difference (interannual versus long-term), crab species (*H. nudus* versus *H. oregonensis*) and their interaction, with a random effect of site (to account for multiple observations for each site). Gaussian models were fitted using the *lmer()* functions in the lme4 package in R [17].

3. Results

We found substantial differences in the presence/absence of the two *Hemigrapsus* species between historical and contemporary sampling (electronic supplementary material, table S1). We analysed only those site–host species combinations where both historical and contemporary sampling successfully yielded at least 10 individuals ($n = 7$ sites for *H. oregonensis*, $n = 7$ sites for *H. nudus*, $n = 11$ sites overall; figure 1).

In total, we dissected 3488 crabs across the historical ($n = 1992$) and contemporary ($n = 1496$) time points. Of these, 2596 were female, and of this subset, 1790 were collected at sites where we had at least 10 individuals of that crab species from both historical and contemporary time points. Among this group, 1035 were *H. oregonensis* and 755 *H. nudus*. Across both the historical and contemporary time points, we observed three parasite taxa: *P. conformis*, larval cystacanths of acanthocephalans and larval metacercariae from trematodes in the family Microphallidae. Of all the crabs sampled (both *H. oregonensis* and *H. nudus*), 36.0% were infected with *P. conformis* (34.8% at the historical time point and 38.4% at the contemporary time point; electronic supplementary material, table S2). We found only one acanthocephalan cystacanth in *H. nudus* across both contemporary and historical time points (electronic supplementary material, table S2); therefore, we analysed change over time in the abundance of acanthocephalans only for *H. oregonensis*, of which 3.7% were infected (4.1% at the historical time point and 2.4% at the contemporary time point). We found only four *H. nudus* infected with metacercariae across both contemporary and historical time points (electronic supplementary material, table S2); therefore, we analysed change over time in the abundance of metacercariae only for *H. oregonensis*, of which 30.2% were infected with microphallid metacercariae (8.4% at the historical time point and 61.8% at the contemporary time point).

In the experiment comparing the detectability of parasites between fresh specimens (i.e. the kind of specimens examined in the historical dataset) and frozen specimens (i.e. the kind of specimens examined in the contemporary dataset), we found few differences. There was no difference in the number of parasites detected between the two treatments for *P. conformis* or acanthocephalan cystacanths (electronic supplementary material, table S3 and figure S1). There was a marginally significant ($p = 0.09$) difference between treatments for metacercariae, because we found more metacercariae in fresh than in frozen specimens (electronic supplementary material, table S3c and figure S1c). However, this bias was conservative with respect to the temporal patterns observed (see below).

Portunion conformis did not change in abundance over time in *H. oregonensis*, and *H. nudus* experienced an increase in *P. conformis* abundance between the historical and contemporary time points (table 1*a* and figure 2*a*). However, the magnitude of this long-term change in *H. nudus* infection with *P. conformis* was commensurate with the magnitude of the short-term variability in abundance that we observed (table 2*a*; electronic supplementary material, figure S2).

Acanthocephalan cystacanths were only observed in *H. oregonensis* and their abundance did not change over time (table 1*b* and figure 2*b*). Because there was no significant change over time, we did not investigate whether the magnitude of long-term change was significantly different than the magnitude of short-term change.

The prevalence of microphallid metacercariae increased over time in *H. oregonensis* (table 1*c* and figure 2*c*). The

