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Quantifying parasite presence in relation to biological parameters of harbour porpoises *Phocoena phocoena* stranded on the Dutch coast

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ABSTRACT: Harbour porpoises are often found to be infected by endoparasites in several organs including the lungs and stomach as well as the heart, liver and ears. Nevertheless there is still little knowledge about the impact, ecology, transmission, and virulence of these parasitic infections. Here, we profile the presence of parasites in 4 frequently infected organs (lungs, stomach, liver and ears) in relation to biological parameters of harbour porpoises stranded along the Dutch coastline between December 2008 and December 2013. We found that parasites were common, with prevalence of 68% in lungs, 74.4% in ears, 26% in stomach and 23.5% in liver. We used generalised linear models to further quantify parasite presence in relation to biological data gathered during necropsy (sex, body length and nutritive condition). Body length (used as a proxy for age) was significant in explaining parasite presence for all organs with increasing probability of having the parasite with increasing body length. For the parasitic infections in the ears and stomach the nutritive condition was an additional significant factor, with a higher probability of parasite presence in porpoises in a poorer nutritive condition. The results of this study can be used as a baseline for assessing parasite presence in harbour porpoises and are a first step towards linking parasite infections to basic biological data gathered during necropsy.

KEY WORDS: Marine mammals · Parasites · Harbour porpoise · Nematode · Trematode · North Sea · Strandings · Generalised linear model

INTRODUCTION

Parasites are an important factor influencing the health of many animal species (McCallum & Dobson 1995). For terrestrial mammals, extensive descriptions of lesions and diseases attributed to parasite presence are available and some parasites are even considered to regulate host populations (e.g. Gulland 1992, Nettles et al. 2002, Barnes et al. 2010). Much less is known about parasites in marine mammals (Lehnert et al. 2010) and existing studies have concentrated primarily on parasite taxonomy (Dougherty 1949, Arnold & Gaskin 1975) or directly parasite-related pathology (Dailey & Stroud 1978, Faulkner et al. 1998, Jepson et al. 2000, Siebert et al. 2001). Consequently, there is still little knowledge about the impact, ecology, transmission, and virulence of parasites in marine mammals and their effects on host populations.

The harbour porpoise *Phocoena phocoena* is the most abundant cetacean species in the North Sea and adjacent waters (Hammond et al. 2002) and is

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protected under a number of national and international legislative frameworks, such as the Agreement on the Conservation of Small Cetaceans of the Baltic, Northeast Atlantic, Irish and North Seas (ASCOBANS; www.ascobans.org) and the EU Habitats Directive (EEC 1992). Literature is available on the presence of endoparasites in several harbour porpoise organs, including the lungs and stomach as well as the heart, liver and ears (e.g. Raga et al. 1997, Lehnert et al. 2005). Heavy burdens of the gastric nematode *Anisakis simplex* and gastric trematode *Pholeter gastrophilus* can cause mucosal ulcerations (e.g. Lehnert et al. 2005), gastritis (Lehnert et al. 2014) and have also been associated with granulomatous dermatitis (van Beurden et al. 2015). Hepatic trematodes *Campula oblonga* have been associated with hyperplasia of bile ductular epithelium, inflammation of the bile ducts and periductular fibrosis, and occasionally affect the surrounding liver tissue (Lehnert et al. 2005, Jaber et al. 2013). The nematode *Stenurus minor* infects cranial sinuses and the auditory canal (ears); although it does not appear to cause significant pathology (e.g. Brosens et al. 1996), in cases of severe infections sound perception may be impaired (Faulkner 1995). The lung parasites *Pseudalius inflexus, Torynurus convolutus* and *Halocercus* sp., when present in large numbers, can cause obstruction of the airways (Gibson et al. 1998) and may impede diving ability (Siebert et al. 2001). These lung nematodes can be found both free in the airways and embedded (partially) in the parenchyma, and are frequently observed in the lung vasculature. Additionally, several studies have associated these lung parasites with pneumonia and (secondary) bacterial infection (e.g. Kirkwood et al. 1997, Wünschmann et al. 2001). This is of note as respiratory disease (pneumonia in particular) is the most common disease contributing to mortality in harbour porpoises stranding on coasts along the North Sea (Gibson et al. 1998, Jepson et al. 2000, Siebert et al. 2001, Jauniaux et al. 2002).

