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Population Density of the Northern Sea Otter (*Enhydra lutris kenyoni*) as an Indicator of Strep Syndrome Transmission Mechanism

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Abstract

Population Density of the Northern Sea Otter (*Enhydra lutris kenyoni*) as an Indicator of Strep Syndrome Transmission Mechanism

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The Northern sea otter (*Enhydra lutris kenyoni*) in Alaska, U.S. has recently experienced unusual mortality events due to Strep syndrome caused primarily by *Streptococcus infantarius, Streptococcus bovis,* and *Streptococcus phocae*. The pathogenicity and transmission method of this disease is currently unknown, making it difficult to predict the disease's prevalence and the risk it poses to the species. Preliminary evidence from higher-density populations that have experienced significant mortality suggest that population density may affect transmission. To test this hypothesis, we acquired stranding data from the U.S. Fish and Wildlife Service (FWS), partitioned the population into discrete ranges using QGIS, and calculated the percentage of infected individuals in these areas from 2001 to 2014. Using abundance data from USFWS and the United States Geological Survey (USGS), among other sources, we calculated population density for these regions over time. We then tested the relationship between infection and population density using generalized linear models and generalized linear mixed models. Additional variables considered were infected individuals' age, sex, and body condition. These variables were included to clarify which individuals may be at greatest risk for infection as demographic details have been inconsistent in previous literature. Additionally, we determined the primary physical locations for bacterial infection. Previous literature has noted a high percentage of bacteria in the intestines and we sought to confirm this finding to elucidate the physical characteristics of infection. Results indicate that population density does not significantly influence Strep prevalence, but that males are at greater risk for the disease. These results suggest that Strep may be transmitted in a frequency-dependent manner and influenced by behavior disparities between the sexes. Additionally, the relative frequency at which certain tissues, such as the spleen, test positively for streptococci bacteria contradicts previous literature regarding bacterial presence in the intestines. Therefore, the survival and transport of streptococci bacteria by macrophages requires additional research, as does the proposal of infection via the intestines. Overall, these findings can be used to guide federal agencies in research prioritization and management.

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Introduction

The effects of climate change are far-reaching with drastic consequences for ecosystems and their respective species. As both climate change and anthropogenic factors fragment habitats, shift resource availability, influence intraspecific interactions, and impact innumerable other factors of environmental health, the processes by which organisms can be exposed to disease are altered (Burek, Gulland, & Ohara, 2008). The results of these shifts are apparent in the increasing prevalence of disease-caused mortality for marine mammals in association with warming oceanic and atmospheric temperatures (Kuiken et al., 2006; Harvell et al., 1999; Acevedo-Whitehouse & Duffus, 2009).

Environmental shifts in response to climate change have been particularly extreme and rapid in the polar regions (Hueffer, Parkinson, Gerlach, & Berner, 2013). As these shifts occur, the health of ecosystems' keystone species becomes increasingly important to environmental management. A keystone species is one which disproportionately affects an ecosystem's composition relative to its composition (Paine, 1969). Even a relatively small decline in a keystone species' abundance can have cascading effects on a community's structure and diversity. Thus, as climate change affects environmental processes, it is critical to prioritize the monitoring of these species to track ecosystem health and stability effectively (Harwood, 2001). One such keystone species is the Northern sea otter (*Enhydra lutris kenyoni*) – a keystone predator in the kelp forests of Alaska, United States (Estes & Palmisano, 1974; Estes & Duggins, 1995). Loss of this species results in a trophic cascade in which sea urchins (*Echinoidea*) proliferate and kelp (*Laminariales*) experiences elevated levels of herbivory (Estes & Palmisano, 1974; Paine, 1980).

Strep syndrome has become a research priority due to its notable impact on sea otter health. Caused by *Streptococcus phocae, Streptococcus bovis,* and *Streptococcus infantarius* ss *coli*, a subspecies of the *Streptococcus bovis-equinus* complex (SBEC), this disease has debilitating effects, often resulting in meningoencephalitis, pneumonia, septicemia, endocarditis, and opportunistic wound infection (Rouse, 2018; Imai, Jang, Miller & Conrad, 2009; Barlett et al., 2016; Murray, 2015). Strep syndrome has increased in prevalence since the early 2000s with great consequences for sea otters (Carrasco et al., 2014), including producing an unusual mortality event (UME) for Northern sea otters as determined by the U.S. Fish and Wildlife Service (USFWS) and the National Oceanic and Atmospheric Administration (NOAA) (Gill, 2006). The Marine Mammal Protection Act (MMPA) defines an UME as a "stranding that is unexpected; involves a significant die-off of any marine mammal population; and demands immediate response" (Marine Mammal Protection Act, U.S.C. § 1421h). Since the establishment of the UME program in 1991, only three UMEs have been declared for sea otters (Murray, 2015). The most recent event, which began in 2002 and was declared an UME in 2006, affected the Southwestern sea otter stock and a portion of Southcentral stock (Gill, 2006). Sea otters become stranded for numerous reasons, including disease, starvation, and various forms of trauma. In the four years of this UME, 43% of necropsied animals were determined to have died from Strep syndrome upon physical examination and bacteriological testing (Gill, 2006). A Strep syndrome diagnosis is carried out by initial observation at the stranding site, gross necropsy, histopathology, and confirmed when streptococci bacteria are successfully isolated from the individual. Unfortunately, although observational patterns have been established, much remains unknown about the bacteria's pathogenicity and the factors that enabled Strep syndrome to cause this UME.

While the disease's pathogenicity is still undetermined, recent studies have begun to describe the infection trends and potential transmission sources. One such study isolated *S. infantarius* ss *coli* from mussels, which is a major component of sea otter diets, indicating that the bacteria could be acquired from environmental sources, such as prey or contaminated water (Counihan-Edgar et al., 2012). SBEC bacteria are commensal to the gastrointestinal tract in many mammalian species and a streptococci infection may begin in the intestinal epithelium (Counihan et al., 2015), possibly by means of a contaminated food source (Counihan-Edgar et al., 2012). An additional study found a positive correlation between skin trauma and infection prevalence of *S. phocae* for the Southern sea otter (*Enhydra lutris nereis*), indicating that water contamination may also result in infection (Bartlett et al., 2016). However, in addition to the intestines and skin lesions, the streptococci bacteria are frequently isolated from the cardiovascular system and numerous organs ranging from the heart to the brain (Counihan et al., 2015; Rouse, 2018). As a result, some uncertainty remains as to the predominant physiological region of infection and additional analyses are required to characterize the infection process.

The hypothesis of infection via contaminated environmental factors holds promise. However, a possible transmission method not yet fully explored is that of direct transmission from a bacterial carrier to a susceptible individual. Disease transmission by direct contact can be categorized as density-dependent or frequency-dependent. In the former, the rate of contact between individuals or the rate at which the disease is contracted is dependent upon population density.

Airborne, parasitic, and viral diseases are commonly transmitted in a density-dependent manner (Anderson & May, 1979; Anderson & May, 1981; Thrall, Biere & Uyenoyama, 1995). In a frequency-dependent scenario, the rate of contact is not dependent on density, but rather is determined by another characteristic of the disease. Sexually transmitted diseases and vector-borne diseases are common examples of frequency-dependent diseases since transmission is confined to specific encounters (Thrall, Biere & Uyenoyama, 1995). As sea otters experienced near extinction in the early 1900s and mortality-causing events such as the Exxon Valdez oil spill in 1989, population densities have only recently begun to rise. Simultaneously, stranding reports as well as the proportion of incidents involving Strep syndrome have increased (Johnson & Mayer, 2015; Gill, 2006). Thus, it is reasonable to hypothesize that the rate of contact between infected and susceptible individuals has increased as a result of increased population densities, resulting in the proliferation of Strep syndrome-causing bacteria. Investigation of the relationship between population density and Strep syndrome not only offers insight into a possible transmission method, but also provides additional details regarding factors of susceptibility.