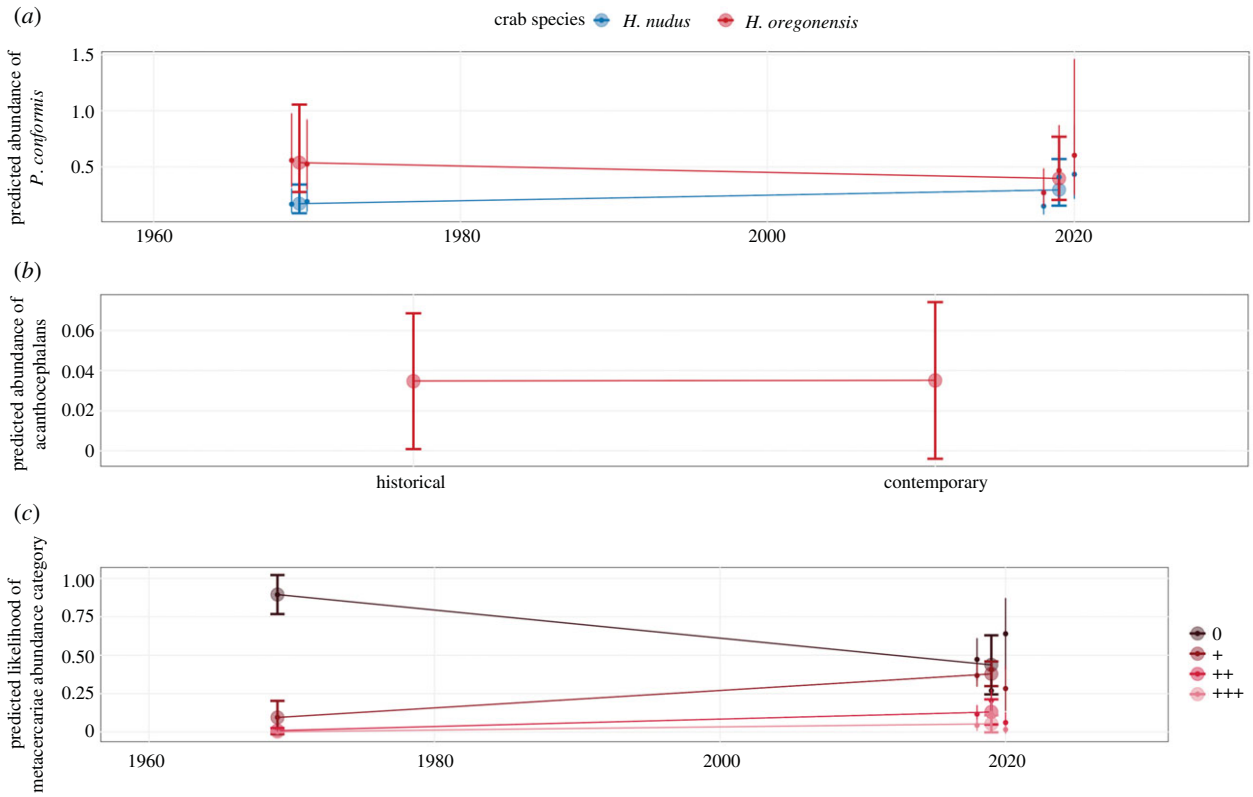

Figure 2. Comparison of the change in (*a*) predicted abundance of *P. conformis*, (*b*) predicted abundance of acanthocephalan cystacanths, and (*c*) predicted likelihood of trematode metacercariae abundance category over the 50 years between our historical versus contemporary sampling for *H. nudus* (blue) and *H. oregonensis* (red). Large, transparent dots represent grand means across all years within a time (i.e. historical versus contemporary), and small dots indicate means for individual years. All error bars represent 95% confidence intervals. In (*c*), metacercariae were classified into ordinal abundance categories (0, metacercariae absent; +, 1–5 metacercariae; ++, 6–25 metacercariae; +++, greater than 25 metacercariae), so results are shown for each abundance category, with lighter red colours indicating higher metacercariae abundance categories. All categories of metacercarial abundance are quantified at the historical time point (year = 1969), but the highest category (+++) is overlapped by the second highest category (++) at a low predicted likelihood ('+++' predicted likelihood = 0.001, '++' predicted likelihood = 0.009).