The severity of the parasite burden, as well as parasite species composition, varies geographically, with harbour porpoises from Greenland coastal waters generally having a lower level of infection compared to porpoises found in the Baltic and North Sea (Jepson et al. 2000, Siebert et al. 2001, Wünschmann et al. 2001). This suggests that the extent, severity and significance of parasite presence are variable on both an individual and a population level. Experimental studies are not ethically accepted in free-living protected marine mammal species, making it difficult to examine the dynamics of host–parasite interactions, or the factors potentially determining the pathogenic capacity of parasitic infections. The investigation of dead stranded animals provides information on the health status and biological parameters such as the nutritional condition, sex, and morphometrics of individuals. Such data allows examination of such parameters in relation to the parasite burden and may provide insight into the mechanisms underlying parasite recruitment and intensity of infections, possibly revealing vital clues about the point and extent to which parasites will be influential on the health of an individual.

This study's objective is to quantify and profile the presence of parasites in the 4 most commonly affected organs (lungs, stomach, liver and ears) in relation to biological parameters of harbour porpoises stranded on the Dutch coast. It aims to provide a baseline for the assessment of parasite presence and step towards a better understanding of parasite–host interactions between endoparasites and harbour porpoises in the southern North Sea.

MATERIALS AND METHODS

Data collection

Between December 2008 and December 2013, necropsies were performed on 873 harbour porpoises that were found dead along the Dutch coastline. Carcasses that could not be studied immediately were stored at −20°C until necropsy. Post-mortem examinations were conducted following an internationally standardised protocol (Kuiken & Garcia-Hartmann 1993). A more advanced state of decomposition can prevent accurate determination of numerous factors including nutritional condition (Bull et al. 2006), and therefore only animals in a relatively fresh condition (complete carcasses, only little decomposition) were included in this study, resulting in a sample size of 410 cases. Stranding date, sex and body length (cm) were recorded. Each individual was assigned a nutritional condition code (NCC), based on evaluation of blubber thickness, muscularity, weight, and the presence of subcutaneous and pleural fat, with NCC1 representing a very good nutritive condition and NCC6 representing an extremely poor nutritive state. In the absence of data on absolute age for the animals in this dataset, individuals were categorised into age groups based on their body length, with animals <90 cm being neonates, 91 to 130 cm considered juvenile, and >130 cm classed as adults.

The lungs, ears, stomach (all compartments assessed collectively) and liver of all necropsied porpoises were routinely macroscopically assessed for the presence of parasites and scored respectively on a binomial scale (1 or 0 for presence/absence). Presence of parasites in the intestines and heart was additionally recorded for some cases, but this was not routinely documented. It was therefore decided to exclude these organs from further analysis. For a number of individuals with parasites present, the severity of the infection was estimated and recorded as minimal, mild, moderate, marked, or severe. These estimations of severity were not systematically reported and estimations were made by several different pathologists. As a result, these observations were likely prone to an observer bias. Consequentially it could not be assumed these followed a true ordinal scale and this data was therefore not used for further analysis.

The biological and necropsy data and individual necropsy reports were documented in a central database, and this information and data were combined and interrogated for the purposes of this study. Co-infection of different parasitic species and types within an organ is known to occur. However such detailed data was not available for the majority of the cases and, given the historical nature of the data, no distinctions could be made between different parasite species and types infesting the same organ in this study.

Data analyses

Data exploration was applied following Zuur et al. (2009) and covariates were examined for outliers, missing values and collinearity. This revealed seasonality in both stranding rate as well as the composition of biological parameters of the stranded population, and it was decided to exclude the stranding date from the analysis to avoid this having a confounding effect on the other variables. Remaining covariates used to estimate the correct predictor function therefore included sex, NCC, and body length. The number of individuals included in each analysis varied per organ due to missing data for some cases.

To ensure a similar sample size and representation of all body lengths in all NCC categories, the levels of NCC were regrouped, with NCC1–2 representing a good nutritive condition (n = 133), NCC3–4 indicating a moderate/poor nutritive condition (n = 149) and NCC5–6 being a highly poor/emaciated condition (n = 115). NCC was treated as a categorical variable. Body length was used as a proxy for age.