A possible role for density in the spread of Strep syndrome is supported by the lack of individuals under the age of one presenting with the disease (Rouse, 2018) who presumably experience less exposure due to limitation of contact. Additionally, preliminary evidence from sites affected by the 2006 UME, such as Kachemak Bay, indicates significant levels of mortality associated with elevated population densities (Verena Gill, personal communication, May 2018). It could be argued that an elevated population density increases disease prevalence by additional intraspecific competition for resources. Sea otters have particularly high metabolic rates and thus any caloric deficit can quickly lead to weight loss and issues regarding energetic demand, possibly resulting in increased disease susceptibility (Murray, 2015). However, this hypothesis is made less likely given information presented in the 2006 UME report (Gill, 2006), which noted that the sex ratio of Kachemak Bay, one of the primary locations in which Strep syndrome was detected in the UME, was inconsistent with the ratio expected of a population at its carrying capacity. This observation indicates that competition for resources is not likely to account for the observed increase in disease prevalence.

To investigate whether population density is associated with increased prevalence of Strep in stranded otters, we used the bacteriology data of the USFWS stranding database and gathered sea otter abundances from USFWS, the United States Geological Survey (USGS), the National Park Service (NPS), and other individual researchers. These abundance data were used to estimate the population densities of eight sea otter populations from which stranded individuals had tested positively for the streptococci bacteria. We hypothesized that those regions with elevated population densities would demonstrate a greater infection prevalence than those with low densities, indicating that direct contact may cause disease transmission.

Additional characteristics of infection explored include the demographics of those otters at greatest risk of infection. Some studies have found no significant association between age class, sex ratio, or nutritional state and infection (Carrasco et al., 2014; Barlett et al., 2016). However, Bartlett et al. (2016) found that immature individuals experience greater risk of infection. Additionally, chronic cases of Strep syndrome have been linked to poor body condition, while acute cases often present with no deterioration in nutritional status (Verena Gill, personal communication, March 2019). Due to the variety of descriptors reported to be associated with infection, we sought to characterize the physical location of infection as well as the sex, age class, and nutritional status of infected individuals. By providing additional information, we can further elucidate the demographics of infected individuals and provide additional insights into the effects of infection. Providing federal agencies with a clearer understanding of those populations at greatest risk for infection, as indicated by infection characteristics or population density, offers environmental managers the information necessary to prioritize management efforts. This informed prioritization would result in more efficient resource use and help protect this endangered species from further mortality.

Methods

Database

The database used was developed by the U.S. Fish and Wildlife Service (USFWS) and includes stranding data from 1992 to 2014. Data were most consistently available beginning in the early 2000s, so data from the years 2001 to 2014 are included in the study. The USFWS initiated a response for all reports of stranded sea otters, dead or live, in the southern region of Alaska ranging from Craig to Agattu Island. Every carcass underwent an examination by veterinarians with expertise in marine mammal necropsy or pathology (Gill, 2006). Most otters included in the database were reported by stranding networks throughout Alaska. In addition to these reports, further samples were collected from hunted sea otters. While the MMPA prohibits the harvest of marine mammals, exceptions were made for Alaskan Natives who may, in a non-wasteful manner, hunt otters for the purpose of subsistence and the production of authentic handicrafts (Marine Mammal Protection Act U.S.C. § 1539). All harvested individuals must be tagged and recorded with the USFWS Marking, Tagging, and Reporting Program (MTRP) and it was through this program that hunted otters were incorporated into the database.

Most collected animals were deceased. If an otter was not yet completely decomposed, samples were collected for bacteriology, histopathology, microbiology, and serology testing and physical characteristics were noted. Samples were taken according to a checklist and all sites were sampled if possible (Gill, 2006). Those animals that were still living upon collection were transported to the Alaska Sealife Center (ASLC) for treatment, but none had survived as of 2006 (Gill, 2006). As a result, the possibility of re-stranding is minimal.

The USFWS noted the primary cause of death within the database as well as contributing factors. This system of primary and contributory factors of death was designed to capture the details of the fatality. For example, a sea otter struck by a boat would be logged with trauma as the primary cause of death. However, a strep positive sea otter killed by boat strike would be cataloged with trauma as the primary cause of death and Strep syndrome as a contributing factor as the otter's behavior may have been modified by the infection in such a way as to make a boat strike more likely. If strep was noted in either the primary or subcategories, the disease was noted as having contributed to death.

According to the dataset, otters were collected from a total of 41 sites. Stranding frequency varied widely and many sites reported only a few strandings across the 2001 to 2014 time period. We therefore focused our initial site selection on sites that reported eight or more strandings to support a robust statistical analysis of Strep syndrome as a function of population density (Kain, Bolker, & McCoy, 2015; UCLA: Statistical Consulting Group, n.d.; Austin & Steyerberg, 2015).

The 14 locations with eight or more reported strandings were plotted using QGIS to assess their spatial relationships and determine if sites were spatially independent (Figure 1). Otters were assumed to be capable of dispersing between 3.25 km and 21.25 km from their primary regions. These values were reached by averaging reported minimum and maximum dispersal distances (Bodkin et al., 2002; Alaska Department of Fish and Game, n.d.; U.S. Fish and Wildlife, 2014). The coordinates of the database locations were plotted with the minimum and maximum dispersal distances represented by circles with radii of 3.25 km and 21.25 km, respectively. No locations showed overlap with the minimum dispersal distance. However, three locations demonstrated overlap when plotted with maximum dispersal. These locations were Kachemak Bay, Homer, and Seldovia, which were grouped as a result of this overlap. The decision to collapse these sites was further supported by the fact that USFWS had recommended these locations be treated as one, given their close proximity to each other (Verena Gill, personal communication, July 2018).

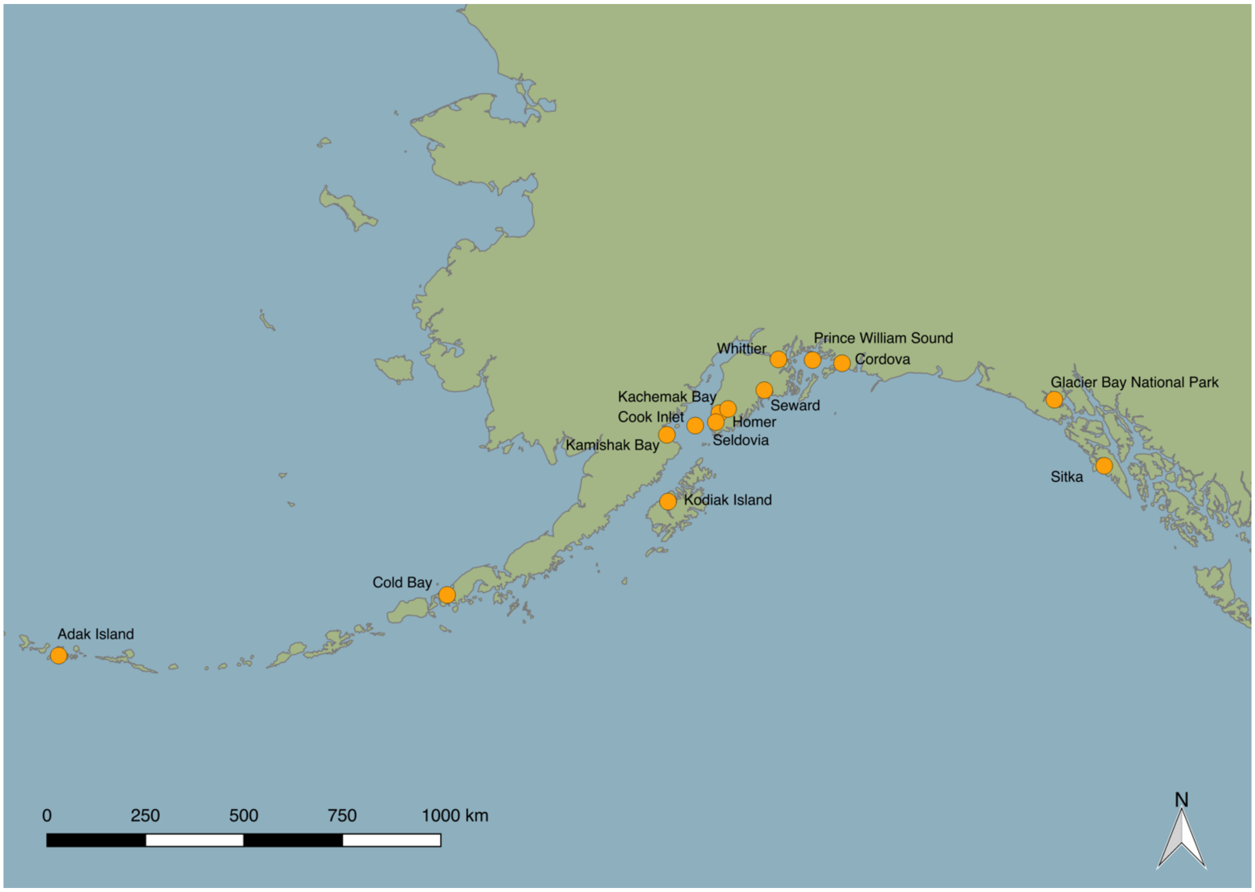

Figure 1. Map of Alaska, United States with the original 14 locations plotted with QGIS.

