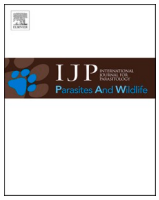
International Journal for Parasitology: Parasites and Wildlife 23 (2024) 100898

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw



Heartworm and seal louse: Trends in prevalence, characterisation of impact and transmission pathways in a unique parasite assembly on seals in the North and Baltic Sea

Insa Herzog [a], Peter Wohlsein [b], Anika Preuss [c], Stanislav N. Gorb [c], Rémi Pigeault [a], Christa Ewers [d], Ellen Prenger-Berninghoff [c], Ursula Siebert [a], Kristina Lehnert [a,*]

[a] Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover, Werftstraße 6, 25761, Büsum, Germany
[b] Department of Pathology, University of Veterinary Medicine, Bünteweg 2, 30559, Hannover, Germany
[c] Department of Functional Morphology and Biomechanics, Zoological Institute of the University of Kiel, Am Botanischen Garten 1–9, 24118, Kiel, Germany
[d] Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, 35392, Giessen, Germany

ARTICLE INFO

Keywords:
Seal louse
Heartworm
Marine mammal parasites
Harbour seals
Grey seals
Host-parasite relationship

ABSTRACT

The ectoparasitic seal louse, *Echinophthirius horridus* infects harbour (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) in the North and Baltic Sea. The endoparasitic heartworm *Acanthocheilonema spirocauda* parasitizes the right heart and blood vessels of harbour seals. The complete lifecycle of the heartworm is not entirely understood although the seal louse is assumed to serve as vector for its transmission. Knowledge about the impact of both parasite species on host health are scarce. In this study, necropsy data and archived parasites of harbour and grey seals in German waters were analysed to determine long-term seal louse (SLP) and heartworm prevalence (HWP) from 2014 to 2021. Histology, microbiology and scanning electron microscopy (SEM) were applied on seal louse infected and uninfected skin to investigate associated lesions and the health impact. During the study period, HWP in harbour seals was 13%, the SLP in harbour seals was 4% and in grey seals 10%. HWP of harbour seals was significantly higher during the winter months compared to the summer. SLP in adults was significantly higher in comparison to juvenile harbour seals. SLP varied significantly between grey seals from the North and Baltic Sea. Filarial nematodes were detected in the haemocoel, pharynx, and intestine of *E. horridus* highlighting the seal louse as vector for heartworms. Alopecia and folliculitis were associated with the attachment posture of *E. horridus* and microbiological investigations isolated bacteria commonly associated with folliculitis.

1. Introduction

Harbour (*Phoca vitulina*) and grey seals (*Halichoerus grypus)* inhabit the North and Baltic Sea of Germany. For both species, population dynamics are characterized by strong fluctuations (Härkönen et al., 2006; Galatius et al., 2020; Silva et al., 2021; Unger et al., 2022) and a drastic decline caused by growing settlements of humans along the coasts and by intensifying of hunting (Reijnders, 1984, 1994; Harding and Härkönen, 1999) over the last centuries. Extensive mortalities due to two epidemic outbreaks of phocine distemper virus (PVD) occurred in 1988 and 2002 (Härkönen et al., 2006). Marine debris (Unger et al., 2017), potentially zoonotic pathogens (Siebert et al., 2007; Waltzek et al., 2012; Postel et al., 2021), noise (Mikkelsen et al., 2019) and contaminants (Bruhn et al., 1999; Green and Larson, 2016; Sonne et al., 2020) still pose a threat to phocid seals in the North and Baltic Sea.

The parasite fauna of pinnipeds in the northern hemisphere is highly diverse (Aznar et al., 2001) and some species share a long evolutionary history with their semiaquatic host (Kim, 1975, 1985; Kim et al., 1975; Leonardi et al., 2021a). The seal louse (*Echinophthirius horridus;* Anoplura, family: Echinophthiriidae) is a permanent ectoparasitic, haematophagous insect (Kim et al., 1975; Leidenberger et al., 2007) and is transmitted directly through physical contact during haul outs of seals (Murray and Nicholls, 1965; Kim, 1975; Raga, 1992). The reproductive cycle of seal lice is completed on land, since seal lice are unable to lay eggs and hatch under water (Scherf, 1963; Murray and Nicholls, 1965; Murray et al., 1965). Severe seal lice infection can cause pruritus, alopecia and anaemia (Durden, 1971; Conlogue et al., 1980) and the damaged skin barrier can constitute a possible entrance for pathogens

* Corresponding author.
E-mail address: kristina.lehnert@tiho-hannover.de (K. Lehnert).

https://doi.org/10.1016/j.ijppaw.2023.100898
Received 5 October 2023; Received in revised form 29 November 2023; Accepted 18 December 2023
Available online 22 December 2023

(Durden, 1971; Raga, 1992; Leidenberger et al., 2007). Systematic analyses of seal louse infected skin potentially causing secondary infection and influencing the skin microbiota are lacking.

Acanthocheilonema spirocauda (Spirurina, family: Onchocercidae) (Anderson and Bain, 1976) is a filarial nematode (Leidy, 1858), which is found in the right ventricle and pulmonary vessels of the heart of phocid seals (Leidy, 1858; Anderson, 2000; Leidenberger et al., 2007; Lehnert et al., 2016). Besides harbour seals (Leidy, 1858; Anderson, 1959) *A. spirocauda* was found in ringed (*Pusa hispida*), harp (*Phoca groenlandica*) and hooded seals (*Cristophora cristata*) (Measures et al., 1997) as well as in monk seals (*Monachus monachus*) (Papadopoulos et al., 2010) and recently in grey seals (*Halichoerus grypus*) (Keroack et al., 2018; Lehnert et al., 2023). Heartworms can cause multiple morphologic alterations like endarteritis (Otto and Jackson, 1969), obstruction of the pulmonary vessels (Stroud and Dailey, 1978), such as verminous emboli (Dunn and Wolke, 1976), and in single cases rupture of the atrium (Lehnert et al., 2007).

Seal lice are believed to serve as vector for heartworms (Wülker, 1929, 1930; Geraci et al., 1981) by ingesting filarial stages during their blood meal, which were released by mature nematodes from the heart (Geraci et al., 1981). Within the seal louse, the first larval stage passes several moults (Geraci et al., 1981), afterwards the infectious third larval stage is emitted during its next blood meal into the circulatory system of the same or a different host (Geraci and Lounsbury, 2001; Leidenberger et al., 2007), in which it develops to a mature heartworm (Raga, 1992). Larval stages found in dissected seal lice (Geraci et al., 1981; Lehnert et al., 2016), detection of heartworm DNA traces in seal lice (Keroack et al., 2018; Hirzmann et al., 2021) and micro-CT-reconstruction with evidence of larval stages in seal lice (Ebmer et al., 2022) support this vector hypothesis.

Little is known about long-term prevalence of the parasite assembly *E. horridus* and *A. spirocauda* in harbour and grey seals (Claussen et al., 1991; Lunneryd, 1992; Lehnert et al., 2016). Monitoring parasite prevalence, intensity (Bush et al., 1997) and parasite associated lesions over decades can provide a useful indicator for population health (Howells et al., 2010; Fiorenza et al., 2020) especially when assessing recurring grey seal populations in the German waters.

This study investigates ecology and health impact of seal louse and heartworm infections on seals in the North and Baltic Sea. Long-term prevalence and intensity of both parasite species were analysed and novel aspects of this study confirm the role of *E. horridus* as vector for heartworm filariae. Lesion caused by attachment postures of parasites were characterized.

2. Material and methods

2.1. Study area and sample collection

All seals included in this study originated from the North or Baltic Sea Coasts of the German Federal State Schleswig-Holstein. Seals were collected from 2014 to 2021 within the stranding network of Schleswig-Holstein. Seals either were found dead or were shot by qualified seal rangers, who are permitted to mercy-kill seals, if their health status does not indicate a survival (Niedersächsisches Jagdgesetz, 2023). Subsequently, these animals were necropsied at the Institute of Terrestrial and Aquatic Wildlife Research (ITAW) in Büsum, Germany, following a standardized protocol (Siebert et al., 2001, 2007; IJsseldijk et al., 2019). Collected animals were dissected shortly after their death or were frozen (−20 °C) and dissected later on. Carcasses were classified into degree of decomposition (DCC) 1 (fresh animals, just died) up to DCC 5 (mummified animal or skeletal remains) (IJsseldijk et al., 2019). Afterwards, histopathological, parasitological, microbiological and virological investigations were conducted (Siebert et al., 2001, 2017). Dissected animals were allocated to three different age groups (AG): animals up to 6 months (young-of-the-year-seal, AG1), animals older than 6 months up to 18 months (yearlings, AG2) and animals older than 18 months (adults, AG3) (Siebert et al., 2007). Dates of finding the carcasses were categorised into seasons: season 1 (winter; December–February), season 2 (spring; March–May), season 3 (summer; June–August) and season 4 (autumn; September–November). Photographs of animals were taken with a Panasonic camera (Model No. DMC – TZ101).

2.2. Parasitology

Seals were examined for ectoparasites during necropsies (Harbour seals, n = 659; Grey seals, n = 106) and ectoparasites were collected with forceps or a louse comb and preserved in 70% alcohol. Heartworm infections were determined after the heart was removed at the base of the large vessels, atria and ventricles were cut open separately (Harbour seals, n = 613; Grey seals, n = 104). Parasites were collected, cleaned and preserved in 70% alcohol. Intensity of infections (Bush et al., 1997) was recorded semi-quantitatively as none, mild, moderate or severe level of infection (Fig. 1) during necropsies (Lehnert et al., 2007). Associated lesions in infected organs were preserved in 10% buffered formalin and underwent histopathological analysis. Parasites were identified based on their morphological characteristics (Anderson, 1959; Geraci et al., 1981; Leidenberger et al., 2007). Recorded prevalence, intensity of infections and parasite-associated lesions were analysed retrospectively based on necropsy protocols (see 2.6.).

2.3. Histological investigation of parasites

Seal lice were collected, washed and preserved in 10% neutral-buffered formalin. Subsequently, seal lice specimens were dehydrated and embedded in paraffin wax according to a standard laboratory procedure, and 3 µm thick sections mounted on a glass slide were stained with haematoxylin and eosin (H&E). In selected cases, additional Giemsa stain was applied. In total, sections of 87 seal lice from six harbour seals were investigated (Table 1). Length and width measurements of 11 filarial stages and photographs were taken (CellSens Entry 3.2, Olympus Corporation, Hamburg, Germany).