absolute value of the magnitude of the long-term change in metacercarial burden (i.e. historical versus contemporary) was significantly greater than the absolute value of the magnitude of short-term changes (table 2*b*; electronic supplementary material, figure S3). Prevalence was consistently high at each year sampled within the contemporary time point (year 1969 = 8.4%, 2018 = 55.5%, 2019 = 75.0%, 2020 = 53.5%; figure 2*c*). Metacercariae were observed at three of seven sites sampled during the historical time point (range of site-level prevalence values = 0.8–12%) and at 9 of 11 sites sampled during the contemporary time point (range of site-level prevalence values = 1.2–77.3%). We found a pattern of increasing metacercarial abundance over time despite the fact that we should have been less likely to find metacercariae in contemporary specimens, which were frozen before dissection, than in historical specimens, which were dissected immediately following euthanasia (electronic supplementary material, table S3c and figure S1c).

4. Discussion

Of the three parasite species that we were able to track through time, one exhibited short-term variability around a stable long-term mean abundance, one remained unchanged and a third increased substantially over the 50 year period of our study. While our ability to attribute these changes to a cause is limited by the correlational nature of our study, we speculate that these complex shifts in parasite abundance might be driven by four general classes of long-term change: (i) shifts in the abundance of *H. oregonensis* and *H. nudus*, (ii) shifts in the encounter rate between *H. oregonensis*/*H. nudus* and parasite propagules, (iii) shifts in the abundance of parasites or parasite reproductive rates in obligately required hosts other than *H. oregonensis*/*H. nudus*, and (iv) shifts in the relative susceptibility of *H. oregonensis*/*H. nudus* to infection.

To identify the most probable drivers of the patterns we observed, we first carefully examined the life cycles of the three parasites, all of which are obligately dependent on multiple host species. *Portunion conformis* is an entoniscid isopod that comes into contact with its definitive crab host via an aquatic, actively searching larval (cryptoniscus) stage [20]. The larva of *P. conformis* pierces the exoskeleton of its host to infiltrate the body cavity and takes up residence in the haemocoel of the crab [20]. When *P. conformis* reaches sexual maturity, its eggs hatch as epicaridium larvae and are released through the crab's gill chamber into the water, where they infect intermediate host copepods [20]. The cryptoniscis larva leaves the copepod to seek a crab final host. *Profilicollis botulus* is the only acanthocephalan identified in *H. oregonensis* and no acanthocephalan has been reported from *H. nudus* [21]. *Profilicollis botulus* uses *H. oregonensis* as an intermediate host, which passes the infection to the definitive host, usually a diving duck or gull, through trophic

Table 2. Results of general linear mixed models comparing the magnitude of the long-term changes we detected in parasite abundance (i.e. historical versus contemporary) against the magnitude of short-term changes (e.g. 1969–1970 or 2018–2019).

	estimate	s.e.	*t*-value	*p*-value
(*a*) *Portunion conformis* (Gaussian; *n* crab species=2, *n* obs=50, *n* sites=11)				
time_difference[long-term]	−0.15414	0.17517	−0.880	0.3789
crab_species[*H. oregonensis*]	−0.32118	0.20692	−1.552	0.1206
time_difference[long-term]*crab_species[*H. oregonensis*]	0.34290	0.23487	1.460	0.1443
(*b*) microphallid trematode metacercariae (Gaussian; *n* crab species=1, *n* obs=26, *n* sites=11)				
time_difference[long-term]	0.31624	0.08351	3.7868	0.0002

transmission [22]. The microphallid trematodes infect crabs when cercariae shed by a first intermediate host snail are released into the water column, where they can penetrate a host crab's carapace and encyst in the body cavity until they reach their definitive host (typically a sea bird) through trophic transmission [23]; once in the intestinal tract of the definitive host, the worms mature and release eggs with bird droppings. With these life-cycle details in mind, we considered four possible classes of long-term change as potential explanations for the patterns observed.