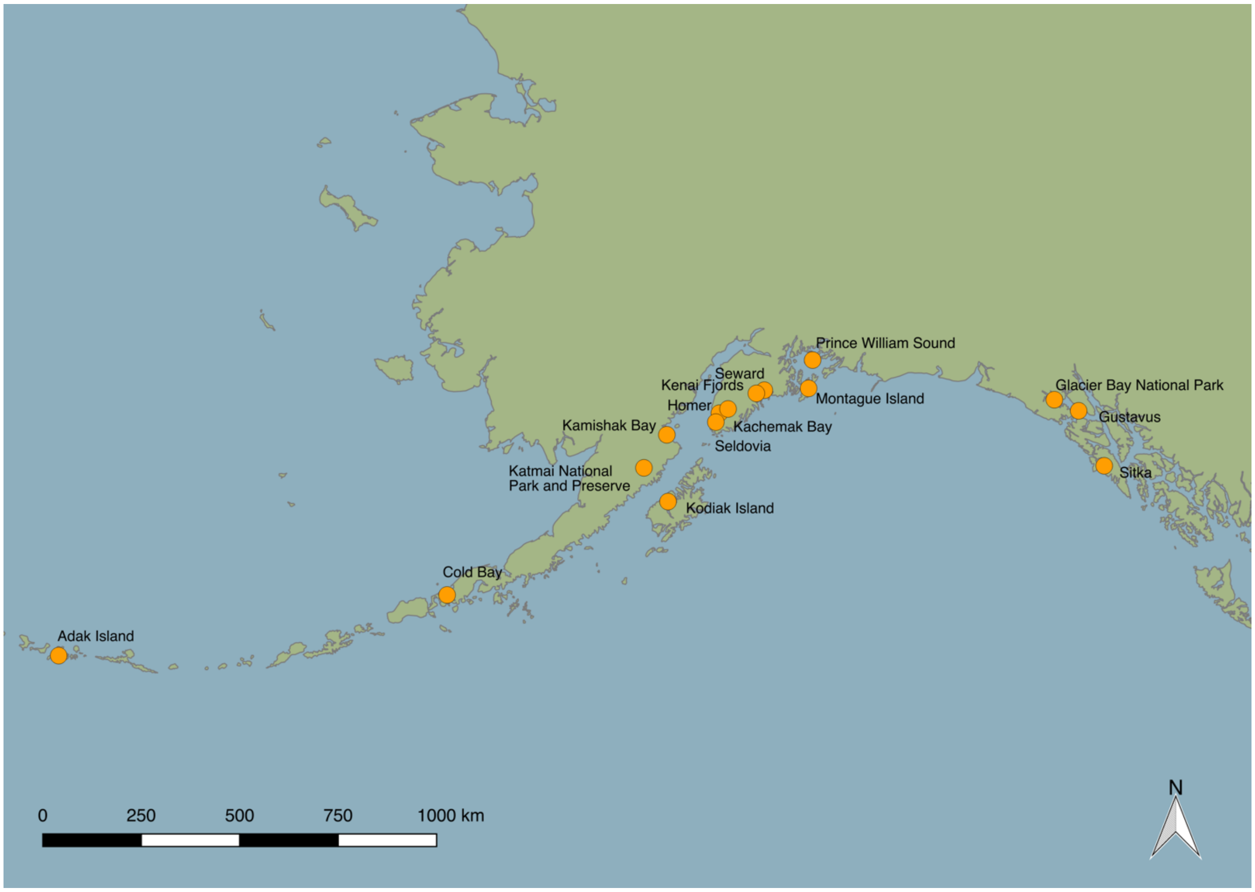

Figure 2. Map of Alaska, United States with the adjusted locations according to spatial relationships and survey areas plotted with QGIS.

Strep as a Function of Density: Defining population densities

Estimations of sea otter abundances were used to calculate population density (otters/km^2) as these data were not included in the database. Abundance values were sourced from surveys conducted by USFWS, the U.S. Geological Survey (USGS), the National Park Service (NPS), and a variety of other researchers (Table A14). Additional sites in the database were grouped with the original 14 locations if they were included in the same abundance survey areas or were in such close proximity (<42 km distance) that they could provide additional information for those locations lacking abundance data. Newly incorporated locations included Montague Island for W. Prince William Sound, Katmai National Park and Preserve for Kodiak Island, and Kenai Fjords National Park for Seward and Gustavus for Sitka (Figure 2).

Most surveyors followed the methods of Bodkin and Udevitz (1999), who used aerial strip transects outlined within a certain shore distance determined by bathymetric data (up to a depth of 40m). Some surveys reported population densities alongside or in lieu of abundance data (Table A11). These densities were used when provided. If multiple density values were reported for a site in a given year, then those densities were averaged. The final compilation of calculated and reported population densities is shown in Table A13.

Overall, those locations considered for inclusion but which were lacking in both abundance data and proximate surveyed areas were excluded from the study as no density approximation could be made. Furthermore, as one of the aims of this study is to analyze Strep syndrome in relation to shifts in population density, it was important to have numerous years of stranding data as well as population densities. To maximize our confidence in the population density trends for each site, those sites with fewer than four years of abundance data available were excluded from analyses which relied on density as a variable. While five years of data would typically be the suggested minimum for our analyses (generalized linear mixed effects model in particular) (NCSS, n.d.), the cutoff was extended to four samples to include a site which had extensive stranding data.

A total of eight sites had a sufficient number of strandings for which strep was tested as well as sufficient population density values (calculated or reported) for the analysis of density as a predictor of Strep. QGIS was used to calculate the population densities using reported sea otter abundances in each of these sites. The survey areas pictured or described in the abundance survey methods were traced and their areas calculated (Figure 3, Figure A7). Then, the sea otter abundances were divided by the surface area of the surveyed region to provide a density (number of sea otters/km^2). While sea otters do occupy a three-dimensional space and often dive for food, all but one survey used correction factors in the abundance value approximation process, which accounted not only for differences between survey methods (such as elevation of the plane used for data collection), but also for unobserved sea otters below the surface. Thus, use of km^2 was deemed to be a reasonable unit of measure for population density. For locations for which densities were provided by agencies or in the literature (Table A11), we recalculated the densities using our own values to ensure that the surface area found in QGIS accurately reflected that which was actually surveyed (Table A11, Table A12).

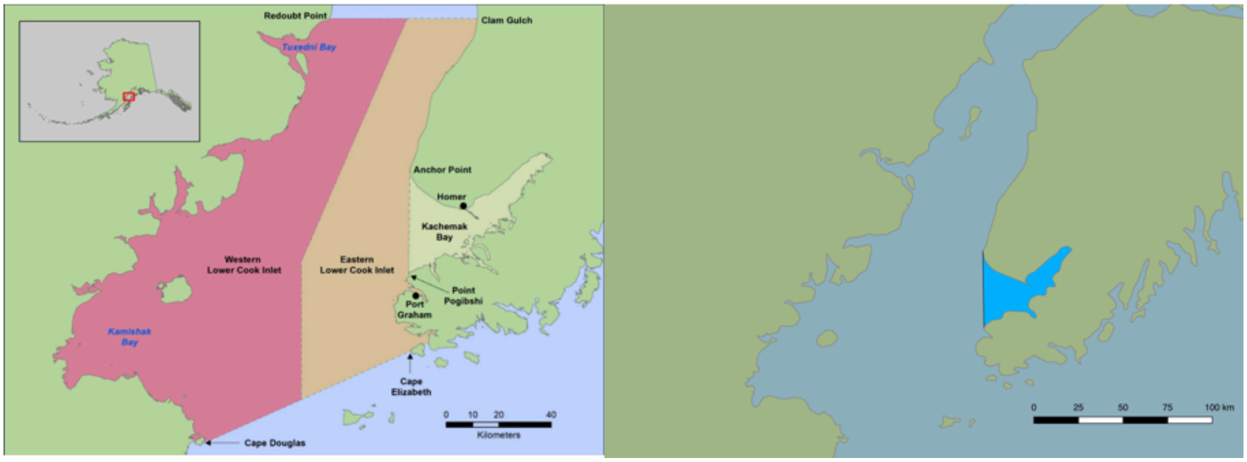

Figure 3. (Left) Figure reprinted from Garlich-Miller et al., (2018). (Right) Survey area of Kachemak Bay outlined in QGIS to mimic image provided by surveyor.