2.4. Pathological and microbiological examination of lice infected skin

In a subsample (see Table 1) of freshly dead animals (DCC 1) additionally to removing ectoparasites, a skin sample (approximately 1 cm × 1 cm) (Fig. 2) was taken from the exact skin area of which the seal louse was removed for histological investigations (n = 17) (Table 1). For comparison, from certain animals an additional sample of macroscopically uninfected skin (but from a similar area of the body) was taken (n = 9) (Table 1). If an animal was infected severely, it was not always possible to find a definite uninfected area of the skin. Skin samples were fixed in 10% neutral-buffered formalin and later routinely embedded in paraffin wax. Tissue sections were cut at 3 µm and stained with H&E. In eleven cases (Table 1), additionally sterile skin samples (approximately 0.5 cm × 0.5 cm) were stored at 4 °C until shipping to the Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University, Giessen for microbiological analysis. Therefore, skin samples were decontaminated superficially by heat and a fresh section was streaked on standard nutrient agar (OXOID, Wesel, Germany) containing 5% defibrinated sheep blood and water-blue metachrome-yellow lactose agar (Gassner agar; Sifin Diagnostics GmbH, Berlin, Germany). Plates were incubated at 37 °C in ambient air and analysed after 24 and 48 h. Additionally, thiosulfate-citrate-bile-salt-sucrose (*Vibrio* selective) agar (OXOID), *Brucella* agar base (Merck, Darmstadt, Germany) with *Brucella* selective supplement (OXOID), modified Kimmig agar (15.0 g peptone l-1, 1.0 g NaCl l-1, 19.0 g D-(+)-glucose l-1, 15.0 g agar agar l-1, 5.0 ml glycerine/l, 50 mg penicillin/l, 25 mg streptomycin/l) with and without supplementary cycloheximide (250 mg l-1, Serva Electrophoresis GmbH, Heidelberg, Germany) for selective culturing of fungi, as well as a selective medium for the isolation of *Erysipelothrix rhusiopathiae*

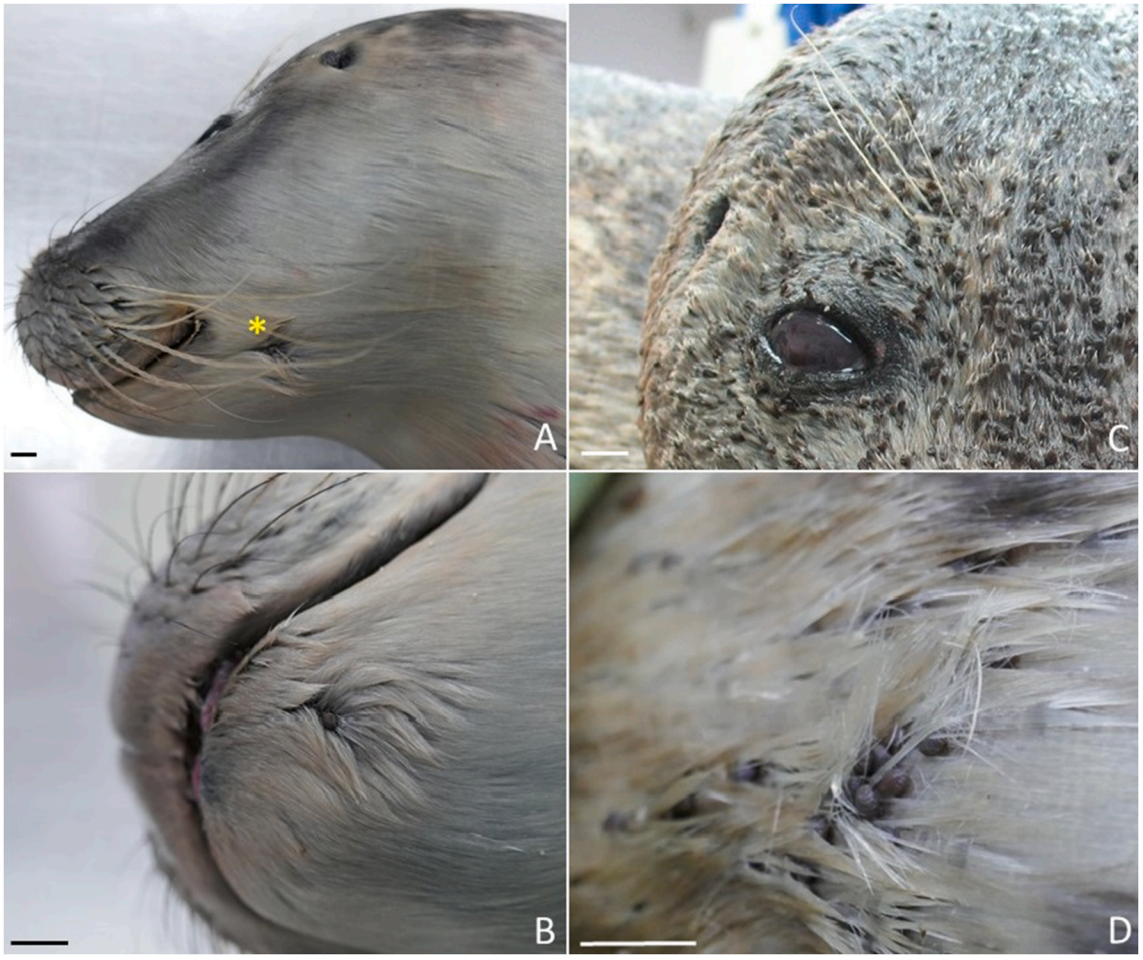


Fig. 1. Levels of infection with E. *horridus* in *P. vitulina*. A: Mild *E. horridus* infection of a harbour seal yearling, asterisk pointing at *E. horridus* B: Close up of *E. horridus* in the head area of a harbour seal C: Severe *E. horridus* infection of a harbour seal D: Close up of severe *E. horridus* infection. Scale bars: A-D 1 cm.

Table 1
Subsample of freshly dead animals, x = sampled, - = not analysed, m = male, f = female.

Marine mammal species	Age group	Sex	Level of *E. horridus* infection	Histological investigation		Microbiological investigation		Histology of seal lice (n)
				E .horridus infected skin	uninfected skin	*E. horridus* infected skin	uninfected skin	
Phoca vitulina	AG 1	m	mild	–	–	–	–	10
Phoca vitulina	AG 1	m	mild	–	–	–	–	10
Phoca vitulina	AG 1	f	moderate	x	–	–	–	30
Phoca vitulina	AG 1	m	mild	x	–	–	–	–
Phoca vitulina	AG 1	m	mild	x	–	–	–	2
Phoca vitulina	AG 1	f	mild	x	–	–	–	1
Phoca vitulina	AG 1	m	mild	x	–	–	–	–
Halichoerus grypus	AG 3	f	severe	x	–	–	–	–
Halichoerus grypus	AG 3	m	severe	x	–	x	–	–
Halichoerus grypus	AG 2	f	severe	x	–	x	–	–
Halichoerus grypus	AG 3	m	mild	x	x	x	x	–
Phoca vitulina	AG 1	m	mild	x	x	x	x	–
Phoca vitulina	AG 1	f	mild	x	x	x	x	–
Phoca vitulina	AG 2	f	mild	x	x	x	x	–
Phoca vitulina	AG 2	f	severe	x	x	x	x	34
Halichoerus grypus	AG 1	m	mild	x	x	x	x	–
Phoca vitulina	AG 1	f	mild	x	x	x	x	–
Phoca vitulina	AG 1	f	mild	x	x	x	x	–
Phoca vitulina	AG 1	f	mild	x	x	x	x	–

(modified by Böhm, 1971), were incubated according to the respective methods required for each organism. For example, *Brucella* selective agar was incubated in a CO_2-incubator with 10% CO_2 for at least 5 d. For the isolation of fungi, Kimmig agar was incubated at 28–30 °C for 3–14 d. Grown colonies were identified utilizing matrix assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS,

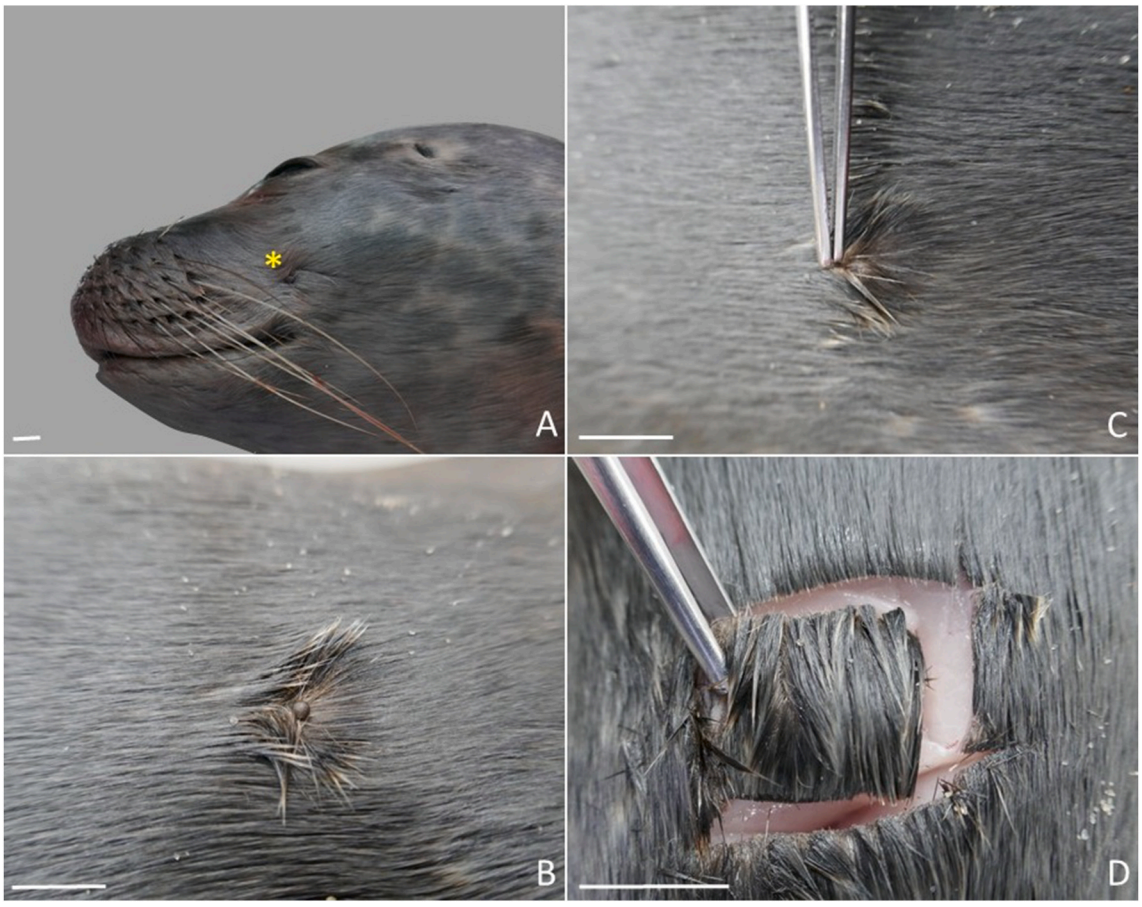


Fig. 2. Sampling routine of *E. horridus* infected seal skin for histological and bacteriological examinations. A: Mild *E. horridus* infection of a harbour seal yearling, asterisk pointing at *E. horridus*. B: Close up of *E. horridus* C: Removing of *E. horridus* D: Cutting and removing of the infected skin with a sterile forceps for further investigations. Scale bars: A-D 1 cm.