Both long-term and short-term shifts in host abundance can influence the abundance of parasites. Increases in the abundance of hosts can 'dilute' the abundance of parasites and decreases in host abundance can 'concentrate' parasites, if parasite abundance is measured as the average number of parasites per host (as it was here) and if change in host density does not lead to change in the total number of parasite propagules available to infect hosts [24]. This pattern is commonly observed in parasites with complex life cycles or parasites that have to seek a host [24], like our three species of interest. All parasites found in this study have complex life cycles and, therefore, pass through hosts of multiple species; any one of these hosts could constitute the 'life-cycle bottleneck' that regulates the abundance of the parasite [25]. If *H. oregonensis* and *H. nudus* are indeed 'life-cycle bottlenecks' for their parasites, long-term stability in the abundance of *P. conformis* and cystacanths (figure 2) could be attributed to *H. oregonensis* and *H. nudus* populations remaining stable. If *H. oregonensis* and *H. nudus* are not 'life-cycle bottlenecks' for their parasites, year-to-year variability in recruitment of these hosts (as seen in [26]) could 'dilute' and 'concentrate' parasites, explaining the short-term variability observed in *P. conformis*. If these crab species are life-cycle bottlenecks for some parasites (e.g. *P. conformis* and acanthocephalans) but are not for others (e.g. metacercariae), changes in crab abundance might explain why the abundance of some parasite taxa changed over time while others remained stable. However, we do not know how abundance of *H. oregonensis* and *H. nudus* have changed over time, and thus cannot explore crab host density as a potential driver of long-term change in parasite burden.

Even without a change in host density, shifts in host behaviour that influence the encounter rate between *H. oregonensis*/*nudus* and parasite propagules could have produced some of the patterns we observed. As *H. oregonensis*/*H. nudus* are intertidal hosts that are infected by parasites of marine origin, any increase in time spent in the ocean should increase parasite exposure. For example, if *H. oregonensis* adjusts its behaviour in response to warming air temperatures to increase the amount of time it spends submerged in water, this change could increase its exposure to the infective stages of microphallid trematodes. In aquatic ecosystems, intertidal organisms have been found to be responsive to rising temperatures associated with climate change [27], with some mobile species able to avoid heat exposure during summer low tides in thermal refugia [28]. Indeed, *H. nudus* can regulate its body temperature by moving between aquatic and terrestrial environments and by seeking refuge under rocks [29]; if small amounts of water are retained under these rocks at low tide, conditions (i.e. low flow, small volume of water, crab is inactive) would be conducive for transmission of trematodes to crabs. Note that this mechanism should theoretically increase the host's likelihood of encountering all the parasite taxa examined here, because the transmissive stages of all three encounter the crab host in seawater. However, if tide pools are used for crab thermoregulation, these habitats would be especially risky with respect to trematode transmission, because they can contain the first intermediate snail hosts that produce trematode life stages infectious to the snails; this could explain why the abundance of metacercariae increased over time, while the abundance of the other two parasites remained the same.

If *H. oregonensis* and *H. nudus* are not 'life-cycle bottlenecks' for their parasites (*sensu* [25]), then some other host obligately required in the complex life cycle will be, and changes in the abundance of this host or the rate at which it produces parasite propagules could change the abundance of parasites observed in *H. oregonensis* and *H. nudus*. For the microphallid trematode metacercariae that increased in abundance in *H. oregonensis*, it is possible that conservation measures adopted in the decades between the early 1970s and the present day have resulted in an increase in the abundance of the sea bird definitive hosts of the trematode. Microphallid trematode abundance is regulated by the abundance of the definitive host in other regions. For example, the prevalence of *Maritrema novaezealandensis* (Family Microphallidae) in snail first intermediate hosts is positively related to the abundance of shorebirds across 12 New Zealand bays [30]. If similar dependence upon bird definitive hosts occurs in our study system, then increases in some seabird populations over the past half-century (e.g. [31]) could be responsible for the observed increase in microphallid metacercarial abundance in *H. oregonensis*. If first intermediate host snails are the life-cycle bottleneck rather than definitive host birds or second intermediate host crabs, perhaps recent