Strep as a Function of Density: Kachemak Bay

One of the primary goals of this study was to ascertain whether sea otter population density is correlated with Strep syndrome prevalence. Kachemak Bay was the only location for which a within-site analysis was run as no other site had sufficient data ($N \geq 20$) across multiple years needed for a robust within-site analysis (Austin & Steyerberg, 2015).

Because population density estimates for Kachemak Bay were only available for four years, but Strep-related data were available for thirteen, we explored whether a density approximation method could be used to include these additional data points in the analysis. An initial approach was to use stranding frequency as a proxy for population density. However, the regression of density versus stranding frequency yielded a poor r-squared value ($r^2 = 0.016$). Subsequently, a linear regression was performed with year as the independent variable and density as the dependent variable, which was deemed appropriate given the tight fit between year and density ($R^2_{adjusted} = 0.89$). This approximation method was used to impute population densities for ten additional years for this site, and these densities were used in the linear model for Kachemak Bay. In this analysis, the Strep prevalence data were logit-transformed and the relationship between prevalence and population density tested in a linear regression. This model was performed using the statistical software R.

In addition to these analyses addressing the relationship between population density and Strep bacteria prevalence, it was also important to assess population density as a factor in Strep acting as a contributing factor to death. Population density values were imputed and data regarding Strep as a factor of death logit-transformed for a linear model to analyze the relationship.

Strep as a Function of Density: Across-Site Analysis

Because the sites included in the study were often limited in the number of years in which population densities were available, a density approximation method was used for the across-site analyses. Those locations with fewer than 20 strandings tested for bacteria were included in the model with an average population density across all years (2001 – 2014), with the assumption that population density does not affect Strep syndrome prevalence over time within sites. The only grouped site with more than 20 strandings (other than Kachemak Bay) was the location encompassing Kodiak Island and Katmai National Park and Preserve. Initially, a linear regression was used to approximate the missing densities for this location, but the r-squared value fell below an acceptable threshold ($R^2 = 0.035$). Thus, the location's reported densities were pooled and averaged for each group (Figure A6a). The first group covered years 2001 to 2007 and the average density was attained from three years of density data. The second group covered years 2008 to 2014 and the average was attained from two years of density data. This approach was utilized in order to provide additional detail regarding density for those locations, while still maintaining an appropriate sample size.

A similar approach was taken for the inclusion of Kachemak Bay in this analysis. The data collected from this site were grouped to maintain a minimum sample size of approximately 20 bacteriologically tested individuals for each density value. The population densities reported for the years included in each group were averaged and it was the resulting eight groups with their corresponding averaged population densities that were included in the GLMM analysis (Figure A6b).

The relationship between Strep prevalence and population density was analyzed across the other seven grouped sites in the database with sufficient stranding data. A generalized linear mixed model (GLMM) with a binomial distribution was run across all sites, with site included as a random effect. This model was performed using the lme4 package in R. A mixed model was used in order to account for pseudoreplication. According to Chaves (2010), most linear models assume absolute independence among the study subjects. However, this assumption is not appropriate for stranding data because strandings were clustered by site. Use of a linear model with this assumption could increase the model's residual variance (Chaves, 2010). A mixed effect model separates the sources of variance, thus accounting for the pseudoreplication of the data collection method (Chaves, 2010).

However, due to the fact that the majority of stranded sea otters were collected from Kachemak Bay ($N_{All} = 321$, $N_{Kachemak} = 251$), the site could overpower any relationship between population density and Strep prevalence apparent in the other sites. In order to give all included sites approximately equal weight, an additional, more condensed model was used in addition to the GLMM. This additional model was a linear model and for this analysis, Strep prevalence data were logit-transformed and Kachemak Bay's density values were pooled according to early and late years (Figure A6c), so that ultimately it contributed only two data points to the analysis. Those densities available for the years 2001 to 2007 were averaged and became the first group and the densities available for 2008 to 2014 were averaged to become the second group.

Just as it was important to assess the affect of population density on the tendency for Strep syndrome to contribute to sea otter death within the site of Kachemak Bay, it was important to do the same in an across-site analysis. A GLMM with a binomial distribution and grouped site as a random effect was run as well as a linear model with logit-transformed cause of death data and pooled population density values. The grouping strategies described above were similarly used in these analyses.

Infection Demographic

An additional aim of this study was to better define those demographics at greatest risk for Strep syndrome. The sites grouped according to abundance survey methods and spatial relationships were included in this analysis (N = 10). No exclusion was made based upon population density data availability as population density was not a factor in our characterization of infection. Within grouped sites, only those individuals that underwent bacteriological testing were included in this study so as to be certain of individuals' infection status. As stated earlier, all otters were tested for bacteria when not completely decomposed.

The characteristics of those individuals infected with Strep were analyzed with a GLMM with grouped site as a random variable, using an Akaike information criterion (AIC) comparison for model selection. In the database, age class had five levels, which were fetus, pup, subadult, adult, and aged adult. Likewise, nutritional state had four levels, including emaciated, thin, normal, and fat. For both of these variables, some categories were grouped as the number of individuals sampled varied greatly between levels, especially for age class (Table 1). Age class levels were collapsed to binary categories: juvenile (fetus, pup, subadult) and adult (adult and aged adult).

Nutritional state was collapsed to poor (emaciated, thin) and good (normal, fat). The original full model included Strep as a binomial response variable, age class, sex, and nutritional state all as interacting, explanatory variables. Non-significant interaction terms were removed if this action improved the AIC score by two points. Only the output for the reduced model was reported.

Table 1. The categories of descriptor information collected for stranded sea otters tested that underwent bacteriological testing. In age class, fetus, pup, and subadult comprise the juvenile category and adult and aged adult comprise the adult category. For nutrition status, emaciated and thin categorizations constitute a poor nutrition state and normal and fat categorizations constitute a good nutrition state.

Sex	**Count**	**Age Class**	**Count**	**Nutrition Status**	**Count**
Female	92	Fetus	1	Emaciated	81
Male	227	Pup	64	Thin	93
		Subadult	68	Normal	94
		Adult	98	Fat	40
		Aged Adult	88		

Physical Location of Streptococci Infection

In addition to the characteristics of the infected population, we also calculated the prevalence of streptococci's physical location across different sampled tissues and organs based on the description of sample sites used for bacteriological testing. Data from all sites were included in this analysis, regardless of population density data availability. The proportion of Strep syndrome-causing bacteria detected in sampled tissues was assessed qualitatively using a proportion table generated in R. Only those tissues sampled from a minimum of ten individuals were included so as to maintain a reasonable sample size for each tissue, although there is a wide margin of error associated with this sample size. Understanding the physical distribution of the bacteria is important to our understanding of the disease and future analyses of these data might include establishing if infection location is a predictor of Strep syndrome contributing to death.

Results

Population Density and Strep Prevalence

Within the site of Kachemak Bay, no significant relationship was detected between population density and Strep prevalence (p=0.10; Table 2). This model was performed with the imputed density values from a linear regression. This conclusion can also be observed graphically (Figure 4), which appears to show a negative linear relationship between strep and population density, but demonstrates a great deal of variance ($R^2 = 0.10$).