Microflex, LT, Compass reference library (version 10.0.0.0), Bruker Daltonics, Bremen, Germany). Due to differing post mortem times at sampling, the quantity of isolated bacteria was not evaluated.

2.5. Scanning electron microscopy

Samples of parasites attached to the skin were collected during necropsies for scanning electron microscopy (SEM) and fixed in 10% neutral-buffered formalin and the seal fur was trimmed to enhance the visibility of the parasite. Afterwards samples were post-fixed in 5% glutaraldehyde solution, dehydrated in an ascending ethanol series, dried using the critical-point-drying method and coated in a sputter-coater (SCD 040; Oerlikon Balzers, Balzers, Liechtenstein) with gold. Digital scanning microscope (DSM 940, Carl Zeiss Jena GmbH) was used to visualize the attachment posture of the seal louse to the skin of their host.

2.6. Statistical analyses

Prevalence was determined according to Bush et al. (1997). To determine the effects of different independent variables on infection of heartworms and lice in harbour seals, as well as lice infection in grey seals, a Generalized Additive Model (GAM) was fitted for each host and for each parasite, based on a binomial distribution with a logit link function, using the *mgcv*-package (v.1.8.34, Wood, 2011). The "presence of parasite infection" (1) was used as response variable. Explanatory variables were "age group" (AG1, AG2, AG3), "sampling month" (1–12), "sampling year" (2014–2021) "location" (North Sea, Baltic Sea), "sex" (male, female) and "degree of decomposition" (1–5). GAMs allow smooth, non-linear functions to be inferred between explanatory variables and response variable, analysing complex relationships within the dataset. Cubic regression splines were used to assess the effects of the "sampling month", "sampling year", "degree of decomposition"; random effects were used for the variables "sex" and "location". The number of knots was set to 4, but was set to lower values when the number of unique values in the variable did not allow for this number of knots (resulting the "degree of decomposition" and "age group" with 3 knots). Models were fitted with the method of the restricted maximum likelihood (REML). The *gratia*-package (v.0.8.1, Simpson, 2023) was used to visualize partial effects of each variable (see Appendix A, Supplementary data).

Level of significance was set at 0.05. All analyses were performed with RStudio (R version 4.2.1, the R Core Team, 2022).

3. Results

3.1. E. horridus and A. spirocauda in harbour seals

Seal louse, *E. horridus* was found in 4% (26/659) of harbour seals between 2014 and 2021 (Table 2). Twenty-four harbour seals were infected mildly with *E. horridus*, one moderately and one severely. Prevalence varied significantly between the sampling years (Tables 2 and 3). No seal louse infection was detected in 2018 and 2019 (Fig. 3). Age affects the seal louse prevalence in harbour seal significantly: older animals (>18 month, AG 3) were more likely to be infected with seal lice (Table 3). Neither sex, nor location, sampling month or decomposition status significantly affected the prevalence of *E. horridus* in harbour seals.

Table 2
Prevalences of *A. spirocauda* and *E. horridus* in grey and harbour seals from 2014 to 2021 in the North and Baltic Sea.

Year		2014	2015	2016	2017	2018	2019	2020	2021
Prevalence in *Phoca vitulina*	*A. spirocauda*	18.5 % (n = 81)	15.9% (n = 82)	15.5% (n = 97)	10.6% (n = 104)	12.2% (n = 49)	3.0% (n = 65)	15.1% (n = 53)	11.3% (n = 82)
	E. horridus	2.3% (n = 86)	9.4% (n = 97)	6.9% (n = 91)	4.5% (n = 111)	0.0% (n = 54)	0.0% (n = 74)	3.4% (n = 59)	1.2% (n = 87)
Prevalence in *Halichoerus grypus*	*E. horridus*	0.0% (n = 12)	0.0% (n = 13)	0.0% (n = 15)	0.0% (n = 11)	0.0% (n = 20)	16.7% (n = 6)	31.3% (n = 16)	38.5% (n = 13)

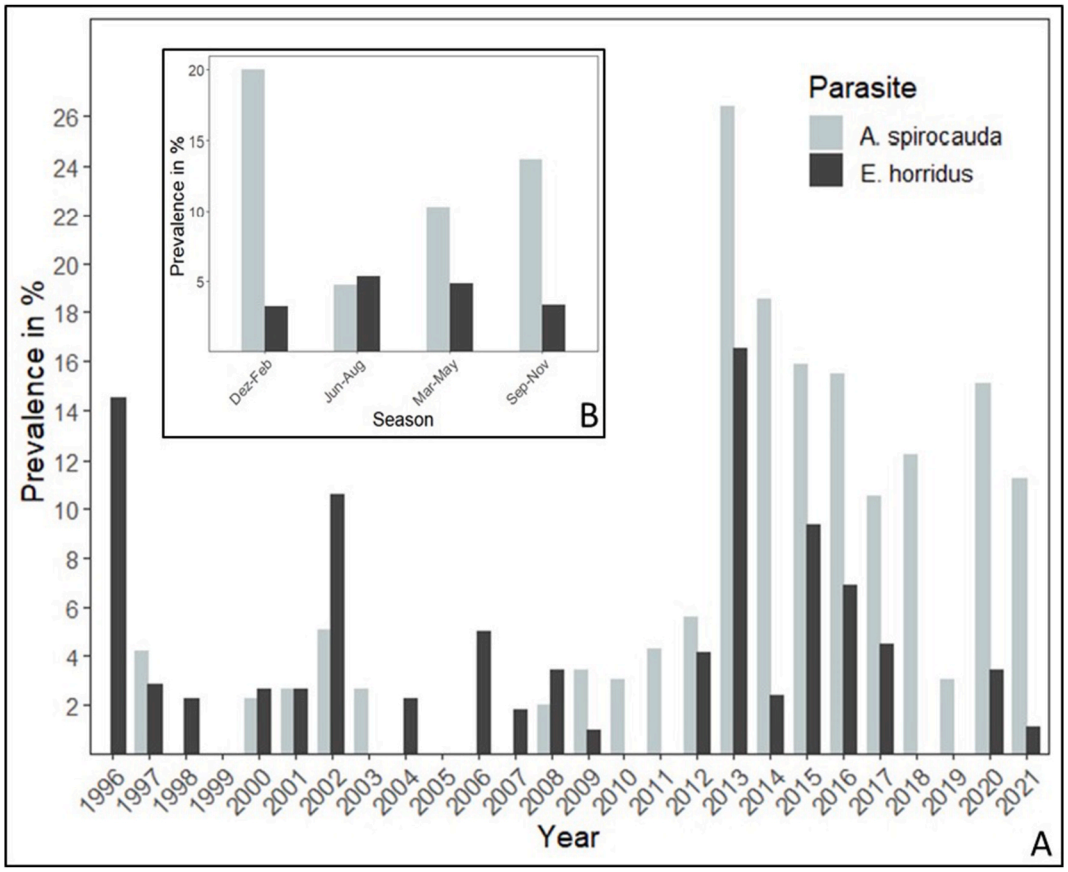


Fig. 3. A: Prevalence of *A. spirocauda* and *E. horridus* in harbour seals in the North and Baltic Sea from 1996 to 2021, data from 1996 to 2013 according to Lehnert et al. (2016). B: Prevalence of *A. spirocauda* and *E. horridus* in harbour seals during the seasons in the North and Baltic Sea from 2014 to 2021.

Table 3
Significance of the variables used in Generalized Additive Model (GAM), the presence/absence of heartworms (left) and lice (right) in the harbour seals. *P*-values are estimated by the Wald-test, integrated in the *mgcv* R-package (version 1.8–40).

		Habour seals investigated for *A. spirocauda* (2014–2021), n = 613				Harbour seals investigated for *E. horridus* (2014–2021), n = 659			
Model	Independent variable	edf	Ref. df	Chi.sq	p-value	edf	Ref.df	Chi.sq	*p*-value
GAM	sampling month	1.685e+00	2	12.558	0.000571	2.642e-01	2	0.324	0.2793
GAM	sampling year	1.302e+00	3	5.016	0.025252	1.589e+00	3	6.032	0.0208
GAM	sex	1.796e-04	1	0.000	0.336954	3.444e-05	1	0.000	0.3714
GAM	location	2.129e-03	1	0.000	0.386247	3.486e-06	1	0.000	0.9721
GAM	degree of decomposition	1.129e-03	2	0.001	0.345611	4.892e-05	2	3.533	0.8111
GAM	age group	1.479e-04	2	0.000	0.374968	8.343e-01	2	0.000	0.0304

A. spirocauda was found in 13% (79/613) of harbour seals between 2014 and 2021. Fifty-eight harbour seals were infected mildly with *A. spirocauda*, 20 moderately and one severely. The prevalence of *A. spirocauda* in harbour seals varied significantly (Table 3) over the eight-year time span (Fig. 3, Table 2). In each study year, *A. spirocauda* prevalence in harbour seals was above 10% with the exception of 2019 whereas prevalence was at 3%. Additionally, a significant difference was found in the prevalence regarding sampling months. The highest prevalence occurred in harbour seals sampled during the winter months (20%), followed by autumn (14%), spring (10%) and summer (5%) (Fig. 3). The implemented model did not show an effect caused by the age class, sex, and degree of decomposition or location. Two harbour seals showed a coinfection with *E. horridus* and *A. spirocauda*. In one harbour seal with severe lice infection alopecia and dermatitis were

Table 4
Significance of the variables used in a Generalized Additive Model (GAM), the presence/absence of lice in the grey seals. *P*-values are estimated by the Wald-test, integrated in the *mgcv* R-package (version 1.8–40).

		Grey seals investigated for *E.horridus* (2014–2021), n = 106			
Model	Independent variable	edf	Ref.df	Chi.sq	*p*-value
GAM	sampling month	1.602e+00	2	12.558	0.01463
GAM	sampling year	1.206e+00	3	5.022	0.02513
GAM	sex	3.950e-06	1	0.000	0.84565
GAM	location	9.144e-01	1	7.856	0.00231
GAM	degree of decomposition	1.377e-05	2	0.000	0.67640
GAM	age group	9.134e-01	2	7.633	0.07942

recorded. No macroscopical lesions associated with *A. spirocauda* infections were diagnosed in harbour seals (n = 79) investigated between 2014 and 2021.

3.2. *E. horridus and A. spirocauda in grey seals*

E. horridus was found in 10% (11/106) of grey seals between 2014 and 2021 (Table 2). Two animals were infected mildly, four moderately and five severely. The GAM model showed a significantly higher seal louse prevalence in grey seals from the Baltic Sea (10/33) compared to grey seals from the North Sea (1/71) (Table 4). A significant difference was observed between sampling years, showing an increase in prevalence over the last years. From 2014 until 2018 no seal louse infection was detected, in 2019 the seal louse prevalence was above 10% and in the two following years above 30% (Table 2). The used model revealed a significant difference between the sampling months (Table 4), showing a peak in prevalence during the winter months. No other independent variable affected the presence of lice significantly. In all five severely infected grey seals alopecia was recorded, severe dermatitis and intralesional bacteria were diagnosed histologically. *A. spirocauda* was found in two male adult grey seals in 2018 and 2020 from the North Sea (see Lehnert et al., 2023).

