The diversity and virulence correlates of metastrongyles in marine mammals.

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Abstract

Metastrongyles are common lung worms of mammals including most aquatic species. Given the importance of the respiratory system for diving and food acquisition in marine mammals, metastrongyles could be particularly detrimental in this mammalian group. However, there is conflicting preliminary evidence regarding the health consequences of metastrongyle infections in marine mammals and little understanding on the factors that could drive pathogenic effects. To fill this gap, we conducted a systematic review of the literature to analyze the diversity of this parasite group in marine mammals and the host and parasite factors that could be associated with pathogenic effects. There have been at least 3 families and 40 species of metastrongyles described in 2 orders, 10 families, and 66 species of marine mammals. The Indo-pacific finless porpoise, narrow-ridge finless porpoise, and short-beaked common dolphin harbored the highest diversity of metastrongyle species while *Halocercus hyperoodoni*, *Otostrongylus circumlitus*, *Parafilaroides gymnurus*, *Halocercus brasiliensis*, and *Stenurus minor* were the metastrongyles which parasitized more marine mammal species. Metastrongyle infections are most frequently documented in the lower respiratory system, otic apparatus, and cardiovascular system of marine mammals. For host-parasite relationships where metastrongyles were found in the respiratory system and pathogenic effect was assessed (n=166), respiratory specific pathogenicity such as bronchopneumonia was reported in 80.12% of the cases (n=133). In contrast, for relationships where metastrongyles were found in the otic apparatus and pathogenic effect was assessed (n=26), otic specific pathogenicity such as otitis was reported in only 23.08% of the cases (n=6). Similarly, for relationships where metastrongyles were found in the cardiovascular system and pathogenic effect was assessed (n=11), cardiovascular specific pathogenicity such as vasculitis was reported in 45.45% of the cases (n=5). Finally, metastrongyle species that infected a larger number of marine mammal species of larger size were more likely to induce pathogenic effects on the host such as tissue specific inflammation and mortality. Metastrongyles are important pathogens of marine mammals that can cause significant tissue damage and mortality although pathogenic host-parasite relationships are dominated by a few metastrongyle species with wider host ranges.

Keywords: marine mammals; metastrongyles; lungworms; parasites; pathogenicity; virulence.

Background

Metastrongyloidea (Nematoda: Strongylida), also known as lungworms or metastrongyles, are common parasites of the respiratory, cardiovascular, and rarely the auditory system of mammals [1]. In these tissues, metastrongyles can cause significant inflammation, which could have detrimental effects for animal species that rely on optimal respiratory and auditory function for fitness, such as marine mammals [1–3]. Marine mammals rely on cardiovascular and lung capacity for foraging dives and the otic apparatus of cetaceans is critical for communication and echolocation in the water column [1–3]. Therefore, the parasitism of these host compartments by metastrongyles could be associated with negative health consequences for the host or even stranding and death [1–3]. Nevertheless, metastrongyle nematodes are one of the most diagnosed parasites in marine mammals, and in many circumstances are considered incidental findings of little to no pathologic importance [4]. This conflicting evidence could be related to negative metastrongyle effects associated with specific host-parasite relationships, parasite traits, environmental conditions, or the research methods employed to assess the health impact. Currently, there is lack of systematic assessment of the evidence on the health impact of metastrongyles in marine mammals and the potential factors that could modulate such effects.

Three families of metastrongyles are prominently found in marine mammals, spanning seven genera, with a considerable diversity of species within each genus. A total of 40 unique species of metastrongyles are outlined in this systematic review, traversing 66 unique marine mammal host species. Despite this diversity, all metastrongyles share similar life histories and host exploitation strategies. Most metastrongyle species require an obligate intermediate host, usually an invertebrate, where several larval stages develop until reaching an infective stage. These infective larvae are consumed by the definitive host and then migrate from the gastrointestinal system to the respiratory or cardiovascular system where sexual maturity and reproduction occurs [5]. Different metastrongyle species illicit a similar immune response in their respective hosts, which can be attributed to well conserved physiological and morphological traits within the Metastrongyloidea superfamily [6]. This immune response is usually directed against parasite cuticle, larvae, eggs or death parasite remains and consist in the attraction of macrophages, eosinophils and lymphocytes to the site of infection [7].

The impact of metastrongyle infections on the health of marine mammals is not yet fully understood. Most notably, there is conflicting evidence regarding the connection between metastrongyle infections and cetacean strandings [2]. Currently, there is no consensus on whether inflammation in the otic apparatus, elicited by a presence of metastrongyle parasites, can impair hearing or echolocation to a significant enough degree to be considered a contributory factor in cetacean strandings [4]. Similarly, the presence and feeding behavior of the nematodes in bronchi and lung parenchyma facilitates secondary bacterial infections with conflicting documented outcomes, ranging from little importance for lung physiology to fatal bronchopneumonia, especially when parasites are found in the lower respiratory system [8–12]. This conflicting evidence could be related to several host and parasite traits, although across biological systems, parasite biomass, usually assessed in terms of burden, is a common driver of negative health outcomes [13,14]. Similarly, parasite and host sizes play an important role in assessing total parasite biomass and can sometimes serve as useful proxies of biomass to identify particularly detrimental host-parasite relationships across studies [13]. Another potential factor impacting health outcomes across host-parasite systems is the capacity of certain helminths to parasitize multiple host species. Parasites with a wider host range can potentially become more virulent because decreases in host fitness due to infection can be compensated by infecting other susceptible species [15,16]. However, the role of these host and parasite traits on the health impact of metastrongyles is not well defined.

The objectives of this systematic review were to i) summarize existing literature concerning relationships between marine mammals and metastrongyle parasites, ii) assess the relative host and parasite diversity of infections iii) outline the significant pathologies and health consequences which result from metastrongyle infections in marine mammals, with assessment of the potential drivers of these effects.

Materials and Methods

a. Searching methods and inclusion criteria

A systematic literature review on metastrongyle parasites in marine mammals was conducted on July 6, 2021. The search was conducted using Google Scholar and PubMed, following the best practices outlined by Haddaway and Watson (2016) to conduct an aggregative systematic review in the field of parasitology [17].

The search terms used for the literature search on PubMed were as follows: "((Marine) OR (Cetacean) OR (Delphinidae) OR (Phocidae) OR (Phocoenidae) OR (Otariidae) OR (Monodontidae) OR (Mustelidae) OR (Trichechidae) OR (Odobenidae) OR (Ursidae) OR (Odobenidae) OR (Balaenidae) OR (Neobalaenidae) OR (Eschrichtiidae) OR (Balaenopteridae) OR (Physeteridae) OR (Kogiidae) OR (Ziphiidae) OR (Platanistidae) OR (Iniidae) OR (Lipotidae) OR (Pontoporiidae) OR (Dugongidae) OR (Seal) OR (Porpoise) OR (Otter) OR (Dolphin) OR (Sea lion) OR (Whale) OR (Walrus) OR (Manatee)) AND ((Metastrongyle) OR (Metastrongylus) OR (Metastrongyloidea) OR (Metastrongylidae) OR (Parafilaroides) OR (Stenurus) OR (Halocercus) OR (Pseudaliidae) OR (Filaroididae) OR (Crenosomatidae) OR (Otostrongylus) OR (Skrjabingylus))".

The search terms used for the literature search on Google Scholar were reduced due to limitations in the advanced search software, notably a 256-character limit on searches. The search terms used for Google Scholar were as follows: "((Metastrongyle) OR (Metastrongylus) OR (Metastrongyloidea) OR (Metastrongylidae) OR (Parafilaroides) OR (Stenurus) OR (Halocercus) OR (Pseudaliidae) OR (Filaroididae) OR (Crenosomatidae) OR (Otostrongylus) OR (Skrjabingylus)) AND ((Marine) OR (Cetacean))".

