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Title: Habitat associations between *Streptococcus bovis/equinus* complex (SBEC) and *Streptococcus phocae*, the causative agents of strep syndrome in sea otters, and the marine environment

Running Title: Strep syndrome habitat associations

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Abstract

The bacteria in the *Streptococcus bovis/equinus* complex (SBEC) and *Streptococcus phocae* have caused significant morbidity and mortality in northern sea otters (*Enhydra lutris kenyoni*). In order to illuminate the persistence and possible mechanisms of transmission of *SBEC* and *S. phocae*, the presence and absence of these bacteria were compared with 31 habitat attributes in Kachemak Bay and Resurrection Bay, Alaska. Bay mussels or water were collected at 1,600 meter intervals around the perimeters of each bay and habitat attributes were recorded onsite and/or determined using ShoreZone. PCR was used to confirm the presence of bacteria, and presence was correlated with habitat attributes. Geographic spatial analysis revealed a cluster of low occurrence of both *SBEC* and *S. phocae* in an extremely shallow portion of Kachemak Bay that may be due to drying of the area between tide cycles. A cluster of high occurrence of *S. phocae* on the northeast side of the Kachemak Bay was identified that may be associated with harbor seal presence. No statistically significant clusters were found in Resurrection Bay. Habitat attributes (rockweed, eelgrass, habitat class, soft brown kelp and substrates of rock, sand and boulder) were found to be associated with presence of the target bacteria; however, relationships were not consistent with the bacteria or each bay. This could be due to the complexity of the relationship between *SBEC* and *S. phocae* and their environments, as well as intrinsic differences (such as nearshore temperatures) between Kachemak and Resurrection Bays.

Keywords: Strep syndrome, bay mussels, seawater, sea otter, transmission routes, spatial distribution

Introduction

Sea otters (*Enhydra lutris*) are the smallest known marine mammal, and the United States is home to two subspecies, southern (*Enhydra lutris nereis*) and northern (*Enhydra lutris kenyoni*) sea otters, which inhabit the nearshore waters off California and coastal waters off British Columbia, Washington and Alaska, respectively. Once abundant, sea otters were hunted to near extinction between 1742 and 1911 for the Russian and American fur trades (Kenyon 1969; USFWS 2020). Population numbers have increased since that time; however, sea otters still face significant threats, and the southwestern Alaskan stock of northern sea otters is

currently listed as 'threatened' under the International Union for Conservation of Nature (IUCN) guidelines (USFWS 2005).

In 2006 the United States Fish and Wildlife Service (USFWS) declared an Unusual Mortality Event (UME) after 43% of northern sea otter carcasses, the majority from Kachemak Bay, Alaska, had a cause of death due to infections from bacteria in the *Streptococcus bovis/equinus* complex (SBEC) between 2002 and 2006 (Gill 2006). An investigation of 780 carcasses recovered between 2002 and 2012 revealed that approximately 44% of the northern sea otters died from strep syndrome, a disease characterized by infectious endocarditis, meningoencephalitis and/or septicemia (Burek-Huntington et al. 2021). The UME was officially ended in 2010, but sea otter strandings due to strep syndrome have continued. Bacterial cultures determined that *Streptococcus lutetiensis* (a member of SBEC and previously classified as *Streptococcus infantarius* subsp. *coli*) was the primary causative agent of strep syndrome (60%), with *Streptococcus phocae* causing 10% of cases, and coinfections of S. *lutetiensis* and *S. phocae* responsible for 8% (Burek-Huntington et al. 2021). Non-specified members of SBEC were implicated in 14% of cases (Burek-Huntington et al. 2021).

Members of SBEC have been associated with bacteremia, septicemia and endocarditis in humans, birds, mustelids and ruminants (De Herdt et al. 1995; Muhlemann et al. 1999; Pedersen et al. 2003; Waisberg et al. 2002). *Streptococcus phocae* belongs to the pyogenic streptococci and has been associated with disease in numerous marine animals, including dolphins, porpoises, sea lions, salmon and various species of seals (Skaar et al. 1994; Henton et al. 1999; Vossen et al. 2004; Johnson et al. 2006; Romalde et al. 2008; Imai et al. 2009; Hueffer et al. 2011; Avendano-Herrera and Poblete-Morales 2015; Diaz-Delgado et al. 2017). Although neither *S. lutetiensis* nor *S. phocae* are salt tolerant and likely do not survive long outside a host, studies are needed to evaluate this. The persistence of these bacteria in the environment is unknown.