3.3. *Histological investigations of E. horridus*

In seven seal lice, sampled from one severely with *A. spirocauda* and *E. horridus* infected female, juvenile harbour seal from the North Sea (Table 1), ten filarial stages were visible in histological sections stained with H&E in the pharynx, intestine, haemocoel and head of *E. horridus* (Fig. 4A–C). The length of filarial stages ranged from a minimum of 5.77 µm length and 3.65 µm width up to a maximum of 70.53 µm length and 3.68 µm width. One filarial stage was visible in the haemocoel of the caudal abdomen of *E. horridus*, sampled from a moderately *E. horridus* and mildly *A. spirocauda* infected female, juvenile harbour seal from the North Sea, measuring 91 µm in length and 3.61 µm in width (Fig. 4D and E). All filarial stages were characterized by typical microfilarial features like a septine shape and a variety of visible cells with dark violet stained nuclei filling the body, which appeared partly dispersed and crowded (Fig. 4A–E) (World Health Organization, 1997).

3.4. *Pathological investigation of seal louse infected skin tissue in grey and harbour seals*

Two severely seal louse infected adult grey seals and one severely infected juvenile grey seal were alopecic, one severely infected juvenile harbour seal was not alopecic (Table 5). Alopecia was not diagnosed in grey or harbour seals of any other level of seal louse infection. Severely infected skin of all investigated grey and harbour seals displayed a

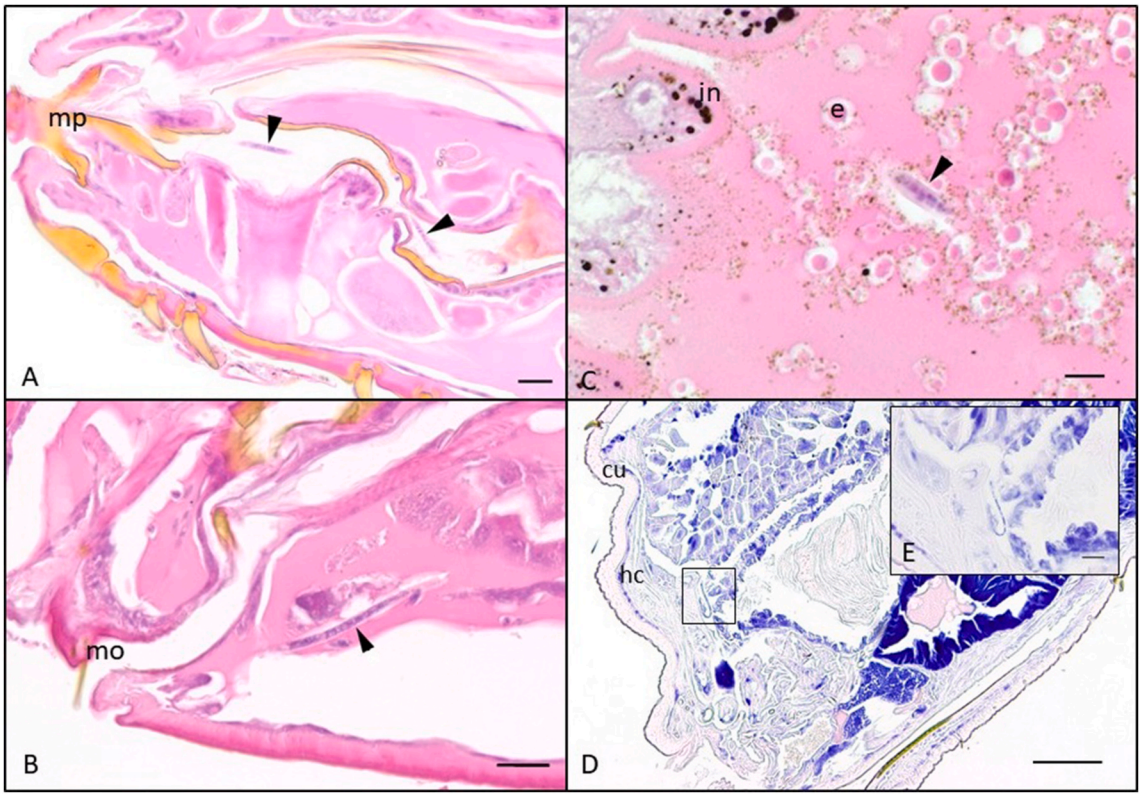


Fig. 4. Histological sections and staining of *E. horridus* revealing filarial stages in *E. horridus*. A: Filarial stages (arrowheads) in the pharynx. bar = 20 µm. B: Filarial stage (arrow) in the mouth region. bar = 40 µm. C: Filarial stage (arrowhead) in the intestine (in) surrounded by erythrocytes (e). bar = 15 µm. D: Filarial stage in the haemocoel (hc) of the abdomen of *E. horridus* (square). E: Close up of filarial stage. mp = mouthparts, mo = mouth, cu = cuticula. A–C: Haematoxylin - Eosin stain, D: Giemsa stain.

Table 5
Histological diagnosis of *E. horridus* infected skin of harbour and grey seals.

Histological diagnosis						
Level of infection	*Orthokeratotic hyperkeratosis*	*Orthokeratotic Hyperkeratosis + Lymphohistiocytic, perivascular dermatitis*	*Purulent folliculitis*	*Intralesional bacteria*	*Pustules*	*Alopecia*
Mild	5/12	4/12	1/12	1/12	0/12	0/12
Moderate	0/1	1/1	0/1	0/1	0/12	0/1
Severe	0/4	4/4	4/4	4/4	3/4	3/4

purulent folliculitis and intralesional bacteria. In one mildly infected harbour seal, a purulent folliculitis was diagnosed. Orthokeratotic hyperkeratosis was seen in one mildly infected grey and four harbour seals. Four mildly infected harbour seals, one harbour seal with moderately infected skin and all four severely infected grey and harbour seals displayed orthokeratotic hyperkeratosis in combination with a mild lymphohistiocytic, perivascular dermatitis. Macroscopically uninfected skin showed mild orthokeratotic hyperkeratosis in one harbour seal and mild orthokeratotic hyperkeratosis with a mild lymphohistiocytic, perivascular dermatitis in three harbour seals (Table 6).

3.5. Microbiological investigation of seal skin samples

In harbour and grey seals, a broad spectrum of bacteria was isolated from seal louse infected (Table 7) as well as uninfected skin (Table 8). In seal louse infected skin, *Psychrobacter* sp. was isolated most frequently, followed by *Pseudomonas* sp., *Streptococcus phocae* and *Staphylococcus pseudintermedius* (Table 7). A similar spectrum of bacteria was detected in macroscopically uninfected skin (Table 8); here *Psychrobacter* sp. and *Pseudomonas* sp. were most frequently identified, followed by *Streptococcus phocae*.

3.6. Scanning electron microscopy

Pictures taken with SEM are showing two tarsal claws of the parasite using the funnel shape of the infundibula of the hair to attach to the root of the hair (Fig. 5 A).

4. Discussion

This study presents *A. spirocauda* and *E. horridus* prevalence in harbour seals and for the first time in the grey seal populations from the North and Baltic Sea based on data from a unique long-term monitoring. A systematic histological and bacteriological examination of *E. horridus* infected skin revealed a variety of lesions in grey and harbour seals associated with seal louse infection of differing severity. SEM images showed the attachment of *E. horridus* not only to the hair shaft but also utilizing skin structures like hair infundibula to secure their clasp. Additionally, histological findings of filarial nematodes within the haemocoel of *E. horridus* clearly indicate the seal louse as a vector of the heartworm.

4.1. E. horridus and A. spirocauda infections in harbour and grey seals

In this study, harbour seals showed an *A. spirocauda* prevalence of 13%, while the prevalence of *E. horridus* was at 4% over the study period of eight years. In comparison to a previous study about harbour seals in the same geographical area between 1996 and 2013, in which the total prevalence of *A. spirocauda* and *E. horridus* accounted to 4.4% and 3.4% respectively (Lehnert et al., 2016), the prevalence of *A. spirocauda* increased and prevalence of *E.horridus* remained low. Thus, the prevalence of *A. spirocauda* within the harbour seal population in the German North and Baltic Sea has almost tripled compared to the previous two decades. In 1988 within a time period of 4 months a prevalence of 24.5% was determined in the Dutch North Sea (Borgsteede et al., 1991). For the same year a prevalence of 11.4% was registered in the Kattegat-Skagerrak and the Baltic region (Lunneryd, 1992), while 32.2% were observed in the Wadden Sea of Lower Saxony (Claussen et al., 1991). All three studies based their investigations on harbour seals, which died during the phocid distemper virus outbreak in 1988/89. This

Table 7
Bacteria isolated from *E. horridus* infected skin (n = 12).

Bacterial organsism	Number of isolates
α-haemolytic streptococci	1
Acranobacterium phocae	1
Escherichia coli	1
Enterobacter hormaechei	1
Enterococcus faecalis	1
Lelliottia amnigena	1
Oceanisphaera sp.	1
Plesiomonas shigelloides	1
Proteus mirabilis	1
Serratia liquefaciens	1
Shewanella baltica	1
Streptococcus dysgalactiae	1
Acinetobacter sp.	2
Atopobacter phocae	2
Vibrio alginolyticus	2
Staphylococcus pseudintermedius	2
Streptococcus phocae	5
Pseudomonas sp.	5
Psychrobacter sp.	7

Table 8
Bacteria isolated from uninfected skin (n = 9).

Bacterial organism	Number of isolates
Aeromonas salmonicida	1
Escherichia coli	1
Morganella morganii	1
Oceanisphaera sp.	1
Plesiomonas shigelloides	1
Proteus mirabilis	1
Vibrio alginolyticus	2
Streptococcus phocae	6
Pseudomonas sp.	7
Psychrobacter sp.	7

Table 6
Histological diagnosis of macroscopically uninfected skin of harbour and grey seals.