The search on PubMed yielded 120 results and the search on Google Scholar yielded 2110 results, excluding citations. As Google Scholar can only display 1000 results per search, only the 1000 articles deemed most relevant by the software were manually reviewed for inclusion in the study, in addition to the 120 articles found via PubMed. To ensure all recently published literature was consulted, a secondary search on google scholar was conducted with the same search terms, filtering to only include articles published from 2017 and onward. This secondary search yielded 380 results, excluding citations, which were also manually reviewed for inclusion in the study. All conference archives (e.g. IAAAM), theses not published in peer-reviewed journals, and posters

were excluded from this study. The full texts for the articles isolated through literature search results were obtained and manually reviewed, being included in the study if they met the following inclusion criteria: i) The host species was any wild marine mammal, with captive wild animals only included if the transmission of parasites was stated to have occurred naturally, prior to their capture. ii) The parasite species belonged to a genus within the Metastrongyloidea superfamily, including studies that described the presence of parasitic eggs and larvae provided they could be positively identified to at least the genus level (studies that described parasites as "lungworms" or "nematodes" without specificity regarding genera were excluded). iii) The article serves as the primary source describing a relationship between a marine mammal host and Metastrongyloidea parasite. Duplicate articles, existing in both the Google Scholar and PubMed searches were manually removed. All non-primary sources which were found through the initial search terms were screened, and citations were followed, leading to the inclusion of 15 additional articles which met the inclusion criteria. Later, the World Register of Marine Species (WoRMS, *https://www.marinespecies.org*), The Society for Marine Mammalogy (*https://marinemammalscience.org*), and the Natural History Museum Host-Parasite database (*https://www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/)* were consulted to ensure all known unique relationships were reported in this study [18]. A total of 35 articles could not be acquired in their full text form for inclusion in data analysis, but the relationships they report are known through prior citations in peer-reviewed journals or available abstracts. The articles which met the inclusion criteria were further evaluated, with significant attributes documented on a master spreadsheet. Many included articles, including the articles not available in full-text form, report multiple different host-parasite relationships, thus independent rows were created on the master spreadsheet for each described host-parasite relationship (n=481), allowing for relationship-specific attributes to be evaluated. The accepted common names and scientific names provided for host species in this study are taken from the Society for Marine Mammalogy Committee on Taxonomy [18].

A total of 207 full text publications met the inclusion criteria for this study, with 114 appearing only on Google Scholar, 6 appearing only on PubMed, 72 appearing on both Google Scholar and PubMed, and 15 coming from other sources. Data was collected on all included full-text publications, including whether they assessed pathogenic effect. An article was deemed to assess

pathogenic effect if it described some biological impact associated with a presence of metastrongyle parasites in the host, or if it explicitly stated no pathogenic effect was associated with the parasitism. Further data was collected on whether full-text publications which met the inclusion criteria mentioned strandings or mortality. For the collection of this data, an article was deemed to mention strandings if it contained the following terms (not including in the citations): "stranded" or "stranding" or "strandings". For an article to have been deemed to mention mortality, it must have contained the following terms (not including in the citations): "mortality" or "mortalities". The inclusion terms for determining which articles mention stranding or mortality were isolated through word searching in adobe acrobat, with technologically non-conforming articles screened manually. Later, the studies mentioning mortality or stranding were assessed to ensure they discussed these effects in the context of the study's findings. The parasite collection methodology was also noted for each included full-text publication. Within the articles which met the inclusion criteria, the geographical location(s) were recorded for 437 documented relationships and the anatomical location(s) of the parasites were recorded in 371 documented relationships. We further calculated the geographical dispersion of the 102 unique relationships between marine mammals and metastrongyles.

For each documented relationship, data was also recorded on any reported pathogenic effects (n=201). Pathogenic effects can be described as some biological impact on the host associated with the presence of metastrongyle parasites. In cases where it was explicitly stated that no pathogenic effect was associated with parasitism, this was recorded as well. Of the 101 articles that assessed pathogenic effect, there were a total of 201 relationships in which attributes describing a pathogenic effect or lack thereof were documented.

Between the 207 full-text publications which met our inclusion criteria, and the additional 35 publications we could not retrieve in full-text form, a total of 481 host-parasite relationships are documented. For each of these relationships, data was collected on their respective species taxonomy. Next, the frequency at which each metastrongyle parasite species appears in the literature, in the context of relationships with marine mammal hosts was recorded. For the purposes of frequency calculations, parasites described only to the genus level were excluded. The frequency at which marine mammal hosts appear in the literature, in the context of relationships

with metastrongyle parasites was recorded in the same manner. For frequency calculations, hosts described only to the genus level were excluded. For the purposes of all diversity calculations, only relationships where both the host and parasite were described to the species level were considered. Finally, host diversity was calculated as a measure of the diversity of host species a metastrongyle species can infect, and parasite diversity was calculated as the diversity of metastrongyle species a marine mammal species can host.

To provide a metric by which the size of a host could be used as a predictor of pathogenic effects, host body mass was calculated by dividing the median weight in kilograms by the median length in centimetres for each host species. Median host measurements were compiled as the median of both genders from a variety of sources based on availability in the following order of priority: 1) World Register of Marine Species (WoRMS, *https://www.marinespecies.org*); 2) Society for Marine Mammalogy (*https://marinemammalscience.org/science-and-publications/species-information/facts/*); 3) National Oceanic and Atmospheric Administration (NOAA, *https://www.fisheries.noaa.gov/species-directory*); 4) Animal Diversity Web (ADW, *https://animaldiversity.org*). In cases where data on host size was not available through those four sources, the median measurements of hosts documented during gross necropsy in articles which met the inclusion criteria of this study were used.

Finally, we produced an up-to-date checklist of all known relationships concerning metastrongyles in marine mammals, which is included as supplementary data, complete with information on geographical and anatomical ranges documented for each relationship. The relationships documented are found within the 207 articles included in this study or within the 35 articles that could not be acquired in their full text form for inclusion in data analysis, provided the relationships they report are known through prior citations in peer-reviewed journals. A complete list of references for all articles included in this review can be found alongside the supplementary data.

b. Data analyses

When assessing the relative host and parasite diversities, corrections were made for literature and intra-study biases which arose from non-uniform sampling efforts. We used a modified approach, based previously published methods [13,19,20], to create a bias penalization index for each host

and parasite species. To do this, we searched scientific names of each host and parasite species individually on Google Scholar and the number of hits was recorded. The number of hits for each species was then multiplied by the number of studies that both met our inclusion criteria and contained mentions of a relationship concerning that respective species. The natural logarithm of these products was then taken, and the result constitutes our penalization index for each species study bias (correction-index).