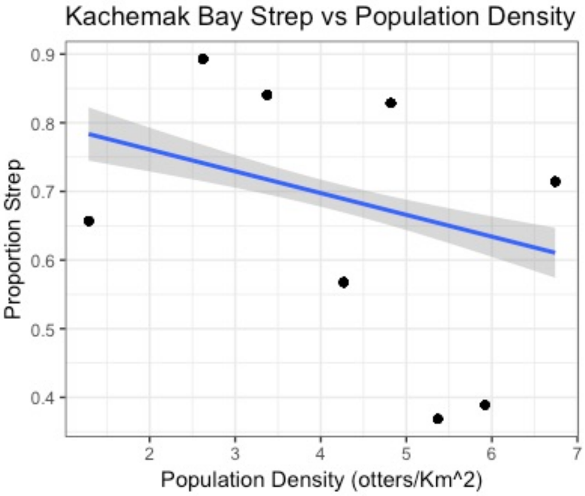

Figure 4. Strep prevalence versus the imputed population density values of Kachemak Bay.

Table 2. Linear model output for Kachemak Bay, assessing population density as a factor in streptococci bacteria prevalence with imputed density values as determined by linear regression.

Variable	**Estimate**	**Std. Error**	**t value**	**p value**	**F-statistic**	**DF**	R^2_{adj}
Intercept	2.41	0.77	3.13	0.011			
Density	-0.32	0.17	-1.81	0.10	3.28	10	0.10

It is important to note the distinction between having Strep syndrome-causing bacteria and having the disease. The relationship between Strep contributing to death and population density also demonstrated a lack of significance with the linear model (Table 3).

Table 3. Kachemak Bay linear model output for the association between population density and Strep as a factor of death using imputed population densities as determined by linear regression.

Variable	**Estimate**	**Std. Error**	**z value**	**p value**	**F-statistic**	**DF**	R^2_{adj}
Intercept	-0.56	2.63	-0.21	0.84			
Density	-1.20	0.59	-2.03	0.07	0.0062	10	0.22

The findings for Kachemak Bay were consistent with those for the across-site analyses. Strep prevalence in relation to population density as assessed via a GLMM demonstrated a lack of significance (Table 4). Additionally, Figure 5 shows a similar trend and level of variance to that observed in Figure 4 ($R^2 = 0.067$).

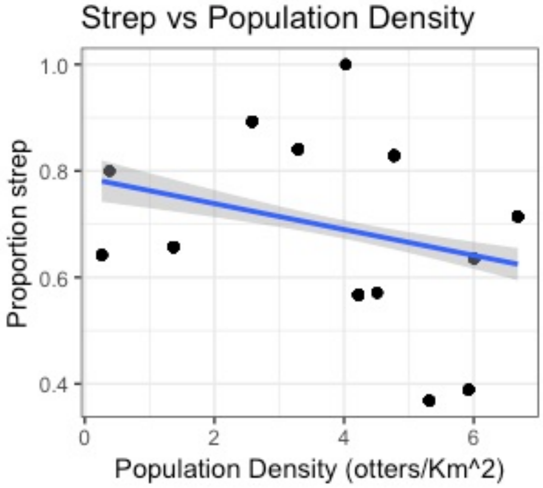

Figure 5. Strep prevalence as a function of approximated population density across all sites. Eight data points originated from Kachemak Bay and two data points originated from Katmai National Park and Preserve and Kodiak Island. See Table 4 for additional information.

When the data were logit-transformed and run in a linear regression for all sites, the relationship between population density and Strep prevalence was similarly non-significant (Table 5). Note that this model treats samples as if they are completely independent, which they are not, making this particular model vulnerable to error.

Finally, the results found in Kachemak Bay regarding the relationship between population density and Strep as a factor of death were supported by the across-site analyses (GLMM and linear), which indicated a non-significant relationship (Table 6, Table 7).

Table 4. Output from across site GLMM, including Kachemak Bay, testing the impact of population density on Strep prevalence, with site and year of collection as random effects.

Variable	**Estimate**	**Std. Error**	**z value**	**p value**	$R^2_{GLMM}(m)$	$R^2_{GLMM}(c)$
Intercept	0.53	0.37	1.43	0.15		
Density	0.04	0.07	0.57	0.57	0.0015	0.105
		Variance	**Var. Std. Dev.**			
Site (random)		3.55e-10	1.88e-05			
Year (random)		0.51	0.71			

Table 5. Output from across site linear model assessing the relationship between population density and bacteria prevalence, with densities for the sites Kodiak Island and Kachemak Bay pooled into early and late years.

Variable	**Estimate**	**Std. Error**	**t value**	**p value**	**F-statistic**	**DF**	R^2_{adj}
Intercept	0.64	0.97	0.67	0.51			
Density	-0.11	0.26	-0.45	0.66	0.198	50	0.229

Table 6. Results of the across-site GLMM with Strep as a factor of death as a response variable, population density as a fixed effect, and year as a random effect.

Variable	**Estimate**	**Std. Error**	**z value**	**p value**	$R^2_{\text{GLMM}}(m)$	$R^2_{\text{GLMM}}(c)$
Intercept	-2.81	1.19	-42.35	0.019		
Density	-0.013	0.25	-0.05	0.96	3.25e-05	0.033

		Variance	**Var. Std. Dev.**
Year (random)		0.63	0.79

Table 7. Output from the across-site linear model assessing Strep as a factor of death in relation to population density.

Variable	**Estimate**	**Std. Error**	**t value**	**p value**	**F-statistic**	**DF**	R^2_{adj}
Intercept	0.042	0.036	1.17	0.247			
Density	0.005	0.009	0.48	0.63	0.23	50	-0.015

Demographic Predictors of Infection

In addition to analyzing Strep prevalence in relation to population density across sites, infection characteristics, sex, age class, and nutritional state also were assessed for infected individuals using a GLMM. The best model (as determined by AIC comparison, (Table 8)), assessed the effect of age class, sex, and nutritional state on bacterial prevalence without interaction. This model was the simplest model with an AIC value approximately 1.99 scores below that of the next simplest model. Due to the closeness of the AIC difference to the 2-score-difference cutoff and the fact that the other interaction terms were not significant, the age class, sex, and nutritional state were included in the model without interaction. There was no significant relationship detected between these characteristics and Strep prevalence, although a p-value of 0.055 was reported for the male sex (Table 9) indicating that males moderately trended toward a high prevalence of Strep bacteria than females.

Table 8. Model selection for predictors of infection according to AIC values. Age class levels include juvenile, sex levels include male and female, and nutritional status includes poor and good.

Parameters	$R^2_{GLMM}(m)$	$R^2_{GLMM}(c)$	**AIC**	**ΔAIC**
Age + Sex + Nutr. State + Site (random)	**0.79**	**0.79**	**383.02**	**0.0**
Age * Sex + Nutr. State + Site (random)	0.56	0.56	385.01	1.99
Age * Sex * Nutr. State + Site (random)	0.39	0.39	385.26	2.24

Table 9. Results of GLMM for age class, sex, and nutritional state. Age class juvenile includes fetuses, pups, and subadults, covering ages zero to three. The age class adult level includes adults and aged adults, covering ages four to ten-plus. A poor nutritional state includes emaciated (muscle wasting) and thin (no subcutaneous fat) individuals, compared to a good nutritional state which includes normal (some subcutaneous fat) and fat (excessive subcutaneous fat) individuals.

Variable	**Estimate**	**Std. Error**	**z value**	**p value**
Age Class Juv.	-0.419	0.2535	-1.656	0.0977
Sex Male	0.5148	0.2683	1.919	0.055
Nutr. State Poor	0.1494	0.2539	0.589	0.5561
Site (random)	**Variance**	**Var. Std. Dev**		
	3.05e-15	5.524e-08		

The frequency at which sampled tissues were positive for either *S. infantarius* ss *coli*, *S. phocae*, *S. bovis*, or *S. bovis/equinus* complex is shown in Table 10. The spleen tested positive with the greatest frequency (43.47% positive), followed closely by heart valve lesions (42.11% positive). Surprisingly, skin trauma and elements of the gastrointestinal system did not test positively as frequently as might be expected given previous literature. For example, no wounds tested positively for strep and only 13.57% of those tissues sampled from the small intestine were positive for the bacteria. However, the stomach mucosa and tonsils exhibited the bacteria more so than the small intestine with 36.36% and 27.52% positive respectively.

Table 10. The proportion of sampled tissues positive for Strep syndrome causing bacteria.