To investigate whether there were relationships between the presence of parasites and any of the explanatory variables, generalised linear models (GLM) using a binomial error distribution and logit link were fitted for each individual organ. These models had the form:

$$P_i \sim Binomial\,(1, \pi_i)\;\; E(P_i) = \pi_i \tag{1}$$

and

$$Var(P_i) = \pi_i\,(1 - \pi_i) \tag{2}$$

where P_i is the presence (1) or absence (0) of parasites in the lungs ears, stomach and liver respectively, and the systematic part is given by:

$$\text{logit}(\pi_i) = \eta(x_{i1}, \ldots, x_{im}) = \alpha + \beta_1 \times x_{i1} + \ldots + \beta_m \times x_{im} \tag{3}$$

where the intercept α plus the pre-specified functions of the explanatory variables x_i comprise the predictor function $\eta(x_{i1}, \ldots, x_{im})$. Body length was centred prior to applying the models so that the intercept is representative of the probability that a harbour porpoise of average length has the parasite, in order to facilitate model interpretation. Average body length was calculated at 116.4 cm based on the 400 individuals for which length was recorded.

Model selection was applied by backward stepwise selection using the Akaike information criterion (AIC; Akaike 1974) to select the optimal model. To account for the potential bias associated with varying number of observations, the AIC of every model was divided by the model's sample size as recommended by Hilbe (2009), and this statistic was used for each model being compared. Model validation was applied to verify the underlying model assumptions by evaluation of calculated dispersion parameters and diagnostic plots using Pearson residuals.

All calculations were conducted using R statistical software (R Core Team 2014) and statistical significance was accepted at $p < 0.05$.

RESULTS

The number of cases included in each analysis varied between organs according to data availability and completeness. Mean length of the harbour porpoises was calculated at 116.4 cm based on the 400 cases for which a length was accurately recorded. Model validation showed no evidence for violation of the underlying model assumptions for any of the individual models. An overview of the parasite severity estimation for each individual organ is given in Table 1.

Table 1. Number of cases per level of severity of parasite infections found in the lungs, liver, ears and stomach of harbour porpoises stranded on the Dutch coastline between 2008 and 2013. n = total number of cases per organ where level of severity of infection was recorded in the necropsy report

Organ	n	Severity				
		Minimal	Mild	Moderate	Marked	Severe
Lungs	265	96	10	110	15	34
Liver	90	46	0	34	3	7
Ears	287	121	12	102	12	40
Stomach	97	58	1	22	1	15

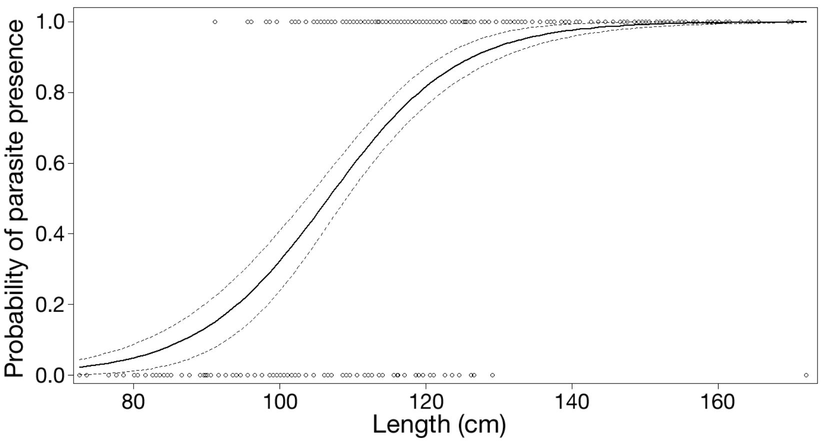

Fig. 1. Probability (95% CI) of parasite presence in the lungs in relation to total length (cm) for examined harbour porpoises stranded on the Dutch coastline between 2008 and 2013 (n = 391)

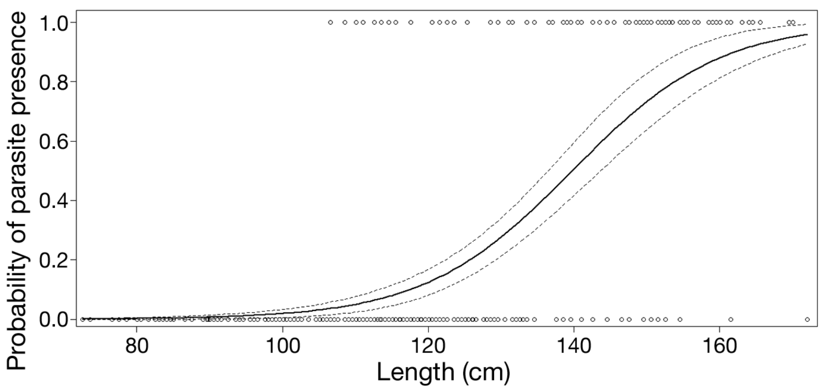

Fig. 2. Probability (95% CI) of parasite presence in the liver in relation to total length (cm) for examined harbour porpoises stranded on the Dutch coastline between 2008 and 2013 (n = 387)