increases in snail abundance have driven increases in transmission of microphallid trematodes to *H. oregonensis*. Nutrient pollution can increase the abundance of freshwater snails by subsidizing their periphyton resources, and this increase in snail abundance has downstream effects on transmission of trematodes to second intermediate host amphibians [32]. In the past 100 years, development of the Salish Sea watershed has led to increases in the amount of nutrient-laden runoff reaching the Sea [33], but to our knowledge, a link between nutrient pollution and the abundance marine snails has not been reported. This is an important research gap, given the possible implications of nutrient-mediated increases in marine snails for the transmission of many marine infectious diseases. However, the abundance of snails need not have changed for transmission from the first intermediate host to have increased over time; increasing temperatures can increase the rate at which first intermediate host snails produce infectious microphallid cercariae [34]. Data from Race Rocks lighthouse in the Strait of Juan de Fuca suggest that surface waters have warmed 1.0°C since 1950 [35]. In one laboratory experiment involving the microphallid trematode *Maritrema subdolum*, increasing mean temperature from 15°C to 25°C caused an 11-fold increase in the rate of cercarial emergence [36].

It is unknown whether *P. conformis* uses only a single species of copepod intermediate host or if it is a generalist at the first intermediate host stage. If *P. conformis* is specialized on one or a few copepod hosts, then variability in the density of the preferred copepod species over time [37] could explain the short-term variability we observed in the abundance of *P. conformis*. If, on the other hand, the parasite is a generalist, then long-term stability in density across multiple copepod species of the northeast Pacific [37] might explain why we observed no long-term trend for *P. conformis* abundance in crab hosts. Additional data on the life history of our focal parasites and long-term change in the abundance of various host species could help to discriminate among these possibilities.

The patterns we observed might also be affected by shifts in host susceptibility. If *H. oregonensis* were experiencing greater physiological stress at the contemporary time point than at the historical time point, an increase in susceptibility could have driven an increase in the burden of trematode metacercariae. For example, elevated temperatures (like those observed in our study region; see above) increase the susceptibility of amphipods (*Paracalliope novizealandiae*) to infection with the microphallid trematode *Maritrema novaezealandensis* [38]. *Hemigrapsus* spp. crabs mount an immune challenge to infection by microphallid trematodes [39] that could be compromised by stressful environmental conditions. By contrast, rising temperatures can also lead to mismatches in host and parasite thermal performance that result in declines of parasite abundance [40].

We made an effort to collect in the same seasons between contemporary and historical sampling bouts, but in some cases, logistical constraints made this impossible. On average across all site–time combinations, we performed contemporary collections within 70 days of the date on which collections were performed in the historical record (electronic supplementary material, table S4). All three of the parasite taxa we quantified are long-lived in their hosts [41] and, therefore, probably do not vary in abundance among seasons. In fact, a comprehensive meta-analysis shows that, in temperate marine fish hosts, the abundances of acanthocephalans and larval trematodes show no consistent summer/winter seasonality (isopods were not tested in this meta-analysis; [42]). Owing to this low seasonality and because Julian dates of collection differed so little (i.e. less than three months on average) between historical and contemporary time periods, we find it unlikely that seasonality would confound our comparisons of parasite abundance between historical and contemporary time periods.