Site Sampled	N	Strep Present
Abscess	32	0.2187
Bladder	10	0.2000
Blood	78	0.2179
Bone Marrow	26	0.0000
Brain	258	0.2364
Colon	257	0.1206
Feces	101	0.0891
Heart	46	0.3260
Heart Blood	178	0.3202
Heart Valve	115	0.2348
Heart Valve Lesion	152	0.4211
Jejunum	159	0.1258
Large Intestine	14	0.0714
Liver	18	0.2778
Loop of Colon	481	0.1392
Loop of Jejunum	456	0.1425
Lung	22	0.2272
Lymph Node	190	0.2578
Nasopharynx	29	0.0344
Pericardial Fluid	11	0.0000
Peritoneum	24	0.0833
Pharynx	35	0.2285
Prepuce	14	0.0714
Rectum	53	0.1321
Small Intestine	140	0.1357
Spleen	23	0.4347
Stomach Mucosa	33	0.3636
Tonsil	218	0.2752
Urine	18	0.1111
Wound	12	0.0000

Discussion

Population Density as a Factor of Strep Prevalence

The primary goal of this study was to determine the relationship, if any, between Northern sea otter population densities and the prevalence of streptococci bacteria and Strep syndrome. This question was raised given observations made in the wake of an unusual mortality event caused by the disease and given the fact that little is known about the ecological factors associated with its proliferation among sea otters. Research regarding Strep syndrome in other species has focused primarily on the physiological and genetic characteristics of the disease (Hueffer, et al 2011; Bethke & Avendano-Herrera, 2017) and not ecological factors such as population density. Thus, there is a clear need for additional knowledge in this area.

Using Northern sea otter stranding data, we found a lack of significance of population density as a predictor of Strep syndrome prevalence, indicating that direct contact between infected and susceptible individuals is not likely to influence the transmission of this disease. Given the results of tests run within the site of Kachemak Bay as well as across sites, it seems that not only does the bacteria's prevalence not change significantly with increased population density, the proportion of otters killed by the disease remains relatively constant as well.

However, the influence of direct contact on disease transmission cannot be entirely discounted as contact may affect transmission in a frequency-dependent manner. For example, otters will often rest with each other in a formation known as a raft (Feinholz, 1998), meaning that the rate of contact between infected and susceptible individuals may remain constant, even as population densities rise. Thus, the results of this study indicate a need for investigation of the possible behavioral components of transmission, as well as further exploration of food and environmental contamination as possible routes of transmission (Counihan-Edgar et al., 2012; Bartlett et al., 2016).

The primary limiting factor of this study was the lack of abundance data available on an extended time scale. In response to this limitation, multiple assumptions had to be made. For instance, in the across-site analyses, it was assumed that population density does not affect Strep syndrome prevalence over time within each site so that an average population density could be used for the site over the time span of interest. This assumption may have masked population density trends within sites, making it difficult to establish a correlation between population density and Strep prevalence. Given additional abundance data, a more precise analysis may have been possible. In this same analysis, those sites with extensive abundance data had to be grouped to fairly weigh all sites included in the model. This was another way in which detail was lost in regards to population density.

Additionally, the majority of stranded sea otters were collected from Kachemak Bay, meaning this site had great influence in the across-site analyses. Compensation for this uneven distribution of samples was attempted, but the approach used was vulnerable to error, thus making any results from this compensation method questionable.

Overall, the data used in these analyses were sparse. The abundance data, although analyzed as a continuous predictor, were course-grain and data availability over the time period of interest was limited. Additionally, the number of stranded and bacteriologically tested sea otters per location was quite unbalanced. The non-ideal nature of the data required substantial data manipulation, altering the analyses performed and their strength. Thus, while the information presented here is of value, it should be considered with circumspection.

Given the limiting effect population estimate data availability had on this study, the continued collection of abundance and stranding data is strongly encouraged. These data can be used to answer many different questions about sea otter health and thus are an effective approach to better understanding this marine mammal. However, abundance surveys and stranding networks are costly and thus the continued support of the agencies that conduct this work, such as the USFWS, USFS, and the NPS, is essential.

Characteristics of Infection

No significant correlation was found between Strep prevalence and age class or nutritional status, which is inconsistent with some previous studies. Rouse (2018) noted the lack of sea otters under the age of one contracting Strep syndrome as an indication that direct contact between individuals may cause disease transmission. This assertion is logical given that pups would experience limited contact with individuals other than their mothers, thus reducing the risk of disease contraction. While our analyses did not have the resolution of age class down to the year, a disparity in bacterial prevalence between the age classes should have been detected if contact was restricted by age. Thus, these results offer evidence from an additional perspective that direct, density-dependent transmission may not be at play.

Alternatively, sex was marginally significant, indicating males tend to demonstrate a higher level of Strep prevalence. Thus, behavioral differences between the sexes, such as differences in mating behaviors could influence disease prevalence. For example, while females typically only mate with one male (Kruuk, 2006), males will repeatedly interact with multiple members of a female group in search of a receptive partner (Fisher, 1939). Once the number of receptive females diminishes, males may leave the females to join a male group (Jameson, 1989). These behaviors could increase the frequency at which males encounter infected individuals, thereby increasing their likelihood of contracting Strep syndrome.

In regards to the physical details of infection, certain results of this study contradict previous literature. While tissues and organs from the digestive tract overall did test positively for strep relatively frequently in relation to other tissues sampled, the intestinal system was surprisingly lacking in the bacteria. According to Counihan (2015), the bacteria, specifically *S. infantarius* ss *coli,* may begin infection in the epithelia of the intestines and subsequently spread throughout the body. If this hypothesis is true and if contaminated food is indeed a method by which the bacteria are introduced, as suggested by Counihan (2012), then one would expect the bacteria to be most consistently detected in the intestines. This is not the case, indicating that the contaminated food hypothesis may require further investigation.

Instead of the intestines, the spleen and heart valve lesions tested positively for Strep with greatest frequency. The spleen plays a critical role in the immune system and harbors many types of macrophages (Bronte & Pittet, 2013). *S. infantarius* ss *coli* and other streptococci species can evade an animal's immune response and survive in macrophages for extended periods of time (Counihan et al., 2015), offering an explanation as to why Strep bacteria would commonly be present in the spleen. Macrophages are also commonly found in the heart valves (Sridhar, Pham, Gee, Hua & Butcher, 2018), further supporting the concept that streptococci bacteria are surviving via macrophages. However, it should be noted that bone marrow, another area in which macrophages reside, exhibited none of the bacteria. Therefore, while macrophages do seem to influence streptococci survival, they are most likely not the only determinant of bacteria presence in tissues.

Future Directions

Understanding that population density is likely not a factor in the transmission of Strep syndrome is an important step towards increasingly focused research on Strep syndrome. Future research should center on further detailing and confirming the contamination hypothesis. If Strep syndrome is contracted by contaminated water or food sources, then environmental managers may be able to test areas for the relevant bacteria and respond accordingly. However, if neither direct transmission nor environmental contamination are able to explain trends in Strep syndrome, genetic analyses of the Northern and Southern sea otters may be advisable. These species were hunted to near extinction by the fur trade with populations dwindling to the thousands by the end of 19th century (Bodkin, 2015). The resulting reduction in genetic diversity has been established by the comparison of microsatellite markers before and after the fur trade (Larson, Jameson, Etnier, Fleming & Bentzen, 2002). This lack of genetic diversity could lead to inbreeding depression, making sea otters more vulnerable to diseases such as Strep syndrome. Investigation of this hypothesis could answer some of the lingering questions regarding disease contraction in sea otters.

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Appendix

Table 11: Reported densities (otters/km^2) for locations of interest. (1) Densities reported in otters/km of coastline. (2) Preliminary data. (3) Density reported as an average over time period.

Surveyed Locations	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**Source**
Adak Island[1]	1.69	1.53	1.07	0.95	0.69	0.90	0.70	USFWS, 2013
W. Lower Cook Inlet		1.13						Bodkin, Monson & Esslinger, 2003
E. Lower Cook Inlet		0.38						Bodkin, Monson & Esslinger, 2003
Glacier Bay		2.3						Esslinger & Bodkin, 2009
Kachemak Bay		0.57					2.34	Garlich-Miller, Esslinger & Weitzman, 2018 Gill, Burn & Doroff, 2009
Katmai NPP[3]								
Katmai NPP								
Kodiak Island				1.0	1.0			Newsome et al., 2015
Prince William Sound								
Sitka & Gustavus		0.48						Esslinger & Bodkin, 2009

Table 11 continued.