Lungs. Data on parasite presence in the lungs were available for 391 harbour porpoises, of which 266 (68%) were infected with parasites. The optimal model for parasite presence in the lungs included only body length (df = 1, $p < 0.001$), where the probability of an individual having lung parasites increased with increasing size ($Z = 8.796$, $p < 0.001$) (Fig. 1, Table 2). The model explained 36% of the variation in parasite presence.

Liver. Data on parasite presence in the liver was available for 387 harbour porpoises, of which 91 (23.5%) were infested with parasites. The optimal model for parasite presence in the liver included body length only, where the probability of parasite presence in the liver increased with increasing body length ($Z = 9.583$, $p < 0.001$) (Table 2). This model explained 40.5% of the variation in parasite presence. The probability of having parasites increased with increasing body length (Fig. 2), and was relatively low for animals of average length. Only the larger individuals have a probability of liver parasites of >50%.

Ears. Data on parasite presence in the ears was available for 387 harbour porpoises, of which 288 (74.4%) were infested with parasites. The optimal model for parasite presence in the ears included body length ($Z = 8.796$, $p < 0.001$) (Table 3) and NCC (df = 2, $p = 0.03$) and explained 27% of the variation in parasite presence. Although the probability of having ear parasites increased significantly with increasing body length for all NCC categories, the probability of parasite presence was higher for harbour por-

Table 2. Model outputs for the optimal models of parasite presence in the lungs and liver of harbour porpoises, including length (centred) only, showing model parameter estimates, standard error, *Z*-score and associated p-value

	Lungs				Liver			
	Estimate	SE	*Z*-score	p-value	Estimate	SE	*Z*-score	p value
Intercept	1.491	0.184	8.115	<0.001	−1.950	0.204	−9.551	<0.001
Length (centred)	0.112	0.0127	8.796	<0.001	0.099	0.010	9.583	<0.001

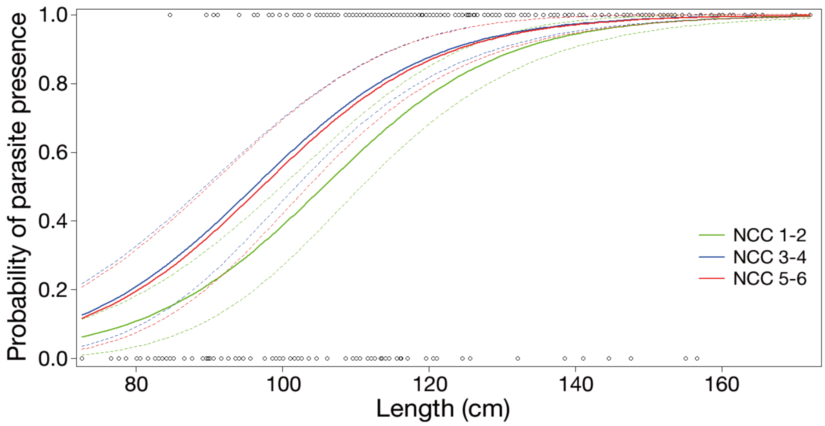

Fig. 3. Probability (95% CI) of parasite presence in ears in relation to total length (cm) and within the different nutritional condition codes (NCC) for examined harbour porpoises stranded on the Dutch coastline between 2008 and 2013 (n = 387)