Histological diagnosis						
Level of infection	*Orthokeratotic hyperkeratosis*	*Orthokeratotic Hyperkeratosis + Lymphohistiocytic, perivascular dermatits*	*Purulent folliculitis*	*Intralesional bacteria*	*Pustules*	*Alopecia*
Mild	1/9	3/9	0/9	0/9	0/9	0/9

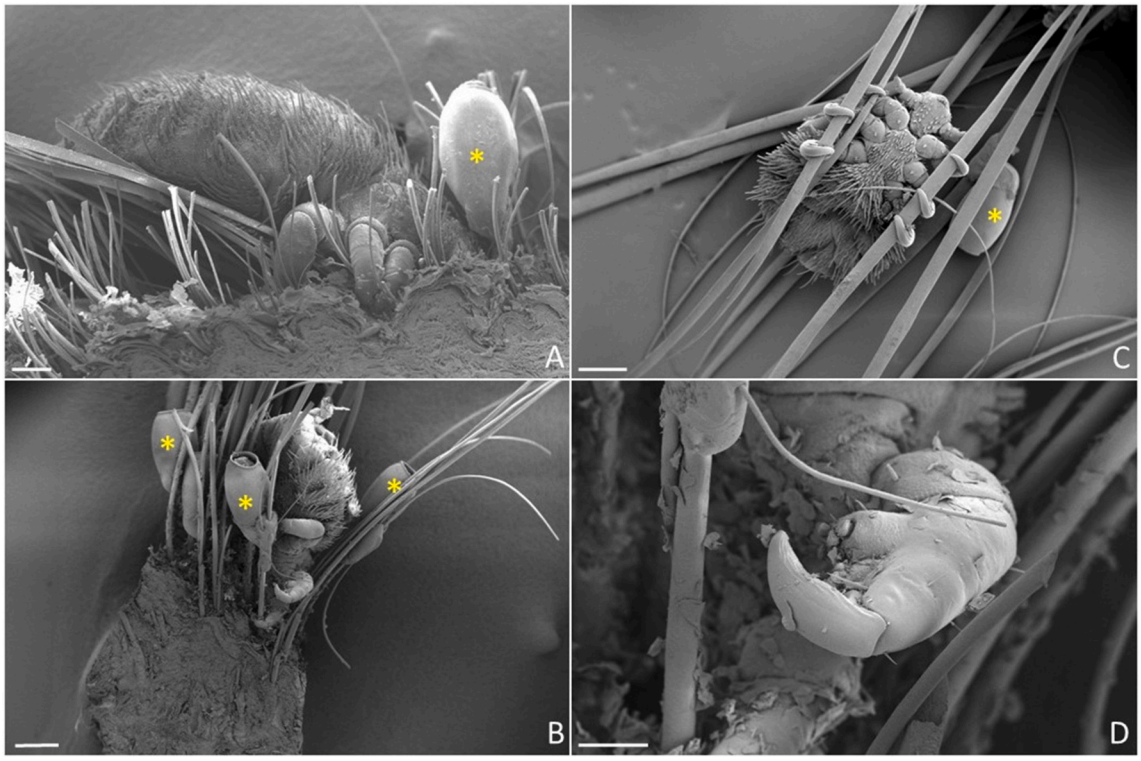


Fig. 5. Scanning electron microscopic images of *E. horridus* attached to seal skin and fur. A: *E. horridus* utilizing a hair follicle of harbour seal skin. B: *E. horridus* attached to seal hair with the head pointing towards seal skin. C: *E. horridus* with six claws attached to seal fur. D: Close up of an unattached claw of *E. horridus*. Asterisks positioned on nits of *E. horridus*. Sale bars: A 200 μm, B 400 μm, C 400 μm, D 100 μm.

implies that, prior to the first PDV epidemic in 1988/89, the prevalence of the heartworm infections within the harbour seal population in the Wadden Sea maintained at a higher level. In the following two decades, the harbour seal population decreased drastically due to two PDV outbreaks (1989/99 and 2002) (Müller et al., 2004, Härkönen et al., 2006), since 2002 the population is recovering and increasing, up to a total number of 26,721 adult animals counted in 2021 (Unger et al., 2022). In consequence of the density dependency of parasites (Anderson and May 1979, May and Anderson, 1979), the heartworm prevalence may increase with an increasing host density in the defined geographical area. Growing seal populations in German waters result in higher seal densities on haul-outs (Unger et al., 2022), thereby facilitating transmission of the heartworm by seal lice vectors. Equally to the present study, dependency of season was observed in previous decades, however the highest infection rate occurred in summer at Washington Coast (Dailey and Fallace, 1989), while in this study, winter months showed the highest *A. spirocauda* prevalence. Transmission patterns of other vector-borne parasites such as the heartworm of dogs (*Dirofilaria immitis*) is influenced by seasonal temperature changes (Ledesma and Harrington, 2011). To discover transmission patterns for *A. spirocuada*, further investigations regarding prevalence of mature heartworms as well as monitoring of microfilaria stages in the blood of seals are needed.

In contrary to the prevalence of *A. spirocauda,* no increase in prevalence of seal louse infections was observed over the last eight years. The prevalence of *E. horridus* in the study period remained low, similar to previous years (Lehnert et al., 2016). While no seal lice infection was reported in harbour seals inhabiting the Kattegat-Skagerrak area after the first seal epidemic (Lunneryd, 1992), seal lice prevalence of 39% was found in Scottish waters, based on a four-year time span of sampling live harbour seals (Thompson et al., 1998). In addition, 45.5% of investigated harbour seals were infected at the Washington state coast, USA (Dailey and Fallace, 1989). These notable variations observed in seal louse prevalence in phocid seals may be due to different conditions influencing the different geographic locations. Equally, the sampling bias of ectoparasites has to be taken into account. Ectoparasites may leave the host after death, are eaten by necrophagous birds or lost during the stranding process and transport of the carcass (Thompson et al., 1998; Lehnert et al., 2021; Rohner et al., 2023). Mild infections can be overlooked at necropsy since manual brushing with a louse comb might not cover the complete fur of the animal and therefore lead to inaccurate prevalence of ectoparasites (Ignoffo, 1958). The highest prevalence of *E. horridus* was observed in adult harbour seals, contrary to a previous study in which the prevalence was highest in yearlings in the German Wadden Sea (Lehnert et al., 2016) and in juvenile harbour seals on the Scottish coastline (Thompson et al., 1998). Vertical transmission from mother to pups is considered the most important way of transmission (Murray et al., 1965; Murray and Nicholls, 1965; Kim, 1975; Leidenberger et al., 2007; Leonardi et al., 2013). This would again require a similarly high infection rate in adult seals, as seen in the present study. In contrary to previous studies (Dailey and Fallace, 1989; Thompson et al., 1998), no seasonal component of *E. horridus* infection was observed.

It is striking that grey seals are twice as often and more severely infected with seal lice compared to harbour seals, while *A. spirocauda* usually seems to occur only in harbour seals (Leidenberger et al., 2007). Infections with *E. horridus* were described in grey seals (Durden and Musser, 1994), but studies investigating the long-term prevalence and dynamics of ectoparasitic seal lice in grey seals are missing. This study showed a significant increase of seal louse infections in grey seals over the last three years (Table 2). All infected grey seals, except for one, originated from the Baltic Sea. Recent recolonization of the southern Baltic Sea with steadily increasing numbers of grey seals on haul outs sites (Galatius et al., 2020) could be linked to higher chances of seal louse transmission rates between grey seals. Due to continued increase of eutrophication, exposure to hazardous substances and marine litter (HELCOM, 2023) environmental conditions may affect the skin microbiome and immune status (Sehnal et al., 2021) influencing susceptibility for ectoparasitic infections.

The different infection patterns of heartworm and seal louse in grey and harbour seals may derive from the genetic constitution of both species. Although both species are closely related, share habitat and dietary preferences (Boyi et al., 2022), immunological traits influencing susceptibility to infectious diseases (Schmid-Hempel, 2003) differ between the two hosts. These differences became apparent during the phocine distemper virus epidemic, when grey seals were exposed to the virus and displayed high levels of antibodies but only a small number of animals died due to the virus infection (Cornwell et al., 1992). Heterozygosity as determining factor for fitness and variations in parasite infection (Rijks et al., 2008, Hoffman et al., 2014) are considered as possible factor causing varying interspecific susceptibility to parasites in harbour and grey seals (Lehnert et al., 2023). Genetic exchange within populations is influenced by recent grey seal recolonization of the North (Reijnders et al., 1995) and Baltic Sea (Galatius et al., 2020), as well as foraging and movement patterns of grey seals causing them to travel hundreds of kilometres (D. Thompson et al., 1991) while harbour seals rather stay resident in one geographical area (Stewart et al., 1989; Thompson and Miller, 1990; P.M. Thompson et al., 1991). Additionally, grey seals may be atypical hosts for seal lice and therefore less resistant to seal louse infections, causing more prevalent and severe infection in seal louse-naive individuals (Daszak et al., 2000). Different infection patterns of *A. spirocauda* in harbour and grey seals were also discussed to be caused by insufficient sampling (Measures et al., 1997; Keroack et al., 2018) or high mortality caused by heartworm infections in grey seals (Keroack et al., 2018).

4.2. Histology of seal lice and vector biology

Filarial stages in *E. horridus* specimens were visualized during ingestion or emission in the pharynx region of the seal louse, for the first time. In the case of the canine heartworm, *Dirofilaria immitis* (Leidy, 1858) mosquitos incorporate filarial stages, which migrate from the midgut into malpighian tubule cells, where they become the infectious third larval stage. The third larval stage then migrates into the lumen of malpighian tubules and back to the proboscis of the mosquito, which enables emission of the infectious larvae with the next blood meal (Grassi and Noè, 1900; Kartman, 1953; Angela 1960). Although the knowledge about the anatomy and physiology of internal organs of *E. horridus* is scarce, the results of this study suggest a similar migration route of *A. spirocauda* filarial stages within *E. horridus*. Dissections of lice are time consuming and difficult to integrate into the routines of necropsies and health monitoring (Geraci et al., 1981; Lehnert et al., 2016). In histology, only the sectional plane is assessed, which implies that reliability improves with increasing number of sections made. The histological findings strongly support *E. horridus* as vectors for *A. spirocauda*, as previously suggested by studies finding evidence after dissecting seal lice (Geraci et al., 1981; Lehnert et al., 2016), reconstruction of micro-CT scans (Ebmer et al., 2022) and molecular analysis (Keroack et al., 2018).

4.3. Impact of E. horridus infections on health status

In this study, only seals with severe levels of lice infections displayed alopecia, intralesional bacteria and pustules. Similarly, folliculitis was only diagnosed in macroscopically infected skin, while pathological findings like lymphohistiocytic, perivascular dermatitis and hyperkeratosis were also found in uninfected skin, indicating background pathology unrelated to parasitic infection. Hyperkeratosis can be seen in a variety of conditions in marine mammals (Migaki and Jones, 1983; Lipscomb et al., 2001), perivascular dermatitis is considered as one of the most unspecific patterns of inflammation (Mauldin and Peters-Kennedy, 2016) and could be caused by other factors, e.g. increased hauling out in consequence of illness and weakness. Diagnosis directly linked to arthropod bites is impeded by similarities to other clinical conditions (Steen et al., 2004; Mauldin and Peters-Kennedy, 2016). Severe cases of seal louse infection were accompanied by alopecia and anaemia (Conlogue et al., 1980; Dailey, 2001), supporting the findings of this study, while mild infections only caused irritation (Raga et al., 1997; Leidenberger et al., 2007). In terrestrial wildlife, swamp wallabies (*Wallabia bicolor*) with a moderate chewing lice infection showed similar histological changes to this study, like alopecia, hyperkeratosis and perivascular dermatitis (Portas et al., 2009). However, histological data of wildlife infected with blood sucking lice are scarce. In elephant seals (*Mirounga leonine*), histological investigations revealed a high inflammatory skin reaction caused by the elephant seal louse (*Lepidophthirius macrorhini*) during moulting (Leonardi et al., 2021b). The present study indicates an attachment of lice to not only the hair shaft but also using the infundibula of the hair follicle to secure their grasp close to the hair root. In conclusion, severe seal lice infection can result in inflammatory processes in the skin caused by mouthparts of parasites during a blood meal or claws during attachment and therefore has a considerable impact on the health status of harbour and grey seals. In contrast, mild infections were not associated with inflammatory processes.