Prey choice and habitat use may be important factors affecting exposure to and transmission of *SBEC* and *S. phocae* in the marine environment, and previous research suggests an environmental source. Counihan-Edgar et al. (2012) isolated viable SBEC from mussels collected from the California coast. Mussels are filter feeders and likely acquired the bacteria from the environment, suggesting the bacteria were present in the seawater. Mussels are also a prey item for sea otters, especially northern sea otters, and are most often consumed whole (Doroff et al. 2012). The shell may cause abrasions to the gastrointestinal tract allowing bacterial

entry. SBEC have been frequently isolated from the gastrointestinal tract and affected organs in sea otters with strep syndrome (Counihan-Edgar et al. 2012). Additionally, in laboratory experiments, common sea otter prey (clams, mussels, crab and snails) were able to accumulate SBEC and *S. phocae*, which suggests prey could be an infection source (Rouse et al. 2021). *Streptococcus phocae* has also been shown to colonize sea otters through skin wounds (Bartlett et al. 2016).

The purpose of this study was to determine the spatial distribution of SBEC and *S. phocae* in Resurrection and Kachemak Bays, AK, and illuminate possible environmental attributes that may correlate with bacterial persistence in these bays. Results of this study will help shed light on possible routes of transmission, and provide wildlife managers new information about SBEC and *S. phocae* that is important for management decisions, such as shellfish harvest, risk assessments and determination of rehabilitated or translocated animal release sites.

Methods

Site Selection

Samples were collected around the perimeters of two rural, human-inhabited, glacially-fed bays in South-central Alaska: Resurrection Bay (59.9169° N, 149.4020° W) and Kachemak Bay (59.7257° N, 151.1410° W) (Figures 1 and 2). Study regions were selected based upon the locations where sea otters were found with confirmed SBEC or *S. phocae*-related illness from 2002-2016 as well as habitat features of interest (e.g., presence or absence of human settlements and runoff, presence of sea otters and glacial input).

Sample Collection

Sample collection was performed during June, July and August of 2016. Given their stationary nature, ability to concentrate SBEC (Counihan-Edgar et al. 2012), and ubiquity along the shorelines of both study areas, bay mussels (*Mytilus trossulus*) were collected (ADF&G permit #16-022) and used as an indicator organism to examine the presence or absence of bacteria in the bays. Where mussels were not available, water was sampled instead. Sample points were established at 1,600 m intervals around the perimeter of each bay, resulting in 126 sample points in Kachemak Bay and 36 sample points in Resurrection Bay (n = 162). Thirty mussels were collected from the intertidal region at each sample point from a single continuous patch (similar to how an otter would forage), but if too few mussels were present in a continuous

patch, they were collected over a more dispersed area. If no mussels were found within 250 m of the predetermined sample point, 1L of seawater was collected and filtered through a 0.20 µM pore Supor 450 Pall syringe filter. In Kachemak Bay, mussels were collected at 107 sites and water at 19 sites (n=126). In Resurrection Bay, mussels or water were collected at each of the 36 sites, but 5 samples could not be analyzed. Therefore, a total of 31 samples were analyzed, which included mussels collected at 19 sites and water at 12 sites. Mussels and water filters were stored on cold packs and transferred to -20ºC freezers within 48 hours of collection.

Environmental attributes were recorded at each collection point at the time of sampling. Location was recorded as latitude/longitude in decimal degrees using a handheld eTrex Venture HC GPS (Garmin, Lenexa, KS). Water temperature was measured using a handheld non-digital thermometer. Sediment type was classified as rock or cobble, boulder, sand or manmade (e.g., rip rap) (Bain and Stevenson 1999). The presence of algae, kelp and