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Authors
Dent, Colleen E Shockling
Miller, Melissa A
Batac, Francesca
et al.

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Pathology and epidemiology of nasopulmonary acariasis (*Halarachne* sp.) in southern sea otters (*Enhydra lutris nereis*)

Colleen E. Shockling Dent[a,1], Melissa A. Miller[b,c], Francesca Batac[b], Erin Dodd[b], Woutrina Smith[c,d], Risa Pesapane[d], Janet Foley[d,*]

[a] College of Veterinary Medicine, The Ohio State University, 1900 Coffey Road, Columbus, OH, 43210, USA
[b] California Department of Fish and Wildlife, Marine Wildlife Veterinary Care and Research Center, 151 McAllister Way, Santa Cruz, CA, 95060, USA
[c] One Health Institute, School of Veterinary Medicine, University of California, 1089 Veterinary Medicine Dr., Davis, CA, 95616, USA
[d] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, One Shields Ave., Davis, CA, 95616, USA

A R T I C L E I N F O

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A B S T R A C T

Halarachne sp. nasal mites infest harbor seals *(Phoca vitulina)* and southern sea otters *(Enhydra lutris nereis)* in California, but little is known about the pathophysiology of these infestations, or risk factors for exposure. To investigate these questions, a retrospective case-control study was performed using necropsy data from 70 mite-infested sea otters, and 144 non-infested controls. Case records for sea otters examined by pathologists from February 1999 through May 2015 were examined to assess risk factors for infestation, and lesions associated with nasopulmonary acariasis. Animals with a history of captive care within 10 days of death or carcass recovery were 3.2 times more likely to be infested with nasopulmonary mites than those with no history of recent rehabilitation. Sea otters stranding within 1 km of Elkhorn Slough in Monterey Bay were 4.9 times more likely to be infested with nasal mites than other areas; this site is characterized by high sea otter contact with sympatric harbor seals (a common host for *Halarachne* sp.), and a comparatively large population of rehabilitated and released sea otters. Aged adult otters were 9.4 times more likely to be infested than younger animals, and sea otters with nasopulmonary acariasis were 14.2 times more likely to have upper respiratory inflammation than un-infested animals. Additional findings in otters with nasopulmonary acariasis included lower respiratory tract bacterial infections, presence of medium-sized and/or fresh nose wounds at necropsy (indicators of recent face-to-face interaction between otters during copulation or fighting), and turbinate bone erosion. Our findings, although preliminary, suggest that captive rehabilitation and close contact with harbor seals could facilitate nasopulmonary mite transmission to sea otters. We also identified a high-risk zone for nasopulmonary acariasis in sea otters. We also provide preliminary data to suggest that nasopulmonary mite infestations can cause significant respiratory pathology in sea otters.

1. Introduction

While often commensals or opportunistic pathogens, mites are often under-recognized as contributors to wildlife morbidity and mortality. Among more than 50,000 recognized mite species, at least nine mite families (Cytoditidae, Entonyssidae, Ereynetidae, Gastronyssidae, Halarachnidae, Lemurnyssidae, Pneumocoptidae, Rhinonyssidae and Trombiculidae) contain obligate respiratory parasites of vertebrates (Mullen and O'Connor, 2002). Nasopulmonary mite infestations have been reported in four species of sea lions (*Eumetopias jubatus*, *Zalophus californianus, Neophoca cinerea,* and *Otaria byronia*), fur seals (*Callorhinus ursinus* and *Arctocephalus* spp.), and walruses (*Odobenus rosmarus*). The Caribbean monk seal (*Monachus tropicalis*) hosted its own species of respiratory mite, which went extinct with its host (Furman and Dailey, 1980). Respiratory mite infestation has also been reported in Weddell (*Leptonychotes weddellii*), gray (*Halichoeris grypus*), elephant (*Mirounga angustirostrus*), harbor (*Phoca vitulina*), and spotted seals (*Phoca largha*), and less commonly, sea otters (*Enhydra lutris*) (Ferris, 1925; Newell, 1947; King, 1964; Kenyon et al., 1965; Dailey and Brownell, 1972; Domrow, 1974; Dunlap et al., 1976; Fay and Furman, 1982; Pesapane et al., 2018 in review).

Halarachnid mites infest marine mammals, canids, nonhuman primates and small mammals such as porcupines and squirrels (Krantz and Walter, 2009). *Halarachne* sp. infestations have been reported from

* Corresponding author. School of Veterinary Medicine, Department of Medicine and Epidemiology, University of California, Davis, 95616, USA.
E-mail address: jefoley@ucdavis.edu (J. Foley).
[1] Present address: 1287 Vinewood Drive, Columbus, OH 43229, USA.

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seals (Phocidae) and sea otters (Mustelidae) (Kenyon et al., 1965), while *Orthohalarachne* infestations are more typical for fur seals and sea lions (Otariidae) and walrus (Odobenidae) (Newell, 1947; Furman and Dailey, 1980; Kim et al., 1980).