4.4. Microbiological examination and SEM of the skin samples

The microbiome of *E. horridus* infected and uninfected skin is diverse but common, similar to the microbiome of other tissue from harbour seals (Siebert et al., 2017). It ranges from potential pathogens (e.g. *Pseudomonas* sp., *Psychrobacter* sp., *Streptococcus phocae*) to Enterobacteriales (e.g. *Escherichia* (*E.*) *coli*) as well as bacteria usually isolated from soil (e.g. *Oceanisphaera*). The spectrum of isolated bacteria shows a considerable convergence between infected and uninfected skin. All of the isolated bacteria have already been described in marine animals (Higgins, 2000; Siebert et al., 2017). The genus *Psychrobacter* was present on the skin of Wedell Seals (*Leptonychotes weddellii*) in Antarctica (Mellish et al., 2010) and is considered as core component of the skin microbiome of humpback whales (*Megaptera novaeangliae*) (Apprill et al., 2014). *Streptococcus* (*Sc.*) *phocae* has been described in numerous marine mammals, like harbour and grey seals (e.g. Baker et al., 1980; Baker, 1989; Krogsrud et al., 1990), but also in southern sea otters (*Enhydra lutris nereis*) (Bartlett et al., 2016). Besides septicaemia and pneumonia (Baker et al., 1980; Henton et al., 1999), streptococci are known to cause pyoderma (Stroud, 1979). *Sc. phocae* is not able to penetrate intact skin (Gonzales-Contreras et al., 2011), therefore sea otters showed an increased risk of infection, if some kind of wounds caused by fights, hook entanglement etc. were present (Bartlett et al., 2016). This also applies for the genus *Pseudomonas*, where infections are most likely related to disruption of the skin (Agger and Mardan, 1995). *Staphylococcus* (*St.*) *pseudintermedius* plays an important role in veterinary medicine of domestic animals due to its emerging resistance to antibiotics (Rubin et al., 2011). *St. pseudintermedius* frequently causes otitis, pyoderma or infections in the urinary tract (Rubin et al., 2011). In domestic animals like dogs, a variety of bacteria, such as *Pseudomonas* sp., *Proteus* sp., *Streptococcus* sp., *E. coli,* but most frequently staphylococci are known to cause bacterial folliculitis in combination with predisposing factors like parasitic infestations or local irritants (Hagris and Myers, 2017). Concerning human head lice, bacterial infections occur secondary to lice infection (Dodd, 2001). Findings of this study also indicate that bacterial skin infections probably develop secondary to *E. horridus* infections in harbour and grey seals.

A seal louse digging into the follicle of a hair was visualized using SEM for the first time. Contact and attachment to the host may be achieved by using the hair follicle of the skin. Thus contact between host skin and parasite may be less transitory and not only reduced to the feeding process (Steen et al., 2004), contrary to other lice species like *Haematopinus eurysternus*, found on cattle (Baron and Weintraub, 1987). Seal lice need to outlast a long time during diving periods of the host and remain on fast swimming seals before dispersing on haul-outs (P.M. Thompson et al., 1991; van Neer et al., 2023). Further investigations of

attachment forces and feeding mechanisms are needed. The combination of data on histology, microbiology of the skin and the visualization of the attachment posture clearly indicates that seal louse infections cause damage to the skin barrier, resulting in a portal of entry for opportunistic bacteria, and thereby inducing an inflammatory response in the skin of their host.

5. Conclusion

A strong increase of prevalence of *A. spirocauda* in harbour seals was recorded in the North and Baltic Sea of Germany over the last decade, varying significantly between the seasons. Age-dependent infections for *E. horridus* were observed in harbour seals. For the first time, long-term data of *E. horridus* prevalence in grey seals has been reported. It is remarkable that the prevalence of seal louse infections in grey seals is twice as high as in harbour seals. Prevalence of seal lice infections in grey seals was significantly higher in the Baltic Sea. Over all, different infection patterns between grey seals and harbour seals were notable. The findings in this study strongly support that *E. horridus* is a vector for the filarial *A. spirocauda*. Histology represents a reliable and effective method for the detection of filarial stages in *E. horridus*. The combination of scanning electron microscopy, histology and microbiology of *E. horridus* infected sealskin revealed that attachment mechanism of *E. horridus* most likely cause damage to the skin, which poses a portal of entry for facultative pathogenic bacteria resulting in secondary infections. Long-term data sets and stranding networks are essential for monitoring vulnerable wildlife and environmental changes in their ecosystems. In this context, parasites are useful as indicators for host behaviour, distribution and population dynamics.

Funding

Funding to K.L. by the grant LE 4753/4-1 from German Science Foundation (DFG) within the Special Priority Program (SPP 2332) “Physics of Parasitism”. The investigations were partly funded by the Ministry of Energy Transition, Climate Protection, the Environment and Nature (MEKUN S–H) and the National Park Service of Schleswig-Holstein. The funders took not part in the study design, data collection and analysis, decision of publishing or any preparation of the manuscript.

Declaration of Competing Interest

The authors declare to have no conflict that could influence their work.

Acknowledgements

The authors thank all, who report and collect marine mammals in Schleswig-Holstein, in particular national park rangers Sönke Lorenzen, Thomas Diedrichsen and Rolf Lorenzen, as well as the ITAW colleagues and assistants for their support during necropsies, especially Kornelia Schmidt and Sarah Blauth. This Open Access publication was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—491094227 “Open Access Publication Funding” and the University of Veterinary Medicine Hannover, Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.100898.

References

Agger, W.A., Mardan, A., 1995. *Pseudomonas aeruginosa* infections of intact skin. Clin. Infect. Dis. 20, 302–308.

Anderson, R.C., 1959. The taxonomy of *Dipetalonema spirocauda* (Leidy, 1858) n. comb. (= *Skrjabinaria spirocauda*) and *Dirofilaria roemeri* (Linstow, 1905) n. comb.(= *Dipetalonema roemeri*). Can. J. Zool. 37 (4), 481–493.

Anderson, R.C., 2000. Nematode Parasites of Vertebrates: Their Development and Transmission, second ed. CAB International, Wallingford.

Anderson, R.C., Bain, O., 1976. Keys to the genera of the order Spirurida. Part 3. Diplotriaenoidea, Aproctoidea and Filarioidea. In: Anderson, R.C., Chabaud, A.G., Willmott, S. (Eds.), CIH Keys to the Nematode Parasites of Vertebrates. CAB International, Wallingford, pp. 59–116.

Anderson, R., May, R., 1979. Population biology of infectious diseases: Part I. Nature 280, 361–367.

Angela, E.R., 1960. The development of *Dirofilaria immitis* in the mosquito *Aedes aegypti*. J. Helminthol. 34 (1–2), 27–38.

Apprill, A., Robbins, J., Eren, A.M., Pack, A.A., Reveillaud, J., Mattila, D., Moore, M., Niemeyer, M., Moore, K.M.T., Mince, T.J., 2014. Humpback whale populations share a core skin bacterial community: towards a health index for marine mammals? PLoS One 9 (3), e90785.

Aznar, F.J., Balbuena, J.A., Fernández, M., Raga, J.A., 2001. Living together: the parasites of marine mammals. In: Evans, P.G.H., Raga, J.A. (Eds.), Marine Mammals: Biology and Conservation. Kluwer Academic/Plenum Publisher, New York, pp. 385–423.

Baker, J.R., 1989. Natural causes of death in non-suckling grey seals (*Halichoerus grypus*). Veterinary Rec 125, 500–503.

Baker, J.R., Anderson, S.S., Prime, J.H., Baird, A., 1980. The pathology of the grey seal (*Haliochoerus grypus*). I. Pups. Br. Vet. J. 136, 401–412.

Baron, R.W., Weintraub, J., 1987. Immunological responses to parasitic arthropods. Parasitol. Today 3 (3), 77–82.

Bartlett, G., Smith, W., Dominik, D., Batac, F., Dodd, E., Byrne, A.B., Jang, S., Jessup, D., Chantrey, J., Miller, M., 2016. Prevalence, pathology, and risk factors associated with *Streptococcus phocae* infection in southern sea otters (*Enhydra lutis nereis*), 2004-10. J. Wildl. Dis. 52 (1), 1–9.

Böhm, K.H., 1971. Neue Selektivnährböden für Rotlaufbakterien. Zbl Bakt Hyg I Abt. Orig ,218,, 4.

Borgsteede, F.H.M., Bus, H.G.J., Verplanke, J.A.W., van Burg, W.P.J., 1991. Endoparasitic helminths of the harbour seal, *Phoca vitulina*, in The Netherlands. J. Sea Res. 28, 247–250.

Boyi, J.O., Heße, E., Rohner, S., Säurich, J., Siebert, U., Gilles, A., Lehnert, K., 2022. Deciphering Eurasian otter (*Lutra lutra* L.) and seal (*Phoca vitulina* L.; *Halichoerus grypus* F.) diet: Metabarcoding tailored for fresh and saltwater fish species. Mol. Ecol. 31 (19), 5089–5106.

Bruhn, R., Kannan, N., Petrick, G., Schulz-Bull, D.E., Duinker, J.C., 1999. Persistent chlorinated organic contaminants in harbour porpoises from the North Sea, the Baltic Sea and Arctic waters. Sci. Total Environ. 237–238, 351–361.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al., revisited. J. Parasitol. 82 (4), 575–583.

Claussen, D., Strauss, V., Ising, S., Jäger, M., Schnieder, T., Stoye, M., 1991. The helminth fauna from the common seal (*Phoca vitulina vitulina* Linnè, 1758) of the Wadden Sea in Lower Saxony. Part 2. Nematodes. J. Vet. Med. B 38, 649–656.

Conlogue, G.J., Ogden, J.A., Foreyt, W.J., 1980. Pediculosis and severe heartworm infection in a harbor seal. Vet. Med. Small Anim. Clin. 75 (7), 1184–1187.

Cornwell, H.J.C., Anderson, S.S., Thompson, P.M., Mayer, S.J., Ross, H.M., Pomeroy, P. P., Munro, R., 1992. The serological response of the common seal (*Phoca vitulina*) and the grey seal (*Halichoerus grypus*) to phocine distemper virus as measured by a canine distemper virus neutralisation test. Sci. Total Environ. 115, 99–116.

Dailey, M.D., 2001. Parasitic diseases. In: Dierauf, L.A., Gulland, M.D. (Eds.), CRC Handbook of Marine Mammal Medicine, second ed. CRC Press, Boca Raton, Florida, pp. 367–372.

Dailey, M.D., Fallace, L.S., 1989. Prevalence of parasites in a wild population of the Pacific harbour seal (*Phoca vitulina richardsi*) from Gray’s Harbor, Washington. Bull. Southern California Acad. Sci. 88 (1), 1–10.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife-threats to biodiversity and human health. Science 287 (5452), 443–449.