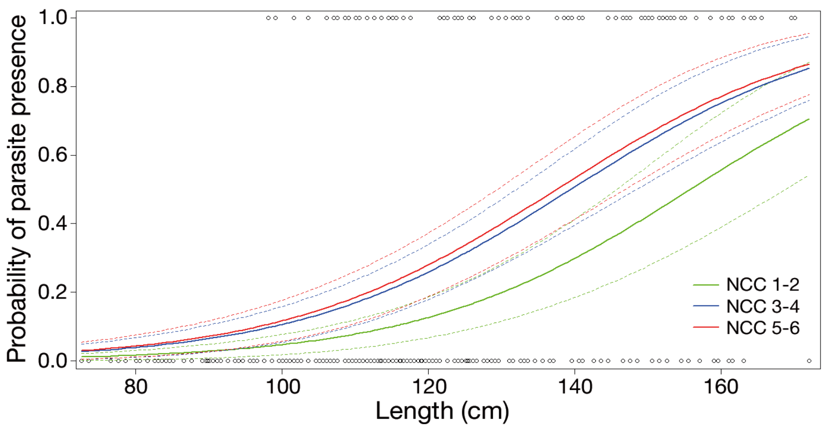

Fig. 4. Probability (95% CI) of parasite presence in stomach in relation to total length (cm) and within the different nutritional condition codes (NCC) for examined harbour porpoises stranded on the Dutch coastline between 2008 and 2013 (n = 392)

poises with a poor nutritive condition (NCC5–6) (Fig. 3). There was a probability of >75% of observing ear parasites in porpoises of average length in all NCC categories.

Stomach. Data on parasite presence in the stomach was available for 392 harbour porpoises, of which 102 (26%) were infested with parasites. The optimal model for parasite presence in the stomach included both body length ($Z = 7.728$, $p < 0.001$) (Table 3) and NCC (df = 2, $p < 0.001$) and explained 21% of the variation. The probability of having stomach parasites increased significantly with increasing body length with only the larger individuals having a probability >50% of having stomach parasites (Fig. 4). Harbour porpoises with poor nutritive condition (NCC5–6) generally had higher probabilities of having stomach parasites than animals in a good nutritive condition.

DISCUSSION

This is the first extensive study investigating parasite presence in 4 major organs (lungs, liver, stomach and ears) of stranded harbour porpoises in relation to biological data (sex, body length and nutritive condition). We demonstrate that body length, here used as a proxy for age, is significant for all organs, with an increasing probability of parasite presence with increasing age. For parasites in the ears and the stomach a significant negative correlation with the nutritional condition was found. With these findings we provide a baseline for parasite presence in harbour porpoises stranded on the Dutch coastline. Parasites are a common finding, with prevalence of 68% in the lungs, 74.4% in the ears, 26% in the stomach and 23.5% in the liver. This is consistent with results from other studies on parasite prevalence in harbour porpoises stranded along the North Sea coasts (e.g. Jepson et al. 2000, Siebert et al. 2001, Jauniaux et al. 2002, Lehnert et al. 2005).

Table 3. Model outputs for the optimal models of parasite presence in the ears and stomach of harbour porpoises, including length (centred) and the nutritional condition code (NCC), showing model parameter estimates, standard error, *Z*-score and associated p-value

	Ears				Stomach			
	Estimate	SE	*Z*-score	p-value	Estimate	SE	*Z*-score	p-value
NCC1–2 (good) (intercept)	1.185	0.239	4.948	<0.001	−1.934	0.274	−7.063	<0.001
NCC3–4 (moderate)	0.778	0.333	2.334	0.019	0.881	0.339	2.600	0.009
NCC5–6 (poor)	0.694	0.354	1.959	0.050	0.988	0.352	2.803	0.005
Length (centred)	0.082	0.010	8.032	<0.001	0.054	0.007	7.728	<0.001

Sex was not a significant parameter in explaining the variation in parasite presence in any of the models. This indicates there are no substantial differences in the presence of parasites between males and females for any of the examined organs, which is supported by Brosens et al. (1996) who also did not observe sex-related differences in parasite presence.