A recent study by our team confirmed southern sea otter (*E. lutris nereis*) infection by *H. halichoeri*, estimated the prevalence of nasopulmonary mites in necropsied otters, and created a DNA reference for mites collected from southern sea otters during necropsy (Pesapane et al., 2018 in review). The current study builds on that effort by investigating potential risk factors for infestation, and assessing lesions associated with nasopulmonary acariasis in necropsied southern sea otters. Light intensities from 1 to 7 mites of *Halarachne* sp. were reported in 3% of necropsied, wild northern sea otters (*E. lutris kenyoni*) from Amchitka Island, Alaska (Kenyon et al., 1965). In contrast, > 3000 *Halarachne* sp. mites were reported from the respiratory tract of a captive sea otter that died with erythematous nasal mucosa, turbinate osteolysis, and pulmonary congestion (Kenyon et al., 1965). During necropsy, we have observed dense *H. halichoeri* infestations in southern sea otters, including stranded animals that had been held in captivity for rehabilitation, and wild otters that stranded in regions with dense populations of sympatric pinnipeds (unpub. Miller). Although both have been postulated as potential sources of nasopulmonary mite infestation in sea otters and other marine mammals (Kenyon et al., 1965; Furman and Smith, 1973; Fravel and Procter, 2016), no epidemiological studies have been reported to assess the strength of these associations. In addition, other than a single anecdotal report (Kenyon et al., 1965), little was known about the significance of varying nasopulmonary mite burdens on sea otters, or potential mite-associated pathology.

As a first step toward addressing these questions, a retrospective case-control study design was used to scan records from 214 southern sea otters necropsied by pathologists from 1999 through 2015 for a defined set of respiratory lesions and other health conditions. We also analyzed each animal's stranding location, age class, sex, and other attributes for potential associations with nasopulmonary mite infestation. Although preliminary, our findings support hypotheses regarding the potential for captive care and phocid contact to facilitate sea otter infection with nasopulmonary mites. Our research also suggest that nasopulmonary mite infestations can cause significant respiratory pathology in sea otters. Findings from this study highlight the importance of controlling nasal mite infestation in captive sea otters, and can inform, rehabilitation, animal translocation, and oil spill response practices.

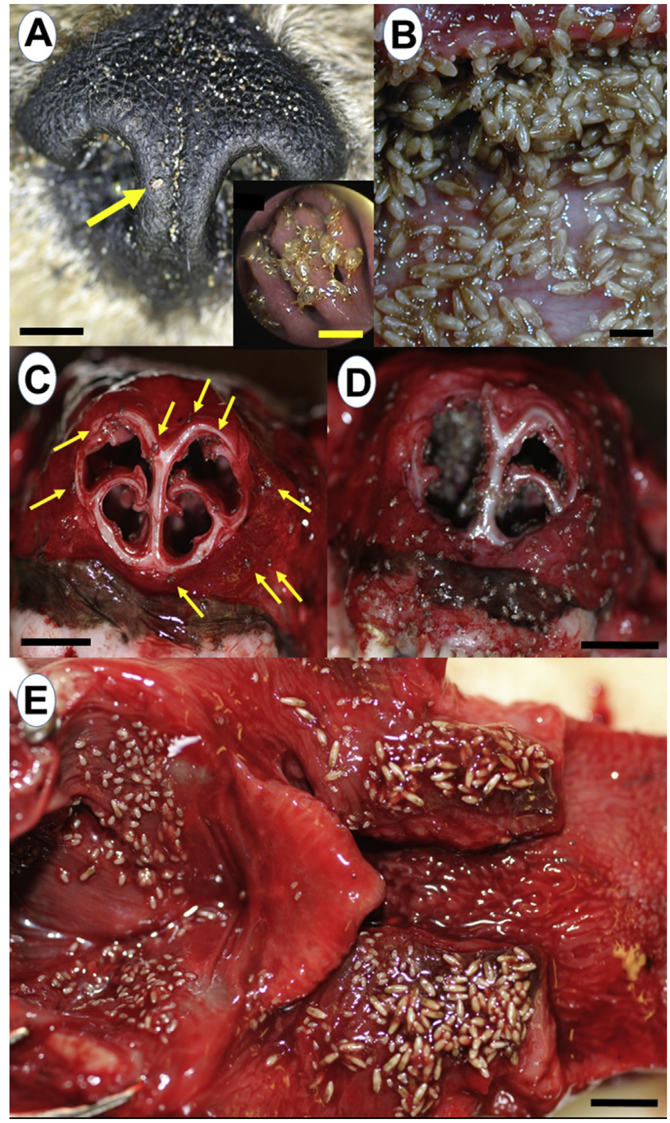

Fig. 1. Nasopulmonary acariasis in southern sea otters (*Enhydra lutris nereis*). **A.** A single hexapod larval nasal mite (*Halarachne* sp.) has wandered out of the nose and is present on the planum nasale (arrow). This highly motile infectious larval stage is most common in the nares and rostral turbinates of the upper respiratory tract (Bar = 6 mm). **Inset**: Rhinoscopic view of a mass of hexapod larval mites (*Halarachne* sp.) crawling on the nasal turbinates of a sedated, live, captive sea otter (Bar = 2.5 mm). Image courtesy of Dr Michael Murray, Monterey Bay Aquarium. **B.** The larger, elongated (cigar-shaped) *Halarachne* sp. adults typically aggregate in the nasopharynx, but can also be found in the oropharynx, trachea and bronchi. Adult mites can become very densely packed in the nasopharynx, as shown here (Bar = 3 mm). **C.** The planum nasale was removed during necropsy, exposing the nasal cartilage and turbinate bones. As refrigerated carcasses warm up, larval mites often exit the nasal cavity and are readily apparent (arrows). This is a moderate infestation. Note the symmetry of the nasal cartilage and underlying turbinates (Bar = 12 mm). **D.** Severe larval mite infestation in a captive sea otter with chronic or recurrent nasopulmonary mite infestation, demonstrating marked asymmetry of the nasal cartilage and underlying turbinates. Severe, diffuse mucosal inflammation and turbinate osteolysis were confirmed on histopathology (Bar = 12 mm). **E.** Sea otter with a heavy intensity of adult *Halarachne* sp. attached to the dorsal soft palate in the nasopharynx, and throughout the larynx. Marked, diffuse mucosal erythema is spatially-associated with areas of mite attachment. Preliminary findings from bacterial culture and histopathology suggest that opportunistic bacterial pathogens, such as beta hemolytic streptococci, are often associated with these regions of mite infestation and respiratory mucosal erythema (Bar = 8 mm).