We explored the predictors of pathogenic effects among different host parasite interactions using binomial generalized linear mixed effects models (GLMMs). We used corrected host diversity, corrected parasite diversity, and host body size as predictors for the presence or absence of pathogenic effects. A random effect variable (Relationship-ID) was added to account for repetitions in unique relationships documented, as various studies documented identical albeit uncorrelated relationships between host and metastrongyle species. For each documented host-parasite relationship, with both the host and parasite specified to the species level and for which pathogenic effect was assessed (n=165), binomial GLMMs for the presence or absence of pathogenic effect were fit. A secondary set of models were fit to exclusively assess the presence or absence of respiratory specific effect (bronchopneumonia or brochities). For the respiratory specific effect models, each documented host-parasite relationship was included if the host and parasite were specified to the species level, pathogenic effect was assessed, and the presence of the parasite was documented in either the upper or lower respiratory system (n=134). We attempted to fit cardiovascular specific effect (DIC, thrombosis, vasculitis, hemorrhage, endocarditis) (n=11) and otic apparatus specific effect (sinusitis, otitis, otic metaplasia) (n=24) models using the same method as for respiratory specific effect but were unable because the small sample size affected model convergence. In both the full and respiratory specific models, we included interaction effects between the corrected host diversity, corrected parasite diversity, and body size predictors terms to account for individual permissiveness of host species to parasite species as well as individual pervasiveness of parasite species to host species.

c. Discrepancies in the literature

Hermosilla, C., et al. (2015) reports *Stenurus sp*. in sei whale (*Balaenoptera borealis*), blue whale (*Balaenoptera musculus*), and fin whale (*Balaenoptera physalus*) as non-primary findings,

however we could not identify a primary source of these relationships [21]. Lehnert, K., et al. (2019) reports *Pharurus alatus* as a parasite of short-beaked common dolphin (*Delphinus delphis*) citing Tomo, I., et al. (2010) [22,23]. Upon reviewing Tomo, I., et al. (2010), it does not appear to include this relationship [22]. The World Register of Marine Species (WoRMS) documents *Halocercus taurica* in of short-beaked common dolphin (*Delphinus delphis*) and *Halocercus hyperoodoni* in northern bottlenose whale (*Hyperoodon ampullatus*), however primary sources could not be identified for either of these relationships [18]. Measures, L.N. (2001) documents *Halocercus pingi* in dall's porpoise (*Phocoenoides dalli*) as a non-primary finding, however we could not find a primary source for this relationship [1]. Three (n=3) additional publications document relationships that were not specified to the genus level [12,24,25].

Characterization of studies describing metastrongyles in marine mammals

The mean publication year of the 207 articles which met the inclusion criteria of this study was 2003, with the distribution of publication years skewed towards the most recent decade (Figure 1). For each of the articles which met the inclusion criteria, a total of 101 (48.79%) assessed pathogenic effect. A total of 163 (78.74%) articles mentioned strandings and a total of 90 (43.48%) articles mentioned mortality. Further, there was a considerable preference for collection of samples via gross necropsy, as performed in 191 (92.27%) studies. Gross necropsy appears to be the only prominent parasite collection methodology for Metastrongyloidea in marine mammals, with the next most common methodology, fecal sampling, occurring in only 13 (6.28%) studies (Figure 2).

A total of 175 relationships were reported in North America (including the commonly referenced Central America region), and 169 relationships were reported in Europe. North America and Europe appear to be the most common locations reported for Metastrongyloidea infections in marine mammals, followed by 54 relationships in South America, 24 in Asia, 23 in Oceania and 11 in Africa (Figure 3). Exactly half of all unique relationships (n=102) are reported in North America (n=51, 50.00%), with 30 (29.41%) of these relationships being reported exclusively in North America (Figure 4).

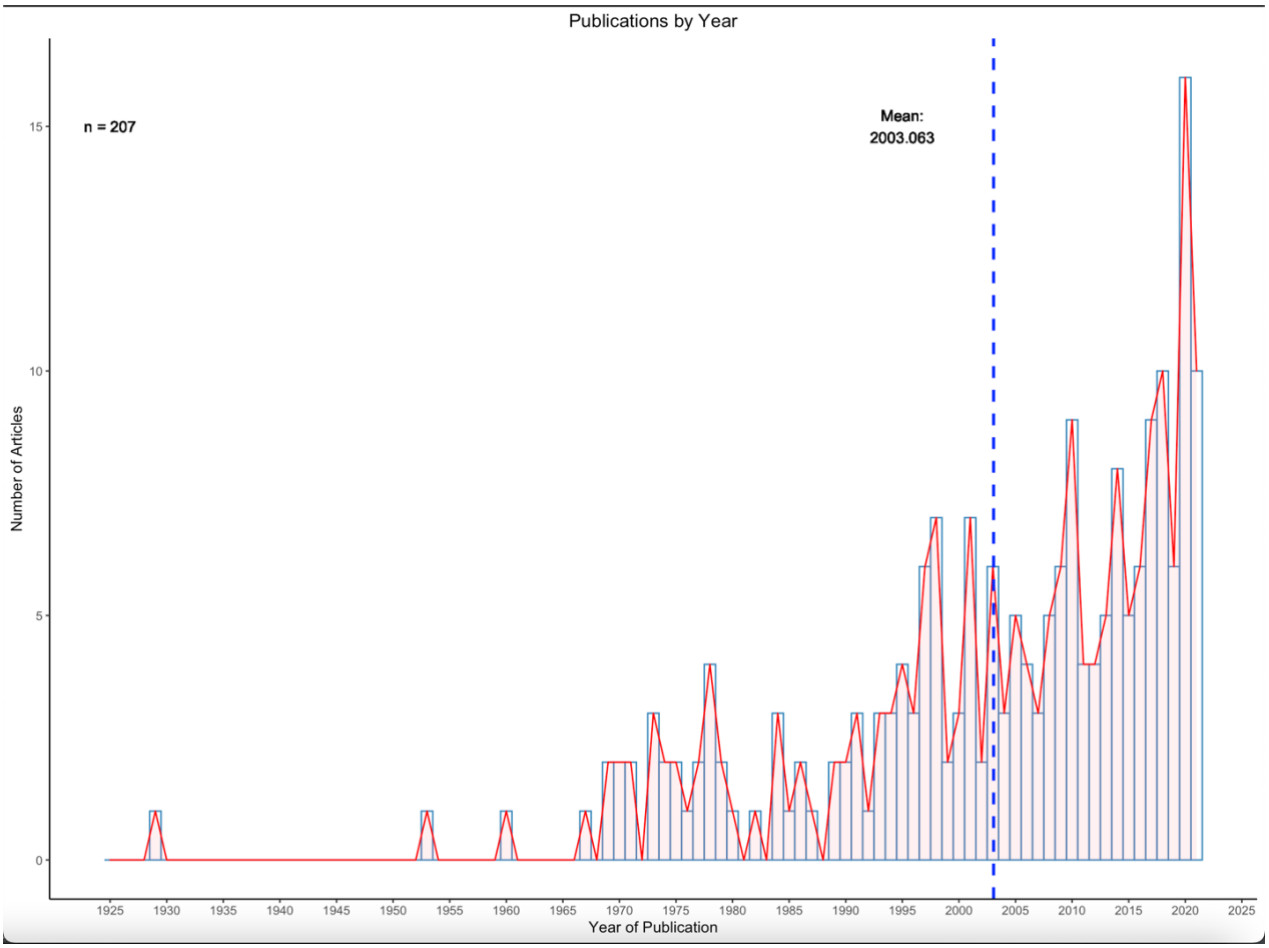

Figure 1: Distribution of publication year for articles which met the inclusion criteria of this study. The mean year of publication was 2003.