As with other studies, the intensity and distribution of parasite infections in harbour porpoises included in this study cohort increased with animal length, a proxy for age. Body length was significant in explaining parasite presence in all organs, with an increasing probability of parasite presence with increasing body length. This is also shown by Siebert et al. (2001, 2006) and indicates that porpoises do not acquire an effective cellular or humoral immune response to parasite infections that enables them to clear infection. Instead, animals appear to tolerate and cope with long-term parasite presence. Neonates (body length <90 cm), that consume milk from their mothers, were rarely infected with parasites in any of the examined organs. This indicates porpoises recruit infection via the ingestion of prey, rather than through vertical pathways. Once weaned, parasite exposure and infection likely occurs through ingestion of an intermediate or paratenic host, a result which is supported by other studies (e.g. Samuel et al. 2001, Lehnert et al. 2010). Nevertheless, the ear parasite *S. minor* was also observed in a few individuals with a body length <90 cm. These particular animals were close to the weaning length and it is possible that these harbour porpoises had already been consuming solid prey (Leopold 2015). However, it does not rule out recruitment via ingestion of colostral or milk or direct transmammary or transplacental transmission of this parasite, as also found by Lehnert et al. (2014). Stomach and liver parasites were significantly more common in larger animals. An explanation for this could be that the intermediate host of these parasite species is a prey species that is more frequently consumed in the later life stages of harbour porpoises. The diet of harbour porpoises consists of a wide variety of pelagic and demersal fish species, crustaceans and polychaetes, and varies according to geographic location, season and age (Santos et al. 2004, Leopold 2015). Parasites in both the lungs and the ears were frequently present in smaller and hence younger animals, from which it can be inferred that the intermediate host for these parasites is a prey species that is already consumed in the earlier life stages; for example the goby (Gobiidae) which is a common species in the diet of harbour porpoises of all ages (Santos et al. 2004, Leopold 2015).

The optimal model for the ears and the stomach included a significant negative correlation with the NCC, showing that porpoises in a poorer nutritive condition have a higher probability of having parasites in these organs. Nutritional condition is a commonly used metric of health (e.g. Wünschmann et al. 2001) and a critical component in the survival of harbour porpoises (Koopman et al. 2002). Their relatively large body surface to body volume ratio imposes very high energetic requirements and even short periods of fasting can rapidly lead to loss of body condition and associated physiological stress (Kastelein et al. 1997, Lockyer et al. 2003). Assuming nutritional condition is a good metric of porpoise health this may suggest that the likelihood of parasite presence is higher in unhealthy and possibly immunosuppressed animals, irrespective of their age (Jepson et al. 2000, Bull et al. 2006). This finding could be of significance for determination of the pathogenic impact of parasites and the causality between the presence of parasites and debilitation and disease in their hosts. Besides a possible direct pathogenic relation between presence of parasites and host health, there may also be an indirect impact of parasite presence. There are several associations and observations of concomitant infections with other pathogens, for example with *Brucella* (Dawson et al. 2008) and *Salmonella* (Davison et al. 2010) among others, yet their relation remains largely unclear (Kirkwood et al. 1997, Jepson et al. 2000, Siebert et al. 2002, Dawson et al. 2008, Davison et al. 2010). It has been suggested that some pathogens may use parasite infections as a transmission route, or have evolved to utilise parasites as a vector (Perkins & Fenton 2006, Dawson et al. 2008). Our findings for the ears and stomach suggests that parasites may only be of significance in unhealthy individuals; however relating the presence of parasites to other potential pathogens was beyond the scope of the study done here. Moreover no such relation was apparent for the lungs and liver, where parasite presence was independent of nutritional condition. This concurs with other studies reporting high parasite prevalence in healthy animals that had died of acute traumatic causes such as bycatch in fishing gear (Siebert et al. 2006), whaling practices (Lehnert et al. 2014) and predation (Leopold et al. 2015).

It should be noted that this study was done based on historical necropsies involving several pathologists and through extracting data from the central database of individual necropsy report findings. This means there were certain limitations to the data, and while further investigation of the severity of parasite infection would provide useful insight into the potential pathogenicity of parasites in their hosts, such analysis was not possible with the data presented here. The development of standardised procedures for parasite severity estimation, and incorporation of these into the internationally accepted necropsy protocol, would eliminate the potential observer bias, making data comparable at an international level, and facilitate future analyses.

The interaction between parasites and harbour porpoises is complex, yet may be influenced by variation in host health and immunity and the presence of other pathogens or disease processes. This study was the first step towards linking the common findings of parasites in harbour porpoises to basic biological data gathered during necropsy. It revealed relations to body length and nutritive condition, but not to sex. Examining and assessing the influence of parasites on the health of harbour porpoises requires a more detailed analysis, where these findings are further related to a scoring of numbers of parasites present, parasite species composition, and the amount and type of tissue reaction and inflammation present within an organ. Additional studies further linking parasite occurrence (and incorporating species identification and the severity of parasite infections) to disease and concomitant infections with other pathogens would make a valuable contribution to the understanding of parasite–host relations of harbour porpoises and other cetacean species.