Dodd, C., 2001. Treatment of head lice: Choice of treatment will depend on local patterns of resistance. BMJ 323 (7321), 1084.

Dunn, J.L., Wolke, R.E., 1976. *Dipetalonema spirocauda* infection in the Atlantic harbor seal (*Phoca vitulina concolor*). J. Wildl. Dis. 12, 531–538.

Durden, L.A., 1971. Lice (Phthiraptera). In: William, S.M., Pybus, M.J., Kocan, A.A. (Eds.), Parasitic Diseases of Wild Mammals, second ed. Iowa State University Press, Ames, IA, pp. 3–17.

Durden, L.A., Musser, G.G., 1994. The sucking lice (Insecta, Anoplura) of the world: a taxonomic checklist with records of mammalian host and geographical distributions. Bull. Am. Mus. Nat. Hist. 218, 1–90.

Ebmer, D., Handschuh, S., Schwaha, T., Rubio-García, A., Gärtner, U., Glösmann, M., Taubert, A., Hermosilla, C., 2022. Novel 3D in situ visualization of seal heartworm (*Acanthocheilonema spirocauda*) larvae in the seal louse (*Echinophthirius horridus*) by X-ray microCT. Sci. Rep. 12 (1), 14078.

Fiorenza, E.A., Wendt, C.A., Dobkowski, K.A., King, T.L., Pappaionou, M., Rabinowitz, P., Samhouri, J.F., Wood, C.L., 2020. It’s a wormy world: Meta-analysis reveals several decades of change in the global abundance of the parasitic nematodes *Anisakis* spp. and *Pseudoterranova* spp. in marine fishes and invertebrates. Glob. Chang. Biol. 26 (5), 2854–2866.

Galatius, A., Teilmann, J., Dähne, M., Ahola, M., Westphal, L., Kyhn, L.A., Pawliczka, I., Olsen, M.T., Dietz, R., 2020. Grey seal *Halichoerus grypus* recolonisation of the southern Baltic Sea, Danish Straits and Kattegat. Wildlife Biol 4, 1–10.

Geraci, J.R., Lounsbury, V.J., 2001. Marine mammals health: Holding the balance in an ever-chaning sea. In: Evans, P.G.H., Raga, J.A. (Eds.), Marine Mammals: Biology and Conservation. Kluwer Academic/Plenum Publisher, New York, pp. 365–371.
Geraci, J.R., Fortin, J.F., Aubin, D.J.S., Hicks, B.D., 1981. The seal louse, *Echinophthirius horridus*: an intermediate host of the seal heartworm, *Dipetalonema spirocauda* (Nematoda). Can. J. Zool. 59 (7), 1457–1459.
Gonzales-Contreras, A., Magarinos, B., Godoy, M., Irgang, R., Toranzo, A.E., Avendano-Herrera, R., 2011. Surface properties of *Streptococcus phocae* strains isolated from diseased Atlantic salmon, *Salmo salar* L. J. Fish. Dis. 34, 203–215.
Grassi, B., Noè, G., 1900. The propagation of the filariæ of the blood exclusively by means of the puncture of peculiar mosquitos. Br. Med. J. 2 (2079), 1306.
Green, A., Larson, S., 2016. A review of organochlorine contaminant in nearshore marine mammal predators. Environ. Anal. Toxicol. 6, 370.
Hagris, A.M., Myers, S., 2017. The integument. In: Zachary, J.F. (Ed.), Pathologic Basis of Veterinary Disease, sixth ed. Elsevier, St. Louis, Missouri, pp. 1071–1075.
Harding, K.C., Härkönen, T.J., 1999. Development in the Baltic grey seal (*Halichoerus grypus*) and ringed seal (*Phoca hispida*) populations during the 20th century. Ambio 619–627.
Härkönen, T., Dietz, R., Reijnders, P., Teilmann, J., Harding, K., Hall, A., Brasseur, S., Siebert, U., Goodman, S.J., Jepson, P.D., Rasmussen, T.D., Thompson, P., 2006. The 1988 and 2002 phocine distemper virus epidemics in European harbour seals. Dis. Aquat. 68 (2), 115–130.
HELCOM, 2023. State of the Baltic Sea 2023, Third HELCOM Holistic Assessment 2016-2021. Balt. Sea Environ. Proc. 197. Available online: http://stateofthebalticsea.helcom.fi/. (Accessed 19 November 2023).
Henton, M.M., Zapke, O., Basson, P.A., 1999. *Streptococcus phocae* infections associated with starvation in Cape Fur seals. J. S. Afr. Vet. Assoc. 70, 98–99.
Higgins, R., 2000. Bacteria and fungi of marine mammals: a review. Can. Vet. J. 41, 105–116.
Hirzmann, J., Ebmer, D., Sánchez-Contreras, G.J., Rubio-García, A., Magdowski, G., Gärtner, U., Taubert, A., Hermosilla, C., 2021. The seal louse (*Echinophthirius horridus*) in the Dutch Wadden Sea: investigation of vector-borne pathogens. Parasit. Vectors 14, 96.
Hoffman, J.I., Simpson, F., David, P., Rijksd, J.M., Kuikene, T., Thorne, M.A.S., Lacy, R. C., Dasmahapatra, K.K., 2014. High-throughput sequencing reveals inbreeding depression in a natural population. Proc. Natl. Acad. Sci. USA 111 (10), 3775–3780.
Howells, M.E., Pruetz, J., Gillespie, T.R., 2010. Patterns of gastro-intestinal parasites and commensals as an index of population and ecosystem health: the case of sympatric western chimpanzees (*Pantroglodytes verus*) and Guinea baboons (*Papio hamadryas papio*) at Fongoli, Senegal. Am. J. Primatol. 73 (2), 173–179.
Ignoffo, C.M., 1958. Evaluation of techniques for recovering ectoparasites. Proc. Iowa Acad. Sci. 65 (1), 540–545.
IJsseldijk, L.L., Brownlow, A.C., Mazzariol, S. (Eds.), 2019. Best Practice on Cetacean Post Mortem Investigation and Tissue Sampling. ASCOBANS/ACCOBAMS, Bonn/Monaco.
Kartman, L., 1953. Factors influencing infection of the mosquito with Dirofilaria immitis (Leidy, 1856). Exp. Parasitol. 2 (1), 27–78.
Keroack, C.D., Williams, K.M., Fessler, M.K., DeAngelis, K.E., Tsekitsidou, E., Tozloski, J. M., Williams, S.A., 2018. A novel quantitative real-time PCR diagnostic assay for seal heartworm (*Acanthocheilonema spirocauda*) provides evidence for possible infection in the grey seal (*Halichoerus grypus*). IJP: Parasites and Wildlife 7 (2), 147–154.
Kim, K.C., 1975. Ecology and morphological adaptation of the sucking lice (Anoplura: Echinophthiriidae) on the northern Fur seal. Rapp. P.-V. Reun. Cons. Int. Explor. Mer. 169, 504–515.
Kim, K.C., 1985. Evolution and host association of Anoplura. In: Kim, K.C. (Ed.), Coevolution of Parasitic Arthropods and Mammals. Wiley, New York, pp. 197–229.
Kim, K.C., Repenning, C.A., Morejohn, G.V., 1975. Specific antiquity of the sucking lice and evolution of otariid seals. Rapp. P.-V. Reun. Cons. Int. Explor. Mer. 169, 544–549.
Krogsrud, J., Evensen, O., Holt, G., Hoie, S., Markussen, N.H., 1990. Seal distemper in Norway in 1988 and 1989. Vet. Rec. 126, 460–461.
Ledesma, N., Harrington, L., 2011. Mosquito vectors of dog heartworm in the United States: vector status and factors influencing transmission efficiency. Top. Companion Anim. Med. 26 (4), 178–185.
Lehnert, K., Raga, J.A., Siebert, U., 2007. Parasites in harbour seals (*Phoca vitulina*) from the German Wadden Sea between two phocine distemper virus epidemics. Helgol. Mar. Res. 61, 239–245.
Lehnert, K., Schwanke, E., Hahn, K., Wohlsein, P., Siebert, U., 2016. Heartworm (Acanthocheilonema spirocauda) and seal louse (*Echinophthirius horridus*) infections in harbour seals (*Phoca vitulina*) from the North and Baltic Seas. J. Sea Res. 113, 65–72.
Lehnert, K., Ijsseldijk, L.L., Uy, M.L., Boyi, J.O., van Schalkwijk, L., Tollenaar, E.A.P., Gröne, A., Wohlsein, P., Siebert, U., 2021. Whale lice (*Isocyamus deltobranchium & Isocyamus delphinii*; Cyamidae) prevalence in odontocetes off the German and Dutch coasts–morphological and molecular characterization and health implications. IJP: Parasites and Wildlife 15, 22–30.
Lehnert, K., Herzog, I., Boyi, J.O., Gross, S., Wohlsein, P., Ewers, C., Prenger-Berninghoff, E., Siebert, U., 2023. Heartworms in *Halichoerus grypus* and first records of *Acanthocheilonema spirocauda* (Onchocercidae; Filarioidea) in two grey seals from the North Sea. Parasitology 150 (9), 781–785.
Leidenberger, S., Harding, K., Härkönen, T., 2007. Phocid seals, seal lice and heartworms: a terrestrial host-parasite system conveyed to the marine environment. Dis. Aquat. Org. 77, 235–253.
Leidy, J., 1858. Contributions to helminthology. Proc. Acad. Nat. Sci. Philadelphia. 10, 110–112.
Leonardi, M.S., Crespo, E.A., Raga, J.A., Aznar, F.J., 2013. Lousy mums: patterns of vertical transmission of an amphibious louse. Parasitol. Res. 112, 3315–3323.
Leonardi, M.S., Crespo, J.E., Soto, F., Lazzari, C.R., 2021a. How did seal lice turn into the only truly marine insects? Insects 13, 46.
Leonardi, M.S., Krmpotic, C., Barbeito, C., Soto, F., Loza, C.M., Vera, R., Negrete, J., 2021b. I've got you under my skin: inflammatory response to elephant seal's lice. Med. Vet. Entomol. 35, 658–662.
Lipscomb, T.P., Mense, M.G., Habecker, P.L., Taubenberger, J.K., Schoelkopf, R., 2001. Morbilliviral dermatitis in seals. Vet. Pathol. 38 (6), 724–726.
Lunneryd, S.G., 1992. *Dipetalonema spirocauda* (Leidy) (Nematoda) and *Corynosoma strumosum* (Rudolphi) (Acanthocephala) infection in harbour seal from the Kattegat-Skagerrak and the Baltic. Sarsia 76, 267–271.
Mauldin, E.A., Peters-Kennedy, J., 2016. Integumentary system. In: Grant Maxie, M. (Ed.), Jubb, Kenedy, and Palmers's Pathology of Domestic Animals, sixth ed. Elsevier, St. Louis, Missouri, pp. 509–678.
May, R., Anderson, R., 1979. Population biology of infectious diseases: Part II. Nature 280, 455–461.
Measures, L.N., Gosselin, J.-F., Bergeron, E., 1997. Heartworm, *Acanthocheilonema spirocauda* (Leidy, 1858), infections in Canadian phocid seals. Can. J. Fish. Aquat. 54, 842–846.
Mellish, J., Tuomi, P., Hindle, A., Jang, S., Horning, M., 2010. Skin microbial flora and effectiveness of aspetic technique for deep muscle biopsies in Weddell Seals (*Leptonychotes weddelli*) in McMurdo Sound, Antarctica. J. Wildl. Dis. 46 (2), 655–658.
Migaki, G., Jones, S.R., 1983. Mycotic diseases in marine mammals. In: Howard, E.B. (Ed.), Pathobiology of Marine Mammal Diseases, vol. II. Taylor & Francis Group, Boca Raton, Florida, pp. 2–25.
Mikkelsen, L., Johnson, M., Wisniewska, D.M., van Neer, A., Siebert, U., Teglberg Madsen, P., Teilmann, J., 2019. Long-term sound and movement recording tags to study natural behavior and reaction to ship noise of seals. Ecol. Evol. 9 (5), 2588–2601.
Müller, G., Wohlsein, P., Beineke, A., Haas, L., Greiser-Wilke, I., Siebert, U., Fonfara, S., Harder, T., Stede, M., Gruber, A.D., Baumgärtner, W., 2004. Phocine distemper in German seals, 2002. Emerg. Infect. Dis. 10 (4), 723.
Murray, M.D., Nicholls, D.G., 1965. Studies of the ectoparasites of seals and penguins. 1. The ecology of the louse *Lepidophthirius macrorhini* Enderlein on the southern elephant seal, *Mirounga leonina* (L). Aust. J. Zool. 13 (3), 437–454.
Murray, M.D., Smith, M.S.R., Soucek, Z., 1965. Studies of the ectoparasites of seals and penguins. 2. The ecology of the louse *Antarctophthirius ogmorphini* Enderlein on the Weddell seal, *Leptonychotes weddelli* Lesson. Aust. J. Zool. 13, 761–771.
Otto, G.F., Jackson, R.F., 1969. Pathology of heartworm disease. J. Am. Vet. Med. Assoc. 154 (4), 382.
Papadopoulos, E., Loukopoulos, P., Komnenou, A., Androukaki, E., Karamanlidis, A.A., 2010. First report of *Acanthocheilonema spirocauda* in the Mediterranean monk seal (*Monachus monachus*). J. Wildl. Dis. 46 (2), 570–573.
Portas, T.J., Crowley, A., Hufschmid, J., 2009. Ectoparasitic dermatitis in free-ranging swamp wallabies (*Wallabia bicolor*) in New South Wales. Aust. Vet. J. 87 (4), 160–162.
Postel, A., King, J., Kaiser, F.K., Kennedy, J., Lombardo, M.S., Reineking, W., de le Roi, M., Harder, T., Pohlmann, A., Gerlach, T., Rimmelzwaan, G., Rohner, S., Striewe, L.C., Gross, S., Schick, L.A., Klink, J.C., Kramer, K., Osterhaus, A.D.M.E., Beer, M., Baumgärtner, W., Siebert, U., Becher, P., 2021. Infections with highly pathogenic avian influenza A virus (HPAIV) H5N8 in harbor seals at the German North Sea coast. Emerg. Microbes Infect 1 (11), 1–32.
Raga, J.N., 1992. Parasitismus bei den Pinnipedia. In: Duguy, R., Robineau, D. (Eds.), Handbuch der Säugetiere Europas, Band 6, Teil II: Meeressäuger (Robben). AULA-Verlag, Wiesbaden, pp. 41–75.
Raga, J.A., Balbuena, J.A., Aznar, J., Fernández, M., 1997. The impact of parasites on marine mammals: a review. Parassitologia 39, 293–296.
Reijnders, P.J.H., 1984. Man-induced environmental factors in relation to fertility changes in pinnipeds. Environ. Conserv. 11 (1), 61–65.
Reijnders, P.J.H., 1994. Historical population size of the harbour seal, *Phoca vitulina*, in the Delta area, SW Netherlands. In: Nienhuis, P.H., Smaal, A.C. (Eds.), The Oosterschelde Estuary (The Netherlands): a Case-Study of a Changing Ecosystem. Developments in Hydrobiology, vol. 97. Springer, Dordrecht, pp. 557–560.
Reijnders, P.J., van Dijk, J., Kuiper, D., 1995. Recolonization of the Dutch Wadden Sea by the grey seal *Halichoerus grypus*. Biol. Conserv. 71 (3), 231–235.
Rijks, J.M., Hoffman, J., Kuiken, T., Osterhaus, A.D.M.E., Amos, W., 2008. Heterozygosity and lungworm burden in harbour seals (*Phoca vitulina*). Heredity 100, 587–593.
Rohner, S., Boyi, J.O., Artemeva, V., Zinke, O., Kiendl, A., Siebert, U., Lehnert, K., 2023. Back from exile? First records of chewing lice (*Lutridia exilis*; Ischnocera; Mallophaga) in growing Eurasian otter (*Lutra lutra*) populations from northern Germany. Pathogens 12 (4), 587.
Rubin, J.E., Ball, K.R., Chirino-Trejo, M., 2011. Antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from various animals. Can. Vet. J. 52 (2), 153–157.
Scherf, H., 1963. Robbenläuse. Mikrokosmos 52 (2), 359–364.
Schmid-Hempel, P., 2003. Variation in immune defence as a question of evolutionary ecology. Proc. Biol. Sci. 270 (1513), 357–366.
Sehnal, L., Brammer-Robbins, E., Wormington, A.M., Blaha, L., Bisesi, J., Larkin, I., Martyniuk, J.C., Simonin, M., Adamovsky, O., 2021. Microbiome composition and function in aquatic vertebrates: small organisms making big impacts on aquatic animal health. Front. Microbiol. 12, 567408.

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., Wohlsein, P., Lehnert, K., Baumgärtner, W., 2007. Pathological findings in harbour seals (*Phoca vitulina*): 1996–2005. J. Comp. Pathol. 137, 47–58.
Siebert, U., Rademaker, M., Ulrich, S.A., Wohlsein, P., Ronnenberg, K., Prenger-Berninghoff, E., 2017. Bacterial microbiota in harbor seals (*Phoca vitulina*) from the north sea of Schleswig-Holstein, Germany, around the time of morbillivirus and influenca epidemics. J. Wildl. Dis. 53, 201–214.
Silva, W.T.A.F., Bottagisio, E., Härkönen, T., Galatius, A., Olsen, M.T., Harding, K.C., 2021. Risk for overexploiting a seemingly stable seal population: influence of multiple stressors and hunting. Ecosphere 12 (1), e03343.
Simpson, G., 2023. Gratia: Graceful ggplot-based Graphics and other functions for GAMs fitted using *mgcv*. R package version 0.8.1 46.
Sonne, C., Siebert, U., Gonnsen, K., Desforges, J.P., Eulaers, I., Persson, S., Roos, A., Bäcklin, B.M., Kauhala, K., Olsen, M.T., Harding, K.C., Treu, G., Galatius, A., Andersen-Ranberg, E., Gross, S., Lakemeyer, J., Lehnert, K., Lam, S.S., Peng, W., Dietz, R., 2020. Health effects from contaminant exposure in Baltic Sea birds and marine mammals: a review. Environ. Int. 139, 105725.
Steen, C.J., Carbonaro, P.A., Schwartz, R.A., 2004. Arthropods in dermatology. J. Am. Acad. Dermatol. 50 (6), 819–842.
Stewart, B.S., Leatherwood, S., Yochem, P.K., Heide-Jørgensen, M.P., 1989. Harbor seal tracking and telemetry by satellite. Mar. Mammal Sci. 5 (4), 361–375.
Stroud, R.K., 1979. Causes of death in marine mammals stranded along the Oregon coast. J. Wildl. Dis. 15, 91–97.
Stroud, R.K., Dailey, M.D., 1978. Parasites and associated pathology observed in pinnipeds stranded along the Oregon coast. J. Wildl. Dis. 14, 292–298.
Thompson, P.M., Miller, D., 1990. Summer foraging activity and movements of radio-tagged common seals (*Phoca vitulina.* L.) in the Moray Firth, Scotland. J. Appl. Ecol. 27 (2), 492–501.
Thompson, D., Hammond, P.S., Nicholas, K.S., Fedak, M.A., 1991. Movements, diving and foraging behaviour of grey seals (*Halichoerus grypus*). J. Zool. 224 (2), 223–232.
Thompson, P.M., Pierce, G.J., Hislop, J.R.G., Miller, D., Diack, J.S.W., 1991. Winter foraging by common seals (*Phoca vitulina*) in relation to food availability in the inner Moray Firth, NE Scotland. J. Anim. Ecol. 60 (1), 283–294.
Thompson, P.M., Corpe, H.M., Reid, R.J., 1998. Prevalence and intensity of the ectoparasite *Echinophthirius horridus* on harbour seals (*Phoca vitulina*): effects of host age and interannual variability in host food availability. Parasitology 117, 393–403.
Unger, B., Herr, H., Benke, H., Böhmert, M., Burkhardt-Holm, P., Dähne, M., Hillmann, M., Wolff-Schmidt, K., Wohlsein, P., Siebert, U., 2017. Marine debris in harbour porpoises and seals from German waters. Mar. Environ. Res. 130, 77–84.
Unger, B., Baltzer, J., Brackmann, J., Brasseur, S., Brügmann, M., Diedirichs, B., Galatius, A., Geelhoed, S.C.V., Huus Petersen, H., Ijsseldijk, L.L., Jensen, T.K., Jess, A., Nachtsheim, D., Philipp, C., Scheidat, M., Schop, J., Siebert, U., Teilmann, J., Thøstesen, C.B., van Neer, A., 2022. In: Kloepper, S., et al. (Eds.), Marine Mammals in: Wadden Sea Quality Status Report. Common Wadden Sea Secretariat, Wilhelmshaven, Germany. Downloaded 03.11.2022. qsr.waddensea-worldheritage.org/reports/marine-mammals.
van Neer, A., Nachtsheim, D., Siebert, U., Taupp, T., 2023. Movements and spatial usage of harbour seals in the Elbe estuary in Germany. Sci. Rep. 13 (1), 6630.
Waltzek, T.B., Cortés-Hinojosa, G., Wellehan Jr., J.F.X., Gray, G.C., 2012. Marine mammal zoonoses: a review of disease manifestations. Zoonoses Public Health 59, 521–535.
Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Soc. Series B Stat. Methodol. 73, 3–36.
World Health Organization, 1997. Bench Aids for the Diagnosis of Filarial Infections. December 11. Geneva, Switzerland. Retrieved September 29, 2023, from: www.who.int/publications/i/item/9241544899.
Wülker, G., 1929. Der Wirtswechsel der parasitischen Nematoden von Meeresfischen. Zool. Anz. 4, 147–157.
Wülker, G., 1930. Über Nematoden aus Nordseetieren. Zool. Anz. 87, 293–302.
§ 27 Niedersächsisches Jagdgesetz, 2023. Retrieved September 26, 2023, from: https://www.ml.niedersachsen.de/.