2. Materials and methods

2.1. Sea otter necropsy, histopathology and mite collection

Necropsies of opportunistically available southern sea otters were performed at the California Department of Fish and Wildlife (CDFW) Marine Wildlife Veterinary Care and Research Center, as previously described (Chinn et al., 2016). During review of records, "Cases" were defined as sea otters where one or more mites were reported in the respiratory tract or oropharynx during postmortem examination, while "controls" did not have mite observations reported. Cases encompassed confirmed-infected, fresh or frozen animals from 1999 to 2015. To minimize the risk of including false-negative animals as controls, only mite-negative fresh, non-frozen carcasses from 2004 to 2015 were included, due to lower precision of respiratory mite screening in frozen-thawed animals, and during gross necropsies performed from 1999 to 2003.

The study population for assessing potential lesion patterns included 214 southern sea otters; 70 cases and 144 controls. The majority of cases (65) were fresh, non-frozen animals (92.9%), 3 (4.3%) were moderately decomposed, and one each were fresh frozen, or advanced decomposition (1.4% each) at necropsy. All (144) controls were fresh, non-frozen animals, as previously described.

Data maintained for necropsied otters included the date of animal or carcass recovery, and coastal stranding location to the nearest 0.5 km. Sea otters found alive were coded as "live stranded", and post-stranding death or euthanasia was also noted. For each animal, the presence or absence of mites, their life stage (larvae or adults, Fig. 1A–E) (Pesapane et al., 2018 in review), their anatomical location at necropsy (turbinates, nasopharynx, trachea, and/or bronchioles), and parasite intensity; low (< 10), moderate (10–50), and heavy (> 50 mites), were recorded. Also assessed subjectively was the overall severity (total burden) of respiratory mite parasitism across all anatomical locations (none, mild, moderate, marked). Mites were collected and saved at −80 C or in 70% ethanol to facilitate morphological and genetic characterization under separate funding (Pesapane et al., 2018 in review).

The following data were routinely collected during sea otter necropsy: sex, age class (pup, immature, subadult, adult, aged adult; estimated age based on total length, dentition, and pelage characteristics), total length (cm), weight (kg), nutritional status (emaciated, poor, fair, good, and excellent), and subcutaneous adipose (none, scant, fair, moderate, and abundant) as reported previously (Chinn et al., 2016). If present, nose wounds were characterized by their size and relative freshness; these wounds are commonly inflicted by other sea otters during copulation or fighting (Chinn et al., 2016), and are indicative of close and sustained facial contact that could result in respiratory mite transmission. During gross necropsy, the upper and lower respiratory tracts were examined, and all major tissues were formalin-fixed, embedded in paraffin, sectioned, stained using hematoxylin and eosin, and examined using compound microscopy. Unfortunately, nares were not always systematically assessed through parasagittal section due to the requirement to keep skulls intact due to needs for collaborating museums and other entities.

Also noted were a defined set of lesions or postmortem conditions that could potentially be related to mite infestation, based on previously published reports (Kenyon et al., 1965; Dunlap et al., 1976; Kim et al., 1980; Fay and Furman, 1982; Alonso-Farré et al., 2012). These included grossly apparent upper respiratory (nostril-larynx) conditions, such as mucopurulent exudate, nasal discharge, erythematous nasopharyngitis, and turbinate lysis. Lower respiratory tract (trachea-lung) conditions that were considered in relation to nasopulmonary mite presence and parasite burden included tracheitis, bronchitis, pulmonary emphysema and/or bullae, pulmonary edema, pulmonary abscesses, bronchopneumonia, and pulmonary pleural scars. Finally, a defined set of extra-pulmonary conditions that could be related to mite infestation were considered, including bacterial septicemia, bacterial endocarditis, and valvular endocardiosis. All available case records, including perimortem stranding data, gross necropsy reports, gross necropsy photos, and histopathology notes were reviewed to define mite infestation status, and presence or absence of potential mite-associated lesions. Also assessed were attributes that could be associated with an enhanced risk of nasopulmonary mite infestation, such as age class, sex, stranding location, and stranding history.

2.2. Data analysis and assessment of risk factors for mite infestation

A retrospective case-control study design was used to evaluate potential risk factors for infestation, and lesions associated with nasopulmonary mite infestation. Descriptive statistics for unmatched case and control sea otters included distribution by sex, age, captivity status, stranding location, carcass condition, nutritional condition, and observed necropsy lesions. Mite infestation was characterized categorically as presence/absence, overall mite intensity, anatomic location, and mite life stages observed. The frequency and prevalence of each condition was calculated for cases, controls, and the overall sample population.