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LITERATURE CITED

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Contr 19:716–723

Arnold PW, Gaskin DE (1975) Lungworms (*Metastrongyloidea*: Pseudaliidae) of harbor porpoise *Phocoena phocoena* (L. 1758). Can J Zool 53:713–735

Barnes TS, Goldizen AW, Morton JM, Coleman GT (2010) Parasites of the brush-tailed rock-wallaby (*Petrogale penicillata*). J Wildl Dis 46:218–228

Brosens L, Jauniaux T, Siebert S, Benke H, Coignoul F (1996) Observations on the helminths of harbour porpoises (*Phocoena phocoena*) and common guillemots (*Uria aalge*) from the Belgian and German coasts. Vet Rec 139:254–257

Bull JC, Jepson PD, Ssuna RK, Deaville R, Allchin CR, Law RJ, Fenton A (2006) The relationship between polychlorinated biphenyls in blubber and levels of nematode infestations in harbour porpoises *Phocoena phocoena.* Parasitology 132:565–573

Dailey M, Stroud R (1978) Parasites and associated pathology observed in cetaceans stranded along the Oregon coast. J Wildl Dis 14:503–511

Davison N, Barnett J, Rule B, Chappell S, Wise G (2010) Group B *Salmonella* in lungworms from a harbour porpoise (*Phocoena phocoena*). Vet Rec 167:351–352

Dawson CE, Perrett LL, Stubberfield EJ, Stack JA and others (2008) Isolation and characterization of *Brucella* from the lungworms of a harbor porpoise (*Phocoena phocoena*). J Wildl Dis 44:237–246

Dougherty EC (1949) The phylogeny of the nematode family Metastrongylidae Leiper, [1909]: a correlation of host and symbiote evolution. Parasitology 39:222–234

EEC (1992) Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora. Off J Eur Union L 206:42. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1992L0043:20070101:EN:PDF

Faulkner J (1995) Study of the cranial sinus nematode, *Stenurus minor* (Metastrongyloidea), in the harbour porpoise, *Phocoena phocoena*. MS thesis, McGill University, Montreal

Faulkner J, Measures LN, Whoriskey FG (1998) *Stenurus minor* (Metastrongyloidea: Pseudaliidae) infections of the cranial sinuses of the harbour porpoise, *Phocoena phocoena.* Can J Zool 76:1209–1216

Gibson DI, Harris EA, Bray RA, Jepson PD, Kuiken T, Baker JR, Simpson VR (1998) A survey of the helminth parasites of cetaceans stranded on the coast of England and Wales during the period 1990–1994. J Zool (Lond) 244: 563–574

Gulland FM (1992) The role of nematode parasites in Soay sheep (*Ovis aries* L.) mortality during a population crash. Parasitology 105:493–503

Hammond PS, Berggren P, Benke H, Borchers DL and others (2002) Abundance of harbour porpoise and other cetaceans in the North Sea and adjacent waters. J Appl Ecol 39:361–376

Hilbe JM (2009) Logistic regression models. Chapman & Hall/CRC Press, Boca Raton, FL

Jaber JR, Perez J, Rotstein D, Zafra R, Herraez P, Carrascosa C, Fernandez A (2013) Biliary cirrhosis caused by *Campula* spp. in a dolphin and four porpoises. Dis Aquat Org 106:79–84

Jauniaux T, Petitjean D, Brenez C, Borrens M and others (2002) Post-mortem findings and causes of death of harbour porpoises (*Phocoena phocoena*) stranded from 1990 to 2000 along the coastlines of Belgium and Northern France. J Comp Pathol 126:243–253

Jepson PD, Baker JR, Kuiken T, Simpson VR, Kennedy S, Bennett PM (2000) Pulmonary pathology of harbour porpoises (*Phocoena phocoena*) stranded in England and Wales between 1990 and 1996. Vet Rec 146:721–728

Kastelein RA, Nieuwstraten SH, Verstegen MWA (1997) Passage time of carmine red dye through the digestive tract of harbour porpoises (*Phocoena phocoena*). In: Read AJ, Wiepkema PR, Nachtigall EP (eds) The biology of the harbour porpoise. De Spil, Woerden, p 265–275

Kirkwood JK, Bennett PM, Jepson PD, Kuiken T, Simpson VR, Baker JR (1997) Entanglement in fishing gear and other causes of death in cetaceans stranded on the coasts of England and Wales. Vet Rec 141:94–98