Surveyed Locations	2008	2009	2010	2011	2012	2013	2014	Source
Adak Island[1]	0.55		0.71					USFWS, 2013
W. Lower Cook Inlet								
E. Lower Cook Inlet								
Glacier Bay								
Kachemak Bay	3.72 5.1				5.92			Garlich-Miller, Esslinger & Weitzman, 2018 Gill, Burn & Doroff, 2009
Katmai NPP[3]	5.2	5.2	5.2	5.2	5.2	5.2	5.2	Coletti et al., 2016
Katmai NPP		7.2			5.96[2]			Newsome et al., 2015; USFWS 2013
Kodiak Island								
Prince William Sound			2.8					Newsome et al., 2015
Sitka & Gustavus								Esslinger & Bodkin, 2009

Table 12. Surveyed locations' population densities (otters/km^2) from 2001 – 2014. No abundance data were reported for 2014, thus that year's column is not shown. These densities were calculated using the abundance data reported in Table 14. The survey area for Cook Inlet was not fully delineated so no area approximation was made for this location.

Surveyed Locations	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Adak Island	1.033	0.936	0.649	0.575	0.409	0.546	0.427	0.315		0.413			
Glacier Bay National Park	1.655	1.827	2.567	3.240	3.450	4.891			5.970	7.170		9.818	
Kamishak Bay		4.025						4.025					
Kachemak Bay, Homer, Seldovia		1.014					4.139	3.441				6.587	
Kenai Fjords, Seward		1.379	1.379	1.379	1.379	1.379	1.379	1.379	1.379	1.379			
Kodiak Island, Katmai NPP	1.316	2.614							6.939			8.454	
Montague Island		0.552	0.926	0.839	1.084		0.532	0.881	0.464		0.990	1.488	
Sitka, Gustavus		0.177									0.346		
W. Prince William Sound		0.466	0.666	0.684	0.696		0.602	0.913	1.002		0.733	0.875	1.082

Table 13: Final collection of population densities (otters/km^2). These values include reported densities, averaged reported densities for those years in which multiple values were reported, and calculated densities in which no values were reported.

Surveyed Locations	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Adak Island	1.033	0.936	0.649	0.575	0.409	0.546	0.427	0.315		0.413			
W. Cook Inlet		1.13											
E. Cook Inlet		0.38											
Glacier Bay National Park	1.655	2.300	2.567	3.240	3.450	4.891			5.970	7.170		9.818	
Kamishak Bay		4.025						4.025					
Kachemak Bay, Homer, Seldovia		0.570					2.340	4.410				5.920	
Kenai Fjords, Seward		1.379	1.379	1.379	1.379	1.379	1.379	1.379	1.379	1.379			
Kodiak Island, Katmai NPP	1.316	2.614		1.000	1.000				7.200			8.454	
Montague Island		0.552	0.926	0.839	1.084		0.532	0.881	0.464		0.990	1.488	
Sitka, Gustavus		0.480									0.346		
Lower Cook Inlet		1.130											
W. Prince William Sound		0.466	0.666	0.684	0.696		0.602	0.913	1.002	2.800	0.733	0.875	1.082

Table 14. All abundance data gathered in preparation for population density calculations. Some data included were not used for the study, but are reported for others' use. The values reported for Cold Bay and Kenai Fjords (2002) include additional survey area. Starred values indicate that the original data source differs from that cited. See the note below for these original sources as well as sources for all abundance values.

Site	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
Kachemak Bay, Homer, Seldovia		912±368[1]					3724±979[1]
Western Cook inlet		6918±368[1]					
Kodiak and Kamishak Peninsula*	22957[3, 4]	22957[3,4]	22957[3,4]	22957[3,4]			
Kodiak Archipelago	5893[3]						
W. Prince William Sound		1849±334[5]	2631±540[5]	2704±315[5]	2751±311[5]		2380±372[5]
Prince William Sound		12385[6]	11989[6]** 9284[7]				
Montague Island		369±48[5]	619±46[5]	561±86[5]	725±105[5]		356±58[5]
Kamishak Bay		6918[3]					
Eastern Cook Inlet		962[9]					
Katmai NPP							
Seward, Kenai Fjords		2673[11] 1211±489[10]	1211±490[10]	1211±490[10]	1211±490[10]	1211±490[10]	1211±490[10]

Site	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**

Glacier Bay National Park	1238±143[12]	1266±196 [12, 13] 1270.1±685.6[15]	1866±458 [12, 14]	2381.25±59 [12, 14]		2785±361[14]	
	1477±600[16]	1555±368[16]	1144±385[16]	1959±771[16]		3117±1141[16]	
	1237±568[16]	1633±502[16]	943±337[16]	2231±890[16]		3535±1348[16]	
	835±347[16]	1008±272[16]	1328±474[16]	4058±1626[16]		2633±767[16]	
	1403±537[16]	1519±532[16]	3229±1183[16]	1277±312[16]		1854±504[16]	
		617±203[16]	2687±1454[16]				
Sitka, Gustavus		1788.6±284.9[15] 1838±307[13]					
Cold Bay	957[3]*						
Adak Island	632[17]	573[17]	392[17]				
	632[18]	573[18]	397[18]	352[18]	250[18]	334[18]	261[18]

Table 14 continued.

Site	2008	2009	2010	2011	2012	2013
Kachemak Bay, Homer, Seldovia	3596±802[1] 3422±919[2] 2344±729[2] 5691±1247[2] 5085±1363[2] 1437±593[2]				5926[1]	
Western Cook inlet						
Kodiak and Kamishak Peninsula*						
Kodiak Archipelago						
Site	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**

W. Prince William Sound	3609±635[5]	3958±653[5]		2896±392[5]	3459±448[5]	4277±638[5]
Prince William Sound						
Montague Island	589±162[5]	310±62[5]		662±224[5]	995±136[5]	
Kamishak Bay	6918[8]					
Eastern Cook Inlet						
Katmai NPP		7095±922[10]			8644±1243[10]	
Seward, Kenai Fjords	1322[10]					
	1211±490[10]	1211±490[10]	1211±490[10]			
Glacier Bay National Park					8508.33±2243[14] 7801.2±2891.3[15] 6765±1405[16] 12960±3046[16] 5800±1251[16]	
Sitka, Gustavus				3543.2±1248[15]		
Cold Bay						
Adak Island	193[18]		253[18]			

[1]Garlich-Miller, Esslinger & Weitzman, 2018, [2]Gill & Burn, 2009, [3]USFWS, 2014b, [4]Doroff, 2014, [5]Bodkin, Ballachey & Esslinger, 2011, [6]Coletti, 2006, [7]Alaska Sea Grant, 2004, [8]Gill, Doroff & Burn, 2009, [9]USFWS, 2014a, [10]Coletti et al., 2016, [11]USFWS, ASC & USGS, 2005, [12]Bodkin et al., 2006, [13]Esslinger & Bodkin, 2009, [14]Weitzman, 2013, [15]Tinker, 2018, [16]Esslinger, Esler, Howlin & Starcevich, 2015, [17]Estes, Tinker & Burn, 2005, [18]USFWS, 2013, *Burn & Doroff, 2005, **Bodkin, Ballachey, Dean & Esler, 2003

Figure 6a. Pooled and averaged population densities for the grouped site of Kodiak Island and Katmai National Park and Preserve. Group 1 includes years 2001 to 2007 and group 2 includes years 2008 to 2014. The value used for group 1 is 1.48 otters/km^2 with a standard deviation of 0.77 and that used for group 2 is 7.83 otters/km^2 with a standard deviation of 0.89.