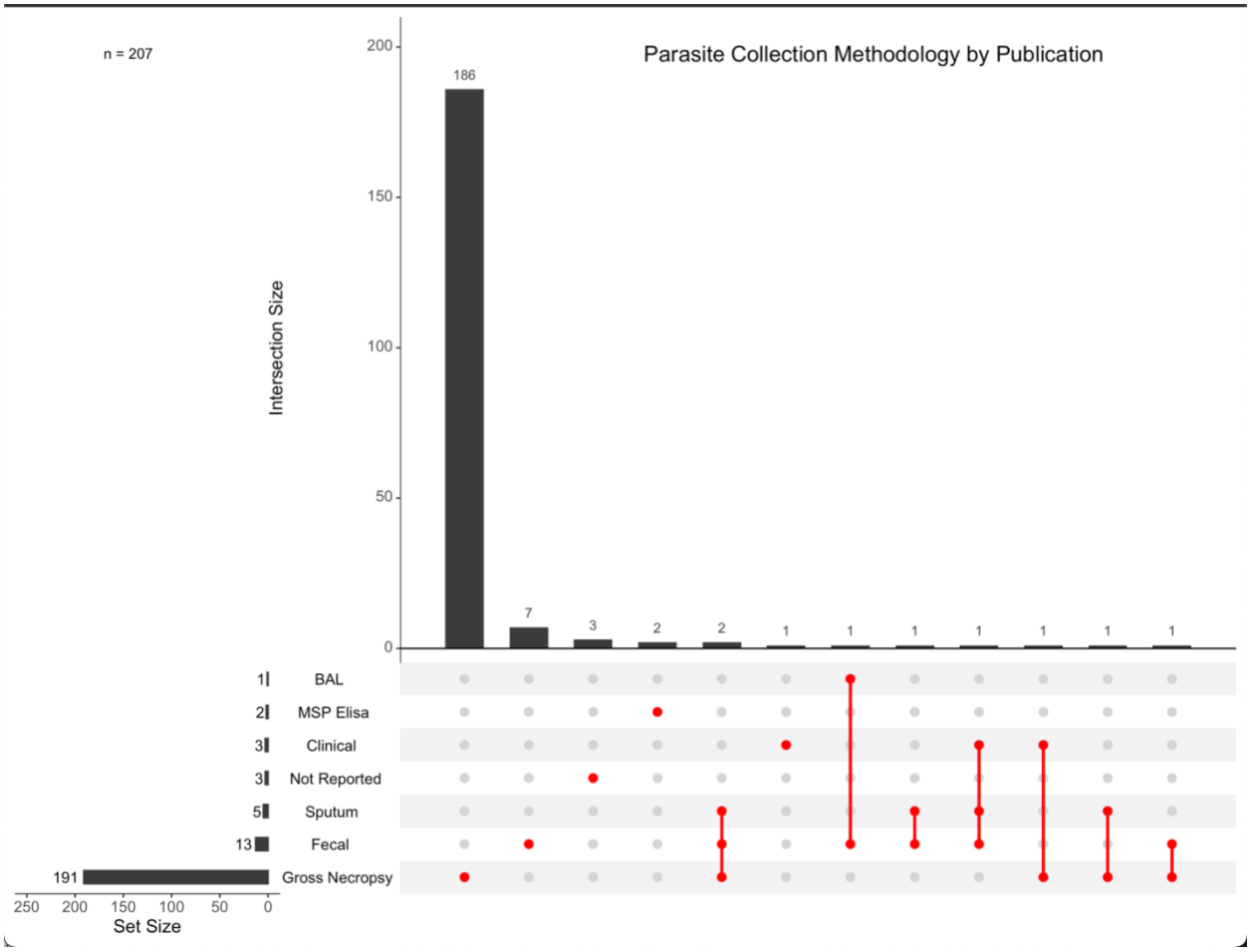

Figure 2: Parasite collection methodologies documented, with intersections between the methodologies shown to represent studies that utilize more than one methodology. Articles that did not report a collection methodology are designated as “Not Reported”.

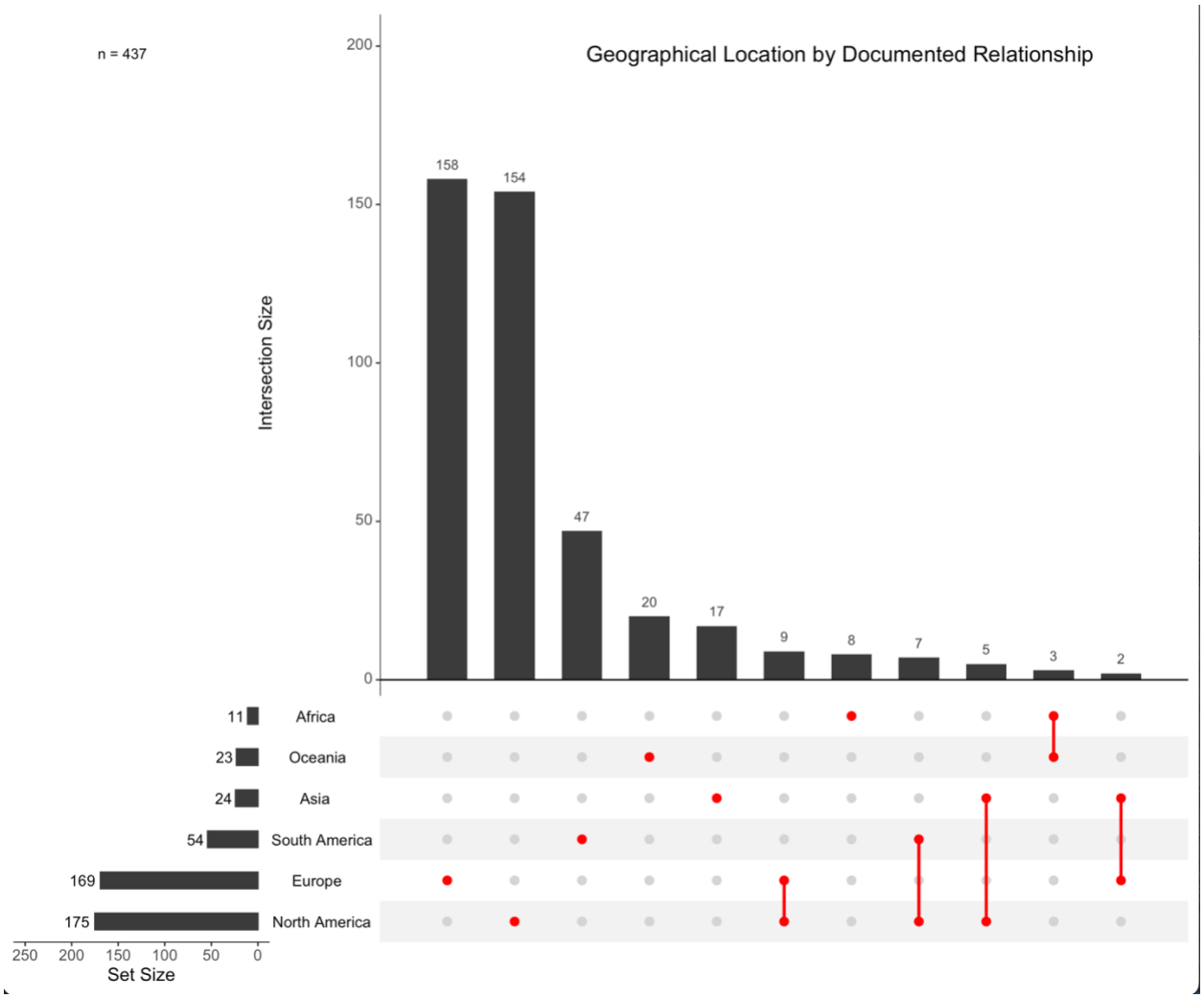

Figure 3: Geographical location(s), denoted by continent, of documented relationships between metastrongyles and marine mammals. Documented relationships include duplicates of unique relationships published independently. Intersections are shown for studies which report individual host species to parasite species relationships occurring in multiple locations.