Koopman HN, Pabst DA, McLellan WA, Dillaman RM, Read AJ (2002) Changes in blubber distribution and morphology associated with starvation in harbour porpoises (*Phocoena phocoena*): evidence of regional differences in blubber structure and function. Physiol Biochem Zool 75: 498–512

Kuiken T, Garcia Hartmann M (eds) (1993) Proceedings of the first European Cetacean Society workshop on cetacean pathology: dissection techniques and tissue sampling. ECS Newsletter 17 (Spec Issue):1–39

Lehnert K, Raga JA, Siebert U (2005) Macroparasites in stranded and bycaught harbour porpoises from German and Norwegian waters. Dis Aquat Org 64: 265–269

Lehnert K, von Samson-Himmelstjerna G, Schaudien D, Bleidorn C, Wohlsein P, Siebert U (2010) Transmission of lungworms of harbour porpoises and harbour seals: molecular tools determine potential vertebrate intermediate hosts. Int J Parasitol 40:845–853

Lehnert K, Seibel H, Hasselmeier I, Wohlsein P and others (2014) Increase in parasite burden and associated pathology in harbour porpoises (*Phocoena phocoena*) in West Greenland. Polar Biol 37:321–331

Leopold MF (2015) Eat and be eaten – porpoise diet studies. Doctoral thesis, Wageningen University. edepot.wur.nl/361729

Leopold MF, Begeman L, van Bleijswijk JDL, IJsseldijk LL, Witte HJ, Gröne A (2015) Exposing the grey seal as a major predator of harbour porpoises. Proc R Soc B 282: 20142429

Lockyer C, Desportes G, Hansen K, Labberté S, Siebert U (2003) Monitoring growth and energy utilisation of the harbour porpoise (*Phocoena phocoena*) in human care. NAMMCO Sci Publ 5:107–120

McCallum H, Dobson A (1995) Detecting disease and parasite threats to endangered species and ecosystems. Trends Ecol Evol 10:190–194

Nettles VF, Quist CF, Lopez RR, Wilmers TJ and others (2002) Morbidity and mortality factors in Key deer. J Wildl Dis 38:685–692

Perkins SE, Fenton A (2006) Helminths as vectors of pathogens in vertebrate hosts: a theoretical approach. Int J Parasitol 36:887–894

Raga JA, Balbuena JA, Aznar J, Fernandez M (1997) The impact of parasites on marine mammals: a review. Parassitologia 39:293–296

R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org

Samuel WM, Pybus MJ, Kocan AA (2001) Parasitic diseases of wild mammals, 2nd edn. Iowa State University Press, Ames, IA

Santos MB, Pierce GJ, Learmonth JA, Reid RJ and others (2004) Variability in the diet of harbour porpoises (*Phocoena phocoena*) in Scottish waters 1992–2003. Mar Mamm Sci 20:1–27

Siebert U, Wünschmann A, Weiss R, Frank H, Benke H, Frese K (2001) Post-mortem findings in harbour porpoises (*Phocoena phocoena*) from the German North and Baltic Seas. J Comp Pathol 124:102–114

Siebert U, Müller G, Desportes G, Weiss R, Hansen K, Baumgärtner W (2002) Pyogranulomatous myocarditis due to *Staphylococcus aureus* septicaemia in two harbour porpoises (*Phocoena phocoena*). Vet Rec 150: 273–277

Siebert U, Tolley K, Víkingsson GA, Olafsdottir D, Lehnert K, Weiss R, Baumgärtner W (2006) Pathological findings in harbour porpoises (*Phocoena phocoena*) from Norwegian and Icelandic waters. J Comp Pathol 134:134–142

van Beurden SJ, IJsseldijk LL, Cremers HJ, Gröne A, Verheije MH, Begeman L (2015) *Anisakis* spp. induced granulomatous dermatitis in a harbour porpoise *Phocoena phocoena* and a bottlenose dolphin *Tursiops truncatus*. Dis Aquat Org 112:257–263

Wünschmann A, Siebert U, Frese K, Weiss R and others (2001) Evidence of infectious diseases in harbour porpoises (*Phocoena phocoena*) hunted in the waters of Greenland and by-caught in the German North Sea and Baltic Sea. Vet Rec 148:715–720

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Statistics for biology and health. Springer, Berlin

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