Although we went to great lengths to match the methods of our contemporary sampling to the historical study, there are still some uncertainties for which we could not adjust. For example, the contemporary and historical studies represent only two time points on a 50 year timeline. We attempted to resolve this issue by conducting contemporary collections at multiple time points and comparing short-term against long-term variability in parasite abundance (figure 2). Some methods differed between the historical and contemporary time points by necessity; for example, crabs were frozen before dissection during contemporary collections, but not during historical collections. To address this potential confounding factor, we conducted an experiment in which we compared the detectability of parasites in frozen versus fresh *H. oregonensis* and *H. nudus* specimens. While we found no difference in the number of parasites detected between the treatments for *P. conformis* or acanthocephalan cystacanths, we did find that there were significantly more trematode metacercariae in the fresh treatment than in the frozen treatment (electronic supplementary material, table S3 and figure S1). This means that—despite the fact that metacercariae were more difficult to detect in contemporary specimens (where all specimens were frozen before being dissected), we still found significantly more metacercariae in contemporary than in historical specimens, suggesting that our estimates in fact underestimate the increase in metacercarial abundance over time. Finally, because of the temporal arrangement of sampling (electronic supplementary material, table S4), our comparison of long-term versus short-term variability might have been confounded by differences in the temporal distance among dates across the long term versus across the short term. However, we find this unlikely because all three of the parasite taxa we quantified are long-lived in their hosts [41] and, therefore, probably do not vary substantially among seasons.

We found that one parasite taxon (microphallid trematode metacercariae) increased substantially over the past 50 years. What ecological consequences might result from this long-term increase in parasite abundance? In other regions, infection by microphallid trematodes can alter the behaviour of crustacean intermediate hosts in ways that facilitate trophic transmission to the parasite's final hosts. For example, infection by the microphallid trematode *Microphallus turgidus* causes grass shrimp intermediate hosts to cease predator avoidance behaviour [43]. If a similar behavioural effect of infection is at play in our host–parasite system, it could mean that *H. oregonensis* and *H. nudus* prey are more available for bird predators today than they were in the mid-twentieth century. Other potential ecological effects of elevated microphallid abundance include increased infection (and resulting castration) of first intermediate host snail populations, increased infection (and resulting intestinal pathology) of definitive host bird populations and non-behavioural infection pathology in the crab second intermediate host.

Few datasets are available to track change in parasite abundance across time [10,11,44], so historical datasets like the one documented here are extremely valuable for

understanding parasite populations in their historical context. Our results suggest that one of the three parasite taxa we detected has increased in abundance over the past half-century, while the abundance of the other two has not changed. From an environmental standpoint, the stasis in abundance of *P. conformis* and the acanthocephalan is noteworthy, because over this 50 year period much has changed in the intertidal ecosystems of the Pacific northwest. This consistency in parasite abundance suggests that the abundance of the various hosts and their transmission dynamic has been resilient, or that there have been compensating changes. While this study documents dramatically increasing trematode abundances over a long time period, we can only speculate as to the cause(s), with the possibilities including enhanced shore bird conservation, increased eutrophication, or increased density or cercarial production of intermediate host snails. Further experimental and observational studies are needed to evaluate the various mechanisms posited here. In the meantime, this study reinforces a major conclusion arising from the growing literature on parasite change through time: parasite responses to environmental change are diverse and non-uniform across parasite taxa. This echoes findings from free-living species, which suggest that, within a given taxon, there are likely to be both 'winners' and 'losers' in a changing world [6].

Ethics. Fieldwork was conducted under Washington State Scientific Collecting Permits WOOD 18-216 and WOOD 19-172 and Oregon Scientific Taking Permits 22323 and 23025.

Data accessibility. The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.mkkwh70zp [45]. The code used to produce the statistical results reported herein is openly available via GitHub at www.github.com/wood-lab/Quinn_et_al_2021_Proc_B.

Authors' contributions. C.L.W. and A.M.K. conceived the study and designed its methodology. J.Q., D.G., A.G. and A.M.K. collected the data. S.L. led data curation, with support from D.G., J.Q. and C.L.W. C.L.W. led the statistical analysis and visualization with support from J.Q. and S.L.. J.Q. wrote the first draft. All authors contributed to later versions of the manuscript and gave final approval for publication.

Competing interests. We declare we have no competing interests.

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