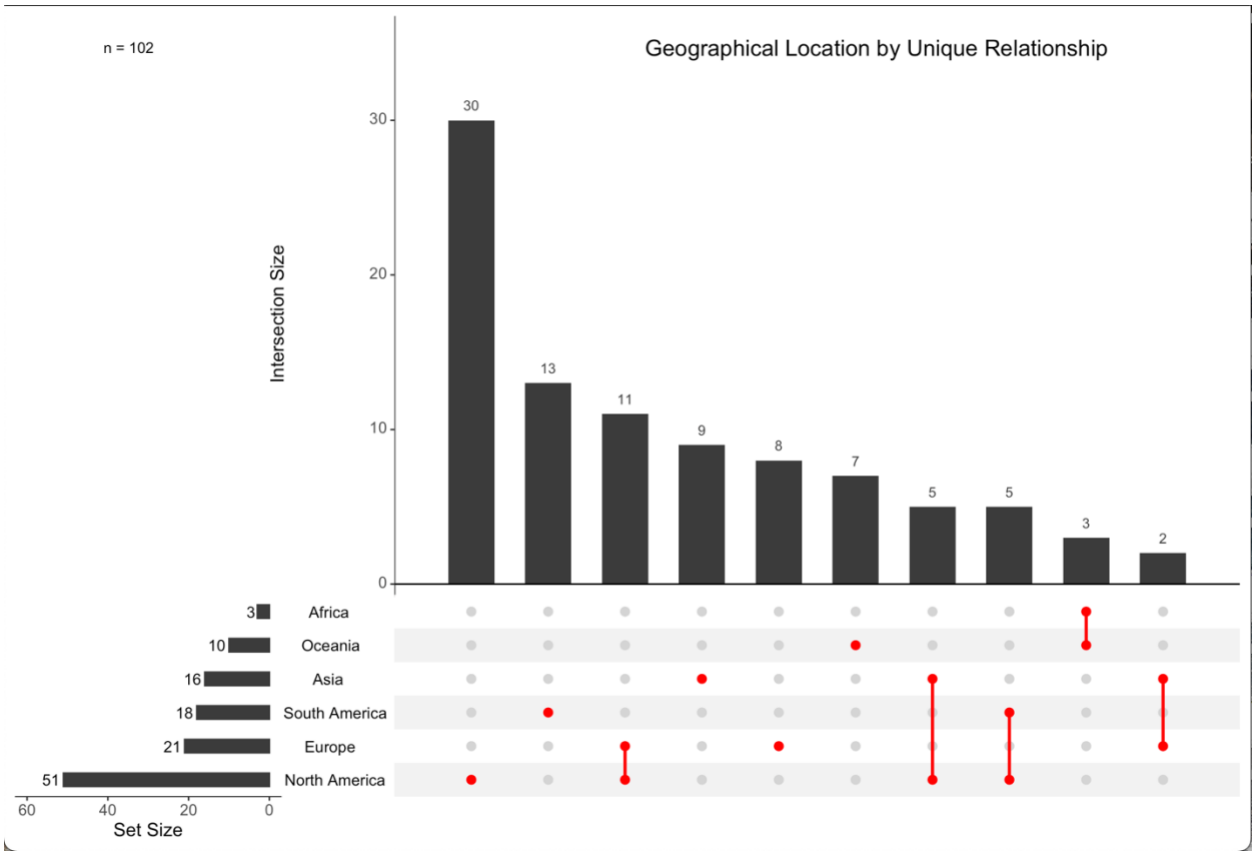

Figure 4: Geographical location(s), denoted by continent, of all unique relationships between metastrongyles and marine mammals. Intersections are shown for studies which report individual host species to parasite species relationships occurring in multiple locations.

Frequencies and diversities of metastrongyles in marine mammals

Pseudaliidae was the most reported metastrongyle family in marine mammals (n=331), followed by Filaroididae (n=90), and Crenosomatidae (n=58). At the host family level, Delphinidae (n=159), Phocoenidae (n=150), and Phocidae (n=107) are most frequently parasitized by Metastrongyloidea. Further, metastrongyles from the Pseudaliidae family are reported in hosts from the Delphinidae (n=158), Phocoenidae (n=148), Monodontidae (n=15), Ziphiidae (n=3), Iniidae (n=2), Balaenopteridae (n=1), Kogiidae (n=1), and Physeteridae (n=1) families. Metastrongyles from the Crenosomatidae family are reported to parasitize hosts from the Phocidae (n=52), Otariidae (n=5), and Phocoenidae (n=1) families. Metastrongyles from the

Filaroididae family are reported to parasitize hosts from the Phocidae (n=54), Otariidae (n=35), and Delphinidae (n=1) families.

Otostrongylus circumlitus (n=52) is the most reported metastrongyle species followed by *Stenurus minor* (n=42) and *Pseudalius inflexus* (n=31) (Supplementary Figure 1). The harbour porpoise (*Phocoena phocoena*) (n=118) is clearly identifiable as the most reported host, followed by harbour seal (*Phoca vitulina*) (n=41) and bottlenose dolphin (*Tursiops truncat*us) (n=23) (Supplementary Figure 2).

Before penalizing for literature and intra-study biases, the host species harbouring the largest number of metastrongyle species (parasite diversity) were: indo-pacific finless porpoise (*Neophocaena phocaenoides*) (n=8), short-beaked common dolphin (*Delphinus delphis*) (n=7), harbour porpoise (*Phocoena phocoena*) (n=7), and bottlenose dolphin (*Tursiops truncatus*) (n=7) (Supplementary Figure 3). After correction for publication bias, indo-pacific finless porpoise (*Neophocaena phocaenoides*) remained the most permissive host (Figure 5.). Before penalization, the metastrongyle species with the largest host diversity were: *Otostrongylus circumlitus* (n=13), *Stenurus mino*r (n=10), *Parafilaroides gymnurus* (n=9), *Halocercus brasiliensis* (n=8), and *Halocercus delphini* (n=8) (Supplementary Figure 4). After correction for publication bias, *Halocercus hyperdooni* emerged as more of a generalist, followed closely by *Otostrongylus circumlitus* and *Parafilaroides gymnurus* (Figure 6.).

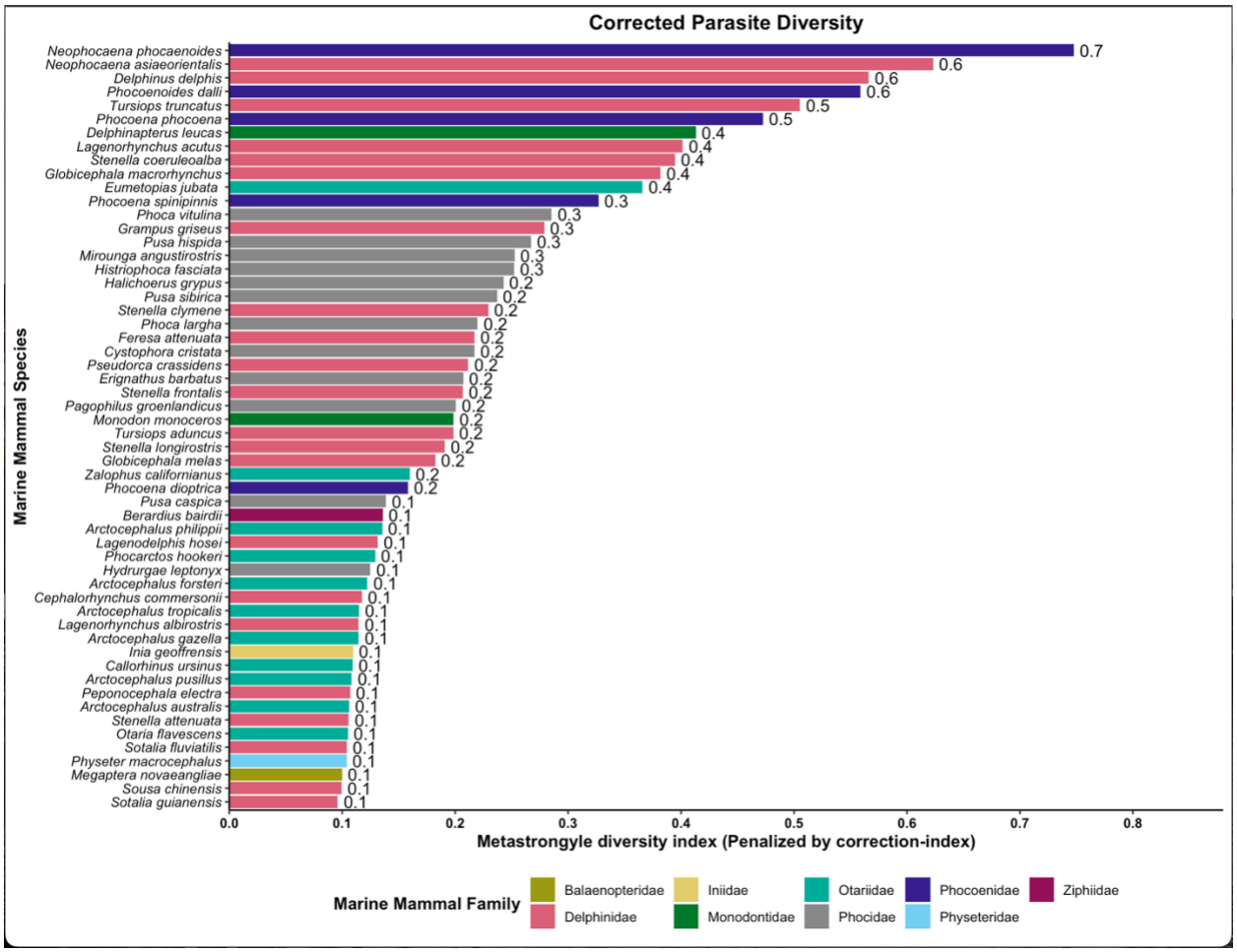

Figure 5: Parasite diversity for metastrongyles in marine mammals, corrected for publication bias (correction-index).

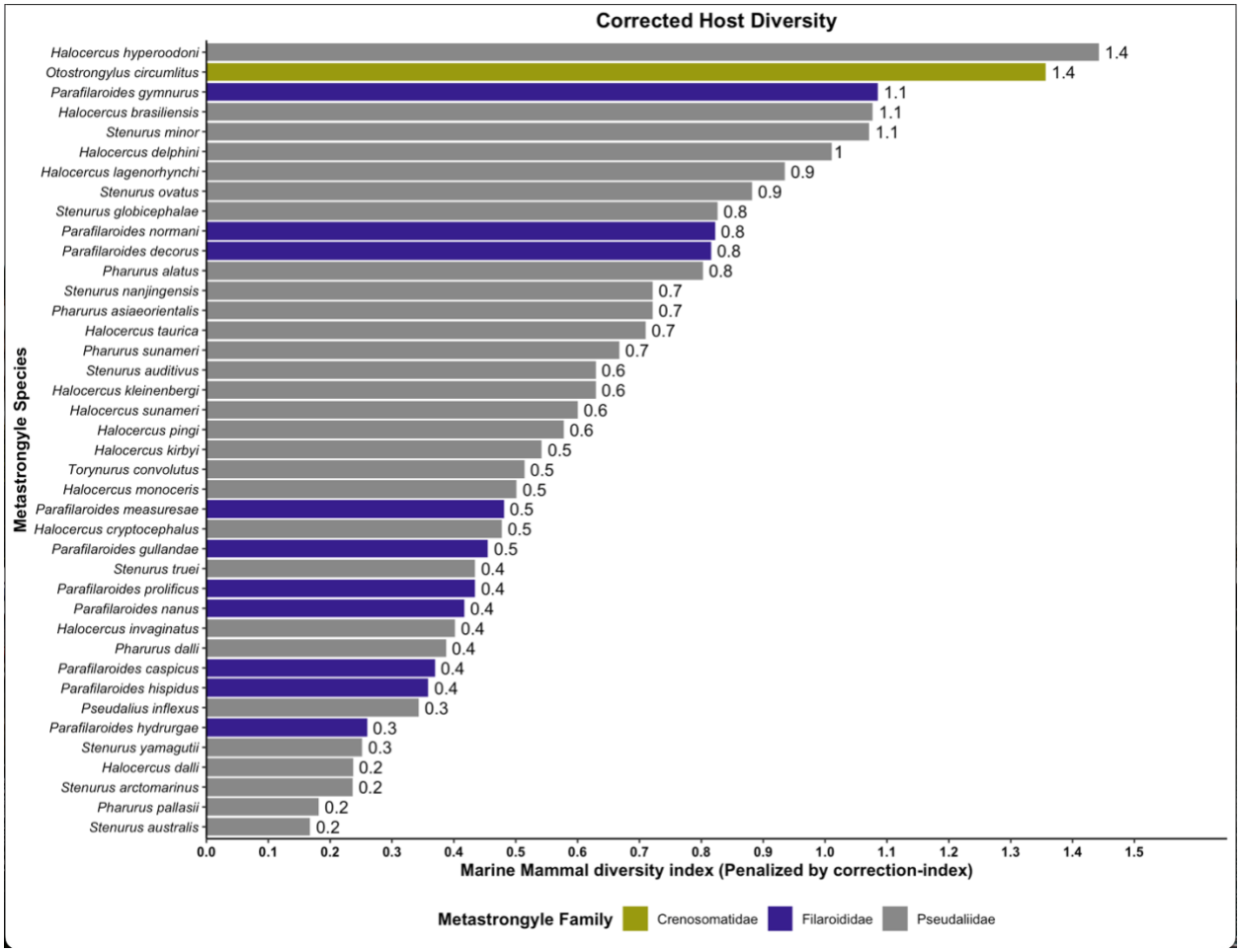

Figure 6: Host diversity for metastrongyles in marine mammals, corrected for publication bias (correction-index).

The impact of metastrongyles on marine mammals

Of the 371 cases which reported an anatomical location, parasites were found in the lower respiratory system in 306 (82.48%) cases, while parasites were found in the otic apparatus in 65 (17.52%) cases. A total of 66 (15.10%) of the 437 documented cases did not report the anatomical location of the identified parasite. In 43 (11.59%) cases, a single species of parasite parasitized an individual host in multiple anatomical locations (Figure 7).

Out of the 201 host-parasite relationships assessed, only 90 (44.77%) mentioned mortality as "possible" or "potential" contributory cause of death and only 21 (10.44%) studies considered metastrongyle infection as the primary cause of death. Of the 201 relationships for which

pathogenic effect was assessed, bronchopneumonia was described in 144 (71.64%) relationships, indicating a high prevalence of bronchopneumonia in hosts infected with metastrongyles (Figure 8). Other reported pathogenic effects included edema (n=22, 10.95%), vasculitis (n=15, 7.46%), thrombosis (n=12, 5.97%), and hemorrhage (n=12, 5.97%). In 33 (16.42%) relationships, it was determined that parasitism resulted in no discernible pathogenic effect on the host.

A total of 166 relationships reported a presence of respiratory parasites and assessed pathogenic effect, with respiratory specific pathology reported in 133 (80.12%) of these relationships. The high prevalence of respiratory specific lesions can be connected to a high degree of parasite presence in the respiratory system. In contrast, a total of 26 cases reported a presence of parasites in the otic apparatus and assessed pathogenic effect, with otic specific lesions reported in only 6 (23.08%) of these relationships.

In the binomial GLMM that assessed pathogenic effect, host body size, corrected host diversity and corrected parasite diversity were all significant predictors of metastrongyle pathogenic effect in marine mammals, with the interaction between host and parasite diversity indexes statistically significant (table 1). When GLMM evaluated respiratory specific effects, corrected parasite diversity and corrected host diversity main effects and their interaction were significant predictors of metastrongyle respiratory specific effect in marine mammals, but host body size was not a significant predictor of respiratory specific effect (table 2).

Table 1: Binomial generalized linear mixed effects model (GLMM) for pathogenic effect. The model used study ID as a random effect.

Group	**Name**	**Variance**	**Std. Dev.**	
Study-ID	(Intercept)	5316	72.91	
Term	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-189.93	65.03	-2.921	0.00349
Corrected Parasite Diversity	523.62	165.18	3.170	0.00152
Corrected Host Diversity	232.05	88.44	2.624	0.00869
Host Body Size	166.90	83.04	2.010	0.04444
Corrected Parasite Diversity : Corrected Host Diversity	-625.90	250.44	-2.499	0.01245
Corrected Parasite Diversity : Host Body Size	-435.38	220.87	-1.971	0.04870
Corrected Host Diversity : Host Body Size	-189.10	131.22	-1.441	0.14953
Corrected Parasite Diversity : Corrected Host Diversity : Host Body Size	581.44	438.86	1.325	0.18521

Table 2: Binomial generalized linear mixed effects model (GLMM) for respiratory pathogenic effects. The model used study ID as a random effect.

Group	**Name**	**Variance**	**Std. Dev.**	
Study-ID	(Intercept)	6728	82.02	
Term	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	0.09403	12.65860	0.007	0.99407
Corrected Parasite Diversity	88.93332	41.43432	2.146	0.03184
Corrected Host Diversity	74.10991	26.73963	2.772	0.00558
Host Body Size	-18.39291	13.61594	-1.351	0.17675
Corrected Parasite Diversity : Corrected Host Diversity	-244.76967	87.16180	-2.808	0.00498
Corrected Parasite Diversity : Host Body Size	8.03858	38.70274	0.208	0.83546
Corrected Host Diversity : Host Body Size	-29.25975	24.54618	-1.192	0.23325
Corrected Parasite Diversity : Corrected Host Diversity : Host Body Size	154.98741	93.04556	1.666	0.09577

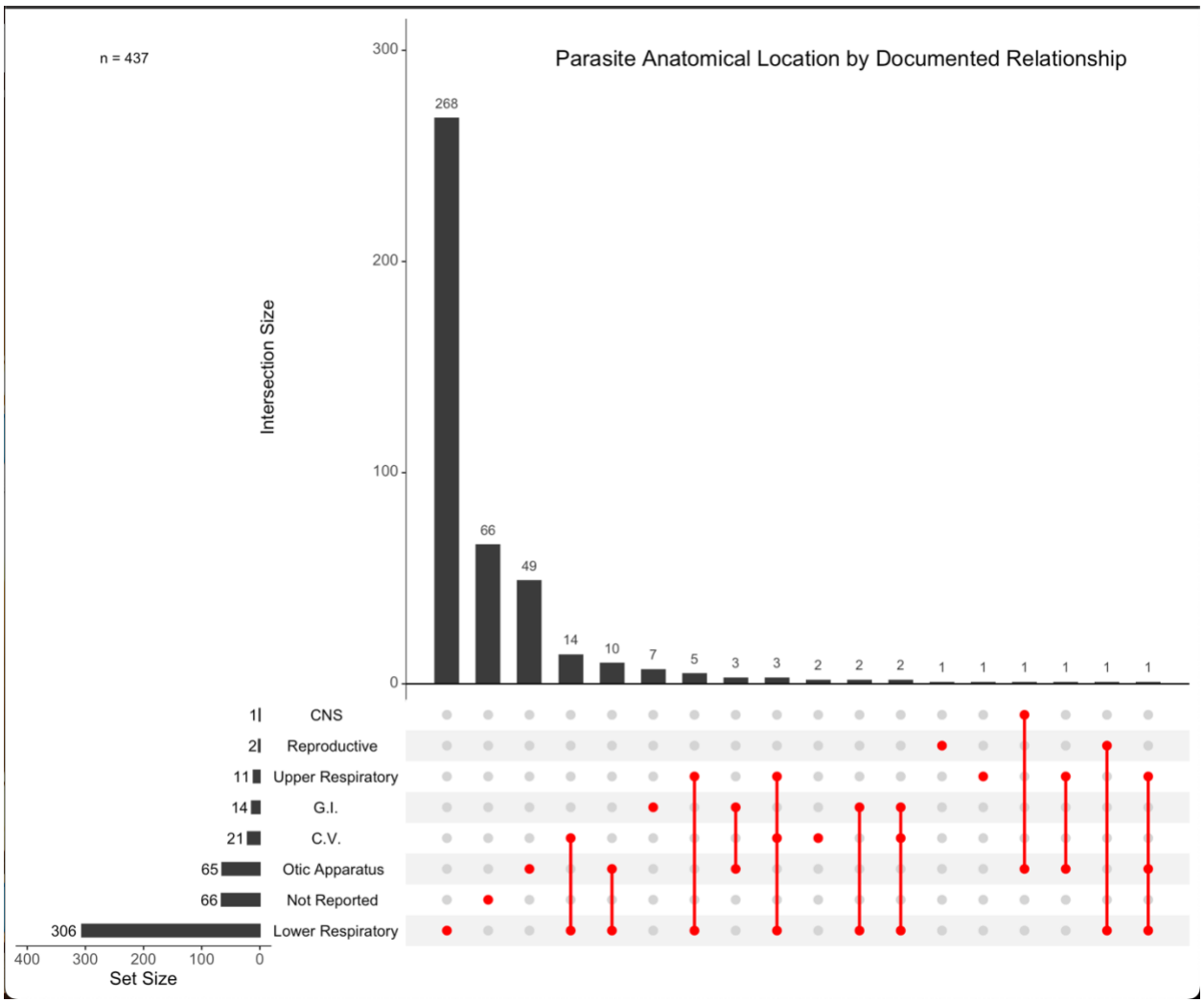

Figure 7: Anatomical location(s) of documented relationships between metastrongyles and marine mammals. Intersections are shown for studies which report individual host species to parasite species relationships occurring in multiple locations. Relationships that did not report an anatomical location are designated as “Not Reported”. (CNS= Central nervous system, G.I= Gastrointestinal tract, C.V= Cardiovascular system).

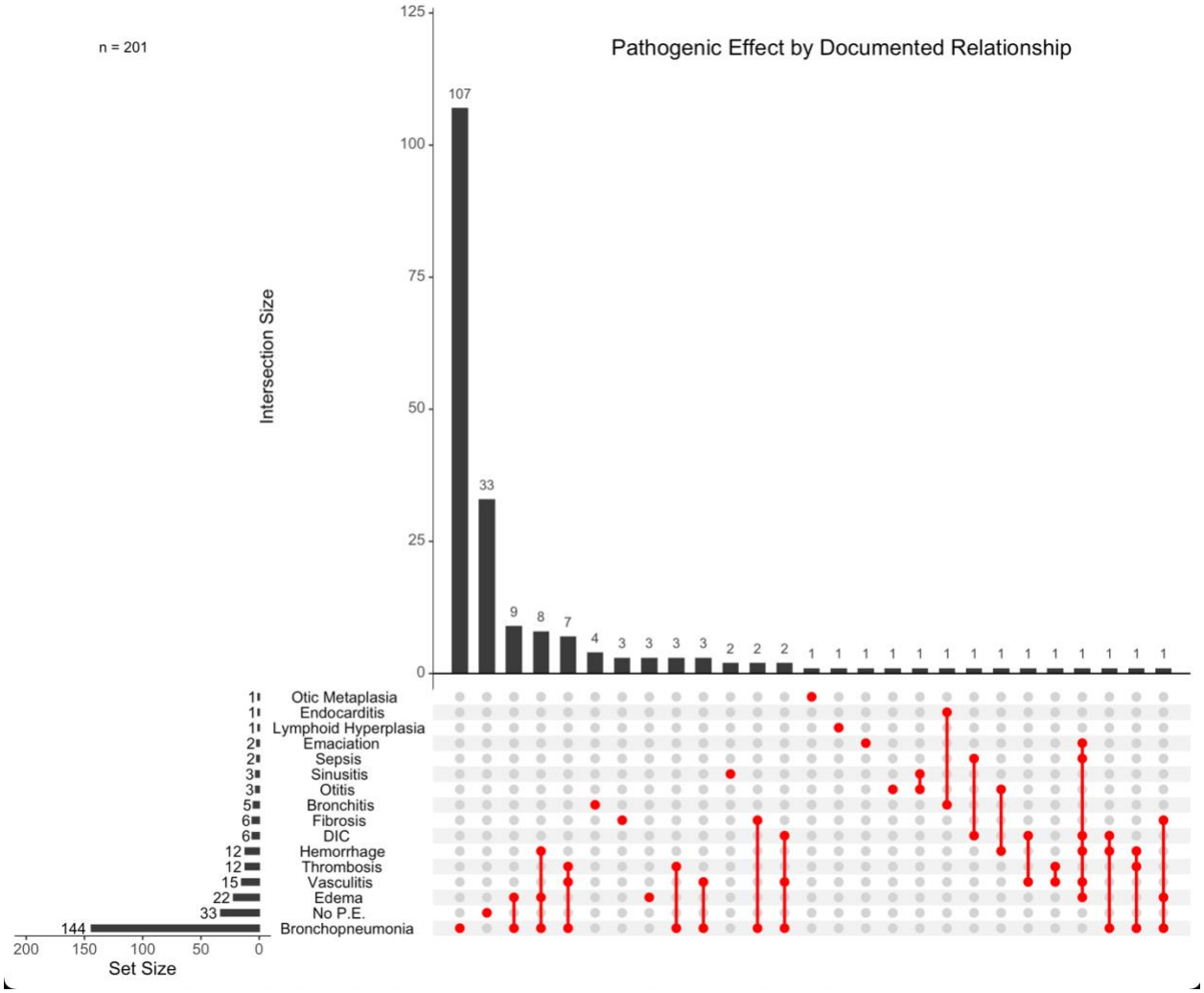

Figure 8: Pathogenic effect(s) of documented relationships between metastrongyles and marine mammals. Intersections are shown for studies which report individual host species to parasite species relationships causing multiple pathogenic effects. Relationships were designated as “No. P.E.” when pathogenic effect was assessed and was determined to be negligible. (DIC= Disseminated Intravascular Coagulation).

Discussion and conclusions

Metastrongyles are common helminth parasites of the respiratory and cardiovascular system of mammals. Given the importance of these body systems for the survival of diving animals, it was expected that the health impacts of metastrongyles would be particularly significant in marine mammals. Our findings confirm that pathogenic effects, mainly bronchopneumonia, are common

in marine mammals infected with metastrongyles, although association of metastrongyle parasites and mortality is usually not definitive. Importantly, our study suggests that pathogenic host-parasite relationships are associated to marine mammal species of larger body size that can host more metastrongyle species (parasite diversity). From the parasite perspective, metastrongyle species with a broader host diversity tend to be more pathogenic. These results emphasize the importance of host and parasite diversity with regards to the virulence of metastrongyles in marine ecosystems.

To date, most parasitology studies in marine mammals have been conducted through the opportunistic collection and examination of stranded carcasses. This probably explains in part why in most cases metastrongyle infections are suggested or considered as a contributory cause of death but rarely confirmed as the primary cause of mortality. Determining cause of death in stranded marine mammals is challenging, amid the rapid rate of postmortem changes in these species and little background information for most cases [26,27]. Additionally, access to carcasses is sometimes difficult (e.g. tides), limiting proper dissection techniques and tissue sampling. As with other host-parasite systems, the use of longitudinal assessments and experimental manipulation of infection could give new perspectives on the impact of metastrongyles in marine mammal health. Recently, the sampling of living species through the collection of feces and body fluids has been implemented in cetaceans [28]. Further, the development of molecular techniques such as major sperm protein (MSP) ELISAs to test for serum antibodies to metastrongyles aid in illuminating metastrongyle seroprevalence [9,11]. The continued use of approaches other than opportunistic necropsies may substantially contribute to the understanding of the drivers and consequences of metastrongyle infection in marine mammals.

As with many wildlife parasites [13,17], most studies on metastrongyles in marine mammals are conducted in North America and Europe, leading to overrepresentation of North American and European marine mammal and metastrongyle species in the literature. Therefore, cosmopolitan coastal marine mammals in these regions such as the harbor porpoise and the harbor seal along with their parasites were among the most assessed species. Despite our intended penalization for these study biases, our results should be interpreted with caution since more studies from

different regions of the world and in cryptic and/or pelagic marine mammal species could influence inferences on the role of host and parasite factors driving pathogenic effects. For instance, PCBs and other persistent organic pollutants (POPs) are found in higher concentrations in marine mammals in more industrialized regions of North America and Europe [29–32]. These immunosuppressive pollutants are thought to increase susceptibility to metastrongyles, facilitating their pathogenic effects [29]. Therefore, more studies from regions of the world with lower POP concentrations or where new or underrepresented host-parasite relationships occur could modify our understanding of the health impacts of metastrongyles in marine mammals.

Regression model results indicate that as parasite diversity and host diversity increase, so does the propensity for a pathogenic outcome. This finding is supported by general hypotheses on parasite virulence that suggests that a parasite species with a greater diversity of potential hosts can evolve to be more pathogenic, as the sustainability of the parasite population is less dependent on the survival of individual hosts [15,16]. Similarly, a host species that harbors a greater diversity of parasites may see a greater propensity for pathogenic outcomes from individual parasite species due to an increase in the stress on the immune and respiratory systems resulting from co-infections. Our results also indicate that larger hosts are more likely to sustain pathogenic outcomes as a result of metastrongyle parasitism. Since marine metastrongyles are transmitted through intermediate hosts that represent food items for marine mammals, this result could indicate that increased food consumption leads to a greater parasitic larval intake and subsequently a higher parasitic burden. Similarly, a larger host body size could correlate with access to different prey items, contributing to a greater diversity of metastrongyle infections.

A comprehensive understanding of the drivers of the negative physiological effects associated with metastrongyle parasitism in marine mammal hosts is challenged by a presumably strong bias towards reporting only positive findings. The available literature clearly elucidates a multitude of host-parasite relationships in which metastrongyles produce a definable pathological effect, howbeit it is inadequate in clarifying why these effects are observed in some species or populations and not in others. However, general host and parasite traits associated with pathogenic outcomes are similar to those of other helminth and microbial parasites, including host permissiveness of infection by multiple species and parasite capacity to infect multiple host

species. In this context, the rapidly changing marine environment with generalized loss of biomass and biodiversity could favor parasites and hosts with a diversified trophic network, potentially selecting for more pathogenic relationships between metastrongyles and their marine mammal hosts.

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Supplementary Data

A complete list of references for articles used in this review as well as a comprehensive checklist of known relationships between metastrongyles and marine mammals is included as supplementary data.

Author's Contributions

J.F and M.S formulated the research questions and designed the study. J.F. collected and analyzed data. J.F and M.S wrote and approved the final version of the manuscript.

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