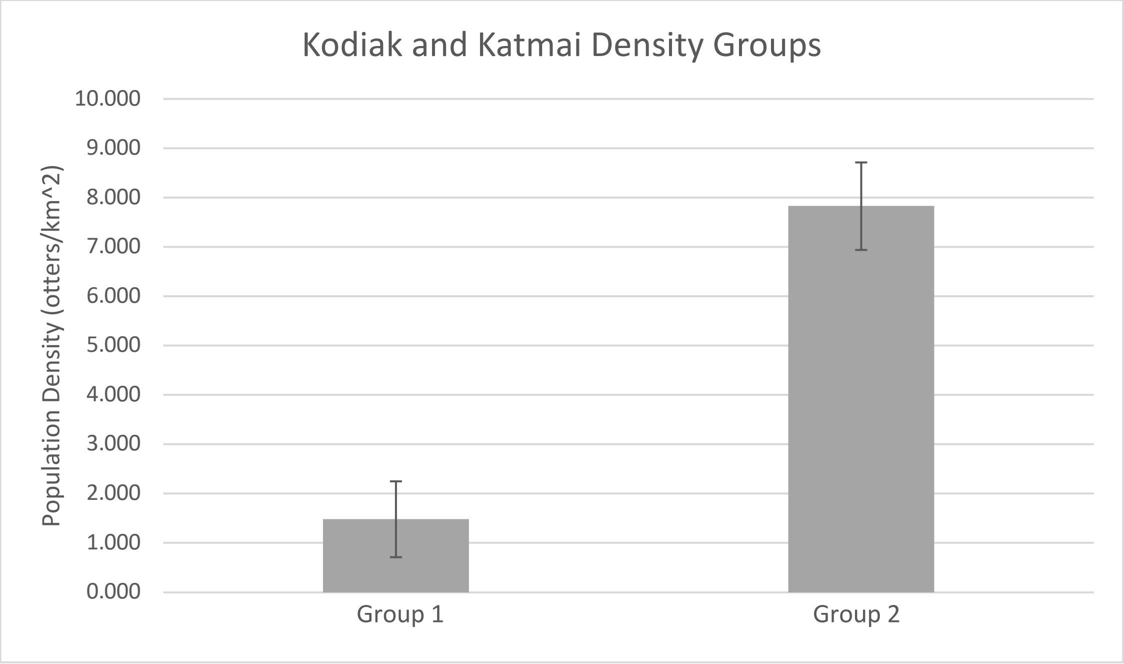

Figure 6b. Averaged sea otter population densities in Kachemak Bay grouped across years to optimize sample size (number of bacteriologically tested sea otters) for each density value. Group 1: 2001 – 2005, N = 35, standard deviation = 0.762; Group 2: 2006, N = 28, standard deviation = 0; Group 3: 2007 – 2008, N = 44, standard deviation = 1.46; Group 4: 2009, N = 37, standard deviation = 0; Group 5: 2010, N = 35, standard deviation = 0; Group 6: 2011, N = 19, standard deviation = 0; Group 7: 2012, N = 18, standard deviation = 0; Group 8: 2013 – 2014, N = 35, standard deviation = 0.388.

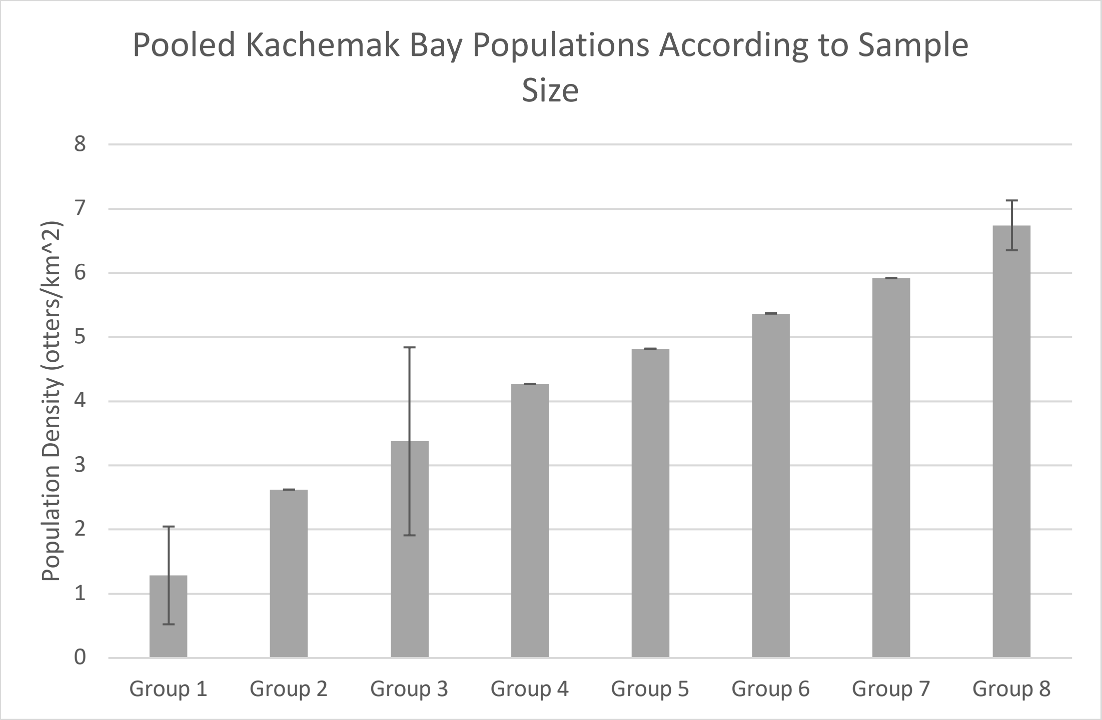

Figure 6c. Pooled and averaged population densities for the grouped site of Kachemak Bay, which in addition to Kachemak Bay includes Homer and Seldovia. Group 1 includes years 2001 to 2007 and group 2 includes years 2008 to 2014. The average density in group 1 is 1.827 otters/km^2 with a standard deviation of 0.81 and that of group 2 is 5.47 otters/km^2 with a standard deviation of 1.05.

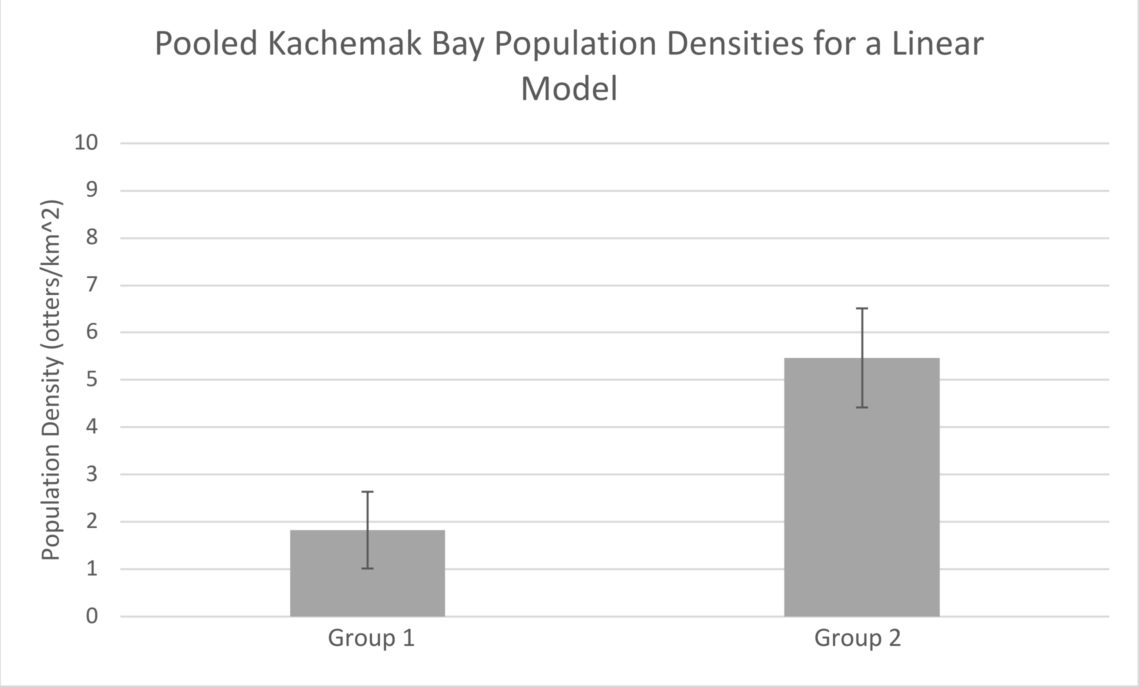

Figure 7. Map of Alaska showing the traced survey areas of grouped sites.