Logistic regression analysis for the case-control study was based on the outcome of nasal mite presence/absence at gross necropsy. Within the case population, mite intensity was also evaluated as an outcome of interest in association with defined exposure factors. Potential risk factors for mite infestation included sea otter sex (male; female), age class (pup/immature; adult; aged adult), nutritional condition (excellent; good/fair; poor/emaciated), coastal stranding location near Elkhorn Slough (≤1 km; > 1 km), stranding near well-defined and common harbor seal haulout sites along the central California Coast (yes/no), history of recent captive care prior to stranding (no prior history of captive care; captive care ≤10 days of death or carcass recovery; or > 10 days between the most recent episode of captive care and sea otter death or carcass recovery), fresh nose wound (red [acute] nose wound suggestive of recent mating or fighting activity; none or older lesion), upper respiratory inflammation from nostrils to larynx (yes; no), and lower respiratory inflammation from trachea to lungs areas (yes; no).

Univariate logistic regression was used to evaluate each potential risk factor for association with mite infestation (presence/absence). Next, risk factors that had been significant at the $P < 0.1$ level were included in a multivariable logistic regression analysis. Using a backwards stepwise approach for selecting final model variables, the strengths of association in the multivariable model were assessed through odds ratios, P-values of ≤0.05, and 95% confidence intervals. Model fit was evaluated using Pseudo R^2 values, and Stata software (College Station, TX) was used for all data analyses.

3. Results

3.1. Distribution of sample population by sex, age, stranding location and other factors

Table 1 shows the distribution of cases and controls by sex, age class, nutritional condition, captivity history and stranding location. Males represented 56.5% of the study population, distributed as 61.4% of cases, and 54.2% of controls. The majority of necropsied sea otters were adults (50.5%) or aged adults (22.9%). Most (94.2%) nasopulmonary mite infestations were observed in adult and aged adult otters (47.1% each), compared with 0, 4.3 and 1.4% of infestations in enrolled pups, immatures and subadults, respectively. Across the sample population, no clear differences were observed between cases and controls with respect to nutritional condition.

Approximately twice as many nasopulmonary mite-infested sea otters (40.0%) had a history of antemortem captive care when compared with controls (19.4%). This same pattern was noted for mite-infested otters with a history of captive care within 10 days of death or stranding (37.1), compared with controls (18.1%), and for mite-infested otters with a history of captive care at any previous point for ≥10 days prior to necropsy (14.3%), compared with controls (7.6%) (Table 1).

The Elkhorn Slough estuary is characterized by continuous, high sea otter contact with sympatric harbor seals. Due to abundant prey, calm water and greater ease of monitoring animals post-release, many rehabilitated sea otters are also released at this location. In the current study, approximately 1/3 (34.3%) of all mite-infested sea otters were recovered from within 1 km of Elkhorn Slough, which represents a very small portion of the > 400 km long coastal range of the southern sea otter.

3.2. Severity of mite infestation, mite life stages and anatomic location

Among 70 southern sea otters with nasopulmonary acariasis, the severity of parasitism, mite life stages and their anatomic distribution are summarized in Table 2. For two cases the nasal mite intensity was not recorded. Due to methods used to record archival data during necropsy, it was not possible to partition mite intensity estimates in the current study to match those of previous reports.

Of 68 otters with mite intensities reported in the case record, 55.7%

Table 1
Distribution by sex, age class, stranding location and other factors for necropsied southern sea otters (*Enhydra lutris nereis*, n = 214) with and without nasopulmonary acariasis.

Category	Subcategory	Cases # (%)	Controls # (%)	Total # (%)
Sex	Female	27 (38.6%)	66 (45.8%)	93 (43.5%)
	Male	43 (61.4%)	78 (54.2%)	121 (56.5%)
Age class	Pup	0 (0%)	9 (6.3%)	9 (4.2%)
	Immature	3 (4.3%)	11 (7.6%)	14 (6.5%)
	Subadult	1 (1.4%)	33 (22.9%)	34 (15.9%)
	Adult	33 (47.1%)	75 (52.1%)	108 (50.5%)
	Aged adult	33 (47.1%)	16 (11.1%)	49 (22.9%)
Nutritional condition	Excellent	19 (27.1%)	23 (16.0%)	42 (19.6%)
	Good	6 (8.6%)	15 (10.4%)	21 (9.8%)
	Fair	10 (14.3%)	15 (10.4%)	25 (11.7%)
	Poor	8 (11.4%)	24 (16.7%)	32 (15%)
	Emaciated	27 (38.6%)	63 (43.8%)	90 (42.1%)
	Unknown	0 (0%)	4 (2.8%)	4 (1.9%)
Captivity	Captive at any point of time prior to death	28 (40.0%)	28 (19.4%)	56 (26.2%)
	Captive at any previous point for ≥10 days	10 (14.3%)	11 (7.6%)	21 (9.8%)
	Captivity > 24 h within 10 days of death	26 (37.1%)	26 (18.1%)	52 (24.3%)
	Captive within 10 days of death for ≥10 days	8 (11.4%)	9 (6.3%)	17 (7.9%)
Proximity to Elkhorn Slough	Within 1 km	24 (34.3%)	11 (7.6%)	35 (16.4%)
	> 1 km	46 (65.7%)	133 (92.4%)	179 (83.6%)

Table 2
Severity of mite infestation, anatomic location of mites, and life stages present in necropsied southern sea otters (*Enhydra lutris nereis,* n = 70) with nasopulmonary acariasis.

Category	Subcategory	Cases # (%)
Nasal mite intensity	Unknown	2 (2.9)
	None	0 (0)
	Mild (< 10)	39 (55.7)
	Moderate (10–50)	12 (17.1)
	Heavy (> 50)	17 (24.3)
Nasal mite location	Rostral nose/turbinates	63 (90.0)
	Nasopharynx	48 (68.6)
	Trachea/bronchi	16 (22.9)
	Rostral nose & nasopharynx	44 (62.9)
	Upper & lower respiratory	16 (22.9)
	Nose, nasopharynx, and lower respiratory	14 (20.0)
Life stage of mites	Larvae	50 (71.4)
	Adults Both	44 (62.9) 37 (53.0)

had < 10 mites, 17.1% had 10-50 mites, and 24.3% had > 50 mites. Most cases had mites in the rostral nose/turbinates and/or nasopharynx (90.0% and 68.6%, respectively) (Fig. 1A–E), while 22.9% had mites in the trachea and bronchi. Nasopulmonary mites were recovered from all locations in the upper and lower respiratory tract for 20% of infested otters. The majority of mite-infested otters (71.4%) were parasitized by larvae (Fig. 1A, C and D), 62.9% had adult mites (Fig. 1B and E), and 53% were infested with both larvae and adults concurrently.

3.3. Potential lesion associations with mite infestation

Lesions reported in necropsy notes for 214 necropsied southern sea otters with and without nasopulmonary acariasis are summarized in Table 3. The majority of cases and controls had either white (chronic) nose wounds (46.3%), or no nose wounds (27.6%). Nose wounds were mainly small for both cases (37.1%) and controls (40.3%), also reflecting lesion chronicity.

Differences of at least 10% prevalence between case and control otters were observed for fresh (red) nose wounds, nasal discharge, mucopurulent respiratory exudate, erythematous nasopharyngitis or bronchitis, focal or multifocal pleural scars, and endocardiosis, all of which were more common in mite-infested otters. Focal or multifocal tiny white to light tan pleural foci were more common in mite-infested sea otters than controls, but were not necessarily observed with concurrent pulmonary bullae. Severe turbinate lysis was noted at gross necropsy for a long-term captive sea otter with a clinical history of chronic or recurrent, heavy mite infestation (Fig. 1D).

3.4. Univariate analyses of risk factors for mite infestation, and mite-associated lesions

Five animals had incomplete data and were excluded from logistic regression analyses, leaving 209 animals (65 cases/and 144 controls) for statistical analysis of potential lesion associations and risk factors for nasopulmonary mite infestation. Variables that were not significant predictors for nasal mite infestation included sea otter sex, nutritional condition, presence of nose wounds, and presence of pulmonary emphysema.

Variables that were significantly associated with nasopulmonary acariasis in univariate analyses are summarized in Table 4. Significant predictors for nasal mite infestation included aged adults (12.9 odds ratio), animals with fresh (red) nose wounds 4.1 odds ratio, and sea otters with presence of upper (30.2 odds ratio) or lower respiratory tract inflammation (22.7 odds ratio, Table 4). Mite-infested animals were more likely (2.8 odds ratio) to have a history of captive care within 10 days of death. Mite-infested otters were also more likely to strand in close proximity to common harbor seal haulouts (2.7 odds ratio), and within 1 km of Elkhorn Slough (5.3 odds ratio), when compared with non-infected controls. All of the above factors were highly significant at $p \leq 0.05$.

3.5. Multivariable analyses of risk factors for mite infestation, and mite-associated lesions

Variables that were significantly associated with nasopulmonary acariasis in multivariable analyses are summarized in Table 5. Significant predictors for nasal mite infestation included aged adult age class (9.4 odds ratio), a history of captive care within 10 days of carcass recovery or death (3.2 odds ratio), animals that stranded within 1 km of Elkhorn Slough (4.9 odds ratio), and sea otters with upper respiratory tract inflammation noted at gross necropsy (14.2 odds ratio). All of the above factors were highly significant at $p \leq 0.05$.

For mite-infested sea otters, factors associated with having a moderate or heavy mite intensity at gross necropsy, when compared to mild

Table 3
Lesions observed in necropsied southern sea otters (*Enhydra lutris nereis*, n = 214) with and without nasopulmonary acariasis.

Category	Subcategory	Cases # (%)	Controls # (%)	Total # (%)
Nose Wound	Yes	54 (77.1%)	97 (67.4%)	151 (70.6%)
	No	15 (21.4%)	44 (30.6%)	59 (27.6%)
	Unknown	1 (1.4%)	3 (2.1%)	4 (1.9%)
Nose wound chronicity	None	15 (21.4%)	44 (30.5%)	59 (27.6%)
	Red	14 (20%)	10 (6.9%)	24 (11.2%)
	Pink	11 (15.7%)	20 (13.9%)	31 (14.5%)
	White	29 (41.4%)	70 (48.6%)	99 (46.3%)
	Unknown	1 (1.4%)	0 (0%)	1 (0.5%)
Nose wound size	None	15 (21.4%)	45 (31.3%)	60 (28.0%)
	Small	26 (37.1%)	58 (40.3%)	84 (39.3%)
	Medium	12 (17.1%)	16 (11.1%)	28 (13.1%)
	Large	16 (22.9%)	25 (17.4%)	41 (19.2%)
	Unknown	1 (1.4%)	0 (0%)	1 (0.5%)
Septicemia	Yes	15 (21.4%)	40 (27.8%)	55 (25.7%)
	No	51 (72.9%)	103 (71.5%)	154 (72.0%)
	Unknown	4 (5.7%)	1 (0.7%)	5 (2.3%)
Mucopurulent respiratory exudate	Yes	8 (11.4%)	1 (0.7%)	9 (4.2%)
	No	54 (77.1%)	142 (98.6%)	196 (91.6%)
	Unknown	8 (11.4%)	1 (0.7%)	9 (4.2%)
Nasal discharge	Yes	16 (22.9%)	10 (6.9%)	26 (12.1%)
	no	47 (67.1%)	132 (91.7%)	179 (83.6%)
	Unknown	7 (10%)	2 (1.4%)	9 (4.2%)
Erythematous or purulent rhinitis	Yes	7 (10%)	1 (0.7%)	8 (3.7%)
	No	54 (77.1%)	142 (98.6%)	196 (91.6%)
	Unknown	9 (12.9%)	1 (0.7%)	10 (4.7%)
Grossly apparent turbinate lysis	Yes	1 (1.4%)	0 (0%)	1 (0.5%)
	No	0 (0%)	0 (0%)	0 (0%)
	Unknown	69 (98.6%)	144 (100%)	213 (99.5%)
Erythematous nasopharyngitis	Yes	16 (22.9%)	2 (1.4%)	18 (8.4%)
	No	47 (67.1%)	141 (97.9%)	188 (87.9%)
	Unknown	7 (10%)	1 (0.7%)	8 (3.7%)
Erythematous tracheitis	Yes	4 (5.7%)	1 (0.7%)	5 (2.3%)
	No	57 (81.4%)	142 (98.6%)	199 (93%)
	Unknown	9 (12.9%)	1 (0.7%)	10 (4.7%)
Erythematous bronchitis	Yes	8 (11.4%)	1 (0.7%)	9 (4.2%)
	No	53 (75.7%)	142 (98.6%)	195 (91.1%)
	Unknown	9 (12.9%)	1 (0.7%)	10 (4.7%)
Pulmonary emphysema	Yes	8 (11.4%)	27 (18.8%)	35 (16.4%)
	No	55 (78.6%)	116 (80.6%)	171 (79.9%)
	Unknown	7 (10%)	1 (0.7%)	8 (3.7%)
Pulmonary edema	Yes	41 (58.6%)	74 (51.4%)	115 (53.7%)
	No	25 (35.7%)	70 (48.6%)	95 (44.4%)
	Unknown	4 (5.7%)	0	4 (1.9%)
Pulmonary abscess	Yes	1 (1.4%)	1 (0.7%)	2 (0.9%)
	No	60 (85.7%)	142 (98.6%)	202 (94.4%)
	Unknown	9 (12.9%)	1 (0.7%)	10 (4.7%)
Bacterial pneumonia	Yes	2 (2.9%)	2 (1.4%)	4 (1.9%)
	No	64 (91.4%)	141 (97.9%)	205 (95.8%)
	Unknown	4 (5.7%)	1 (0.7%)	5 (2.3%)
Pulmonary bullae	Yes	2 (2.9%)	5 (3.5%)	7 (3.3%)
	No	61 (87.1%)	138 (95.8%)	199 (93.0%)
	Unknown	7 (10.0%)	1 (0.7%)	8 (3.7%)
Focal or multifocal pleural scars	Yes	19 (27.1%)	4 (2.8%)	23 (10.7%)
	No	44 (62.9%)	139 (96.5%)	183 (85.5%)
	Unknown	7 (10.0%)	1 (0.7%)	8 (3.7%)
Endocarditis	Yes	5 (7.1%)	5 (3.5%)	10 (4.7%)
	No	57 (81.4%)	138 (95.8%)	195 (91.1%)
	Unknown	8 (11.4%)	1 (0.7%)	9 (4.2%)
Endocardiosis	Yes	32 (45.7%)	39 (27.1%)	71 (33.2%)
	No	30 (42.9%)	104 (72.2%)	134 (62.6%)
	Unknown	8 (11.4%)	1 (0.7%)	9 (4.2%)

Table 4
Univariate analysis of risk factors that were significantly associated with nasopulmonary acariasis in necropsied southern sea otters (*Enhydra lutris nereis*, n = 209).

Risk Factors	Odds Ratio	P value	95% CI
Aged adult age class	12.9	< 0.001	3.4–50.1
History of captive care within 10 days of death	2.8	0.002	1.5–5.5
Stranded within 1 km of Elkhorn Slough	5.4	< 0.001	2.4–12.1
Stranded near harbor seal haulout site	2.7	0.004	1.4–5.5
Fresh (red) nose wound	4.1	0.007	1.5–11.3
Upper respiratory inflammation (nostrils to larynx)	30.2	< 0.001	6.7–135.5
Lower respiratory inflammation (trachea to lungs)	22.7	0.004	2.8–217

Table 5
Multivariable logistic regression analysis of risk factors that were significantly associated with nasopulmonary acariasis in necropsied southern sea otters (*Enhydra lutris nereis*, n = 209).

Significant Risk Factors	Odds Ratio	P value	95% CI
Aged adult age class	9.4	0.004	2.0–44.7
History of captive care within 10 days of death	3.2	0.008	1.2–7.6
Stranded within 1 km of Elkhorn Slough	4.9	0.002	1.8–13.5
Upper respiratory inflammation (nostrils to larynx)	14.2	0.001	2.9–68.3

infestation, included a history of captivity within 10 days of death or stranding (OR 10.3; P = 0.042; 95% CI = 1.1–97.2), female sex (OR 53.2; P = 0.001; 95% CI = 5.5–515.3), and upper respiratory inflammation (OR 46.7; P = 0.003; 95% CI = 3.8–568.7) (data not shown).

4. Discussion

A retrospective case-control study design was used to scan records from 214 southern sea otters necropsied by pathologists for a defined set of respiratory lesions and other health conditions. Necropsy data from 70 mite-infested sea otters, and 144 non-infested controls examined by pathologists from February 1999 through May 2015. We also analyzed each animal's stranding location, age class, sex, and other attributes for potential associations with nasopulmonary mite infestation. All available case records were examined to assess risk factors for infestation, and lesions associated with nasopulmonary acariasis.

Anecdotal reports suggest that respiratory mite infestations can be clinically significant and occasionally, fatal in marine mammals (Kenyon et al., 1965; Van Bree, 1972; Kim et al., 1980). However, risk factors for infestation, mite-associated lesions, and potential co-morbidities are less well characterized. Diverse clinical signs and lesions have been reported in respiratory mite-infested pinnipeds, including sneezing, coughing, nasal discharge, mucosal inflammation and hyperplasia, turbinate erosion, pneumonia, edema, and emphysema (Dunlap et al., 1976; Kim et al., 1980; Fay and Furman, 1982; Baker, 1987; Alonso-Farré et al., 2012). Infested northern fur seals exhibited chronic respiratory mucosal inflammation and congestion, mucosal hyperplasia or erosion, mononuclear and eosinophilic inflammation, bronchiolar and pulmonary edema, pulmonary fibrosis, and pneumonia (Kim et al., 1980). While low-level infestations may be asymptomatic, heavy parasite intensities have been associated with sneezing, nasal discharge, and death (Alonso-Farré et al., 2012).

The life cycle of *H. halichoeri* includes free living, motile hexapod larvae, protonymphs and deutonymphs for very short durations, and less motile adults (Newell, 1947; Pesapane et al., 2018 in review). Transmission appears to be primarily due to larval invasion (Furman and Smith, 1973). In seals, nasal mites were thought to be transmitted by sneezing or coughing, nasal contact, or ‘breath-exchange’ during non-aggressive nosing (Fay and Furman, 1982; Mullen and O'Connor, 2002). In spotted seals and harbor seals, *H. halichoeri* larvae were concentrated in the distal nasal passages among the alar folds of the median septum and in the turbinates, while adults were concentrated posterior to the maxilloturbinates on the walls of the choanae and the dorsal wall of the nasopharynx (Fay and Furman, 1982).

Although *Halarachne* sp. parasites are often called “nasal mites”, mites in about a quarter of the otters were found in tracheae, bronchi, and lungs. Mites have also been recovered during tracheobronchial wash in live animals (Mullen and O'Connor, 2002). It is conceivable but unlikely that this lower respiratory invasion occurred post-mortem because many carcasses were examined fresh, others had been stored in the cold, which impeded mite movement, lesions such as scarring and hemorrhage would be inconsistent with post-mortem invasion, and many of the lower tract mites were adults which are typically sessile. Invading nasal mites could compromise the integrity of the upper and lower respiratory tract by damaging the surface epithelium, possibly increasing susceptibility to other respiratory pathogens (Seguel et al., 2018). In addition, these parasites could act as mechanical vectors to transport bacterial or viral pathogens from the nares or nasopharynx to the lower respiratory tract (Seguel et al., 2018; Pesapane et al., 2018 in review). Finally, the chronic mixed inflammatory response that appears to accompany these infections has the potential to augment tissue damage.

Both larvae and adults were present concurrently in the majority of mite-infested sea otters. The rostral nose and turbinates were the most common sites of parasitism, followed by the nasopharynx, and then the trachea or bronchi. Because motile larvae are the infectious stage, and larvae preferentially colonize the rostral nose and nasal turbinates (Fay and Furman, 1982; Mullen and O'Connor, 2002), future studies could refine estimates of the chronicity of infection by characterizing the parasite's life cycle in sea otters, as well as the life stages, parasite distribution and parasite intensity in infected hosts.

Although mites may also be present in the smaller airways and alveoli, due to their small size they could be easily overlooked during necropsy. Mite-infested sea otters with multifocal tiny (1–3 mm diameter), non-raised white pleural spots have been noted during gross necropsy, but causal associations remain unproven. These spots could represent areas of current or prior mite infestation of the sub-pleural pulmonary parenchyma. Tiny pleural scars have also been described from primates with nasopulmonary mite infestation (Kim, 1978).

In the current study, sea otters with a recent history of captive care, those with upper respiratory tract inflammation noted at necropsy, or animals that stranded near Elkhorn Slough, California were all at significantly higher risk of nasopulmonary mite infestation. With respect to higher parasite intensity, sea otters with moderate or heavy mite intensities were significantly more likely to have a history of captivity within 10 days of death, to be female, and had upper respiratory inflammation reported at necropsy.

Although mite-infested sea otters were 2.7 times more likely to have stranded near a harbor seal haulout in univariate analyses, this factor was not significant in the most parsimonious multivariate model. The portion of the southern sea otter range with the highest and most consistent level of interaction between sea otters and harbor seals is Elkhorn Slough. Elkhorn Slough is a large brackish estuary located near the middle of the Monterey Bay coastline in central California. This slough is protected from large waves and storms, and provides important resting habitat for both sea otters and harbor seals. Elkhorn Slough often contains large numbers of sea otters (USGS annual sea otter surveys) that rest in close proximity to each other, and to sympatric harbor seals. Harbor seals use this same area extensively for resting and pupping, and close physical contact between harbor seals and sea otters is common. Harbor seals have been proposed as a source of nasal mite infestation in sea otters, with shared haul out sites being a site of possible interspecific parasite transmission (Kenyon et al., 1965; Fay and Furman, 1982).

In the current study, otters that stranded in close proximity to harbor seals were more likely to be parasitized by nasal mites on univariate analyses. However, when Elkhorn Slough was incorporated into the multivariate model, the added effect of harbor seals did not significantly increase model fit. This could mean that harbor seal contact poses a lower mite-infestation risk for sea otters than other assessed factors. It could also mean that this risk was accounted for when a high-risk location for harbor seal/sea otter contact (Elkhorn Slough) was incorporated into the model.

As an added complication, Elkhorn Slough also contains a comparatively large population of rehabilitated sea otters with a past history of captive care, and a history of captive care within 10 days of death remained significant as a risk factor in the final multivariate model. Being in captivity is stressful and can be associated with high animal density, and sometimes, multiple species using a shared environment. Preliminary data from a recent study (Pesapane et al., 2018 in review), and studies of captive primates, rodents, birds and other animals (Wallis and Lee, 1999; Forbes, 2008; Mantovani et al., 2018) suggest that the infectious stages of some parasitic mites can survive in captive facilities for extended periods in the absence of potential hosts. If confirmed through subsequent studies, this finding has important implications for captive care and translocation of sea otters and other marine mammals.

Given the relative abundance of food, protection from large waves, and ease of post-release monitoring that this site provides, Elkhorn Slough is a common site for release of rehabilitated sea otters by wildlife care programs. The close proximity of numerous sea otters in this small area could facilitate physical contact, and catalyze aggressive or reproductive activity involving facial contact that could facilitate mite transmission. Presence of fresh nose wounds at necropsy was a significant risk factor for nasopulmonary acariasis in univariate analyses, but not the final multivariate model. Studies employing a larger sample size and different sampling strategies could help clarify the relative importance of captive care and harbor seal contact as risks for nasopulmonary mite infection in southern sea otters.

Older age was one of the most important predictors of nasal mite presence. This could represent higher risk due to differences in behavior for older animals, or accumulated risk of infestation over a long lifespan. Sick or older males may be likely to be recipients of significant intraspecific aggression by other male sea otters (unpub. necropsy observations, Miller). Older otters also commonly have one or more comorbidities that could influence immune competence, such as dental and cardiac disease. Finally, older animals may be less fit to compete for preferred food resources. Subdominant otters might gather together into groups or rafts, could have nutritional deficiencies that could impair resistance to mite infestation, or develop other unknown risk factors for infestation with age. For other marine mammals, young animals appeared at greater risk: grey seal pups were infested after weaning (Baker et al., 1998), although (Fay and Furman, 1982) found no associations between mite infestation and host age in various species of pinnipeds.

Otters with nasopulmonary acariasis were 14.2 times more likely to have upper respiratory inflammation at necropsy than un-infested otters. Although both upper and lower respiratory tract conditions were significantly associated with mite infestation in univariable analysis (Table 4), the most parsimonious multivariable model only retained upper respiratory tract conditions. This should not imply that lower respiratory disease is not associated with mites. Rather, upper and lower tract disease are likely correlated, and inclusion of lower tract disease in the model did not significantly improve model explanatory power to warrant its inclusion. For individual cases, we were confident that nasopulmonary mite infection was associated with concurrent bacterial nasopharyngitis, tracheobronchitis, pneumonia, and potentially, emphysema, bullae and pleural scars. Postmortem lesions associated with mite infestation ranged from mild to severe, and included bacterial septicemia, catarrhal exudate, nasal discharge, rhinitis, turbinate lysis, nasopharyngitis, tracheobronchitis, pulmonary emphysema and bullae, pulmonary edema, pneumonia, pulmonary abscesses, and pleural scars. However, these associations were not significant across the larger sample set in this retrospective study. Studies employing a prospective lesion scoring system, and a larger sample size could improve our collective understanding of lower respiratory comorbidities. This study likely underestimated the frequency of turbinate lysis and other turbinate lesions in necropsied sea otters, because this site is rarely examined during gross necropsy.

5. Conclusion

Our study has confirmed associations of sea otter morbidity with nasopulmonary acariasis. Sea otters with necropsy-confirmed nasopulmonary acariasis were 14.2 times more likely to be diagnosed with upper respiratory inflammation at necropsy than non-infested otters. While not statistically significant across the entire sample population, individual cases were also identified with mite-associated pathology, including nasal turbinate erosion, catarrhal tracheobronchitis, and concurrent upper and lower respiratory bacterial infections. We also identified significant predisposing factors for nasopulmonary mite infestation, including older sea otter age, recent history of captive care, and being in a high-risk location along the California coast. Otters with moderate or heavy mite intensities were significantly more likely to have a history of recent captivity, to be female, and have upper respiratory inflammation reported at necropsy. Our research suggests that nasopulmonary mite infestations can cause substantial respiratory pathology in sea otters. Our findings also support hypotheses regarding the potential for captive care and phocid contact to facilitate sea otter infection with nasopulmonary mites. Future work should consider the contribution of acariasis in concert with other causes of morbidity and mortality as negative impacts on otters, and examine additional features of otter acariasis such as seasonality and cross-species transfer. Findings from this study highlight the importance of controlling nasal mite infestations as part of captive care and rehabilitation, and can also inform oil spill response and animal translocation practices.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2019.03.009.

6. Declarations of interest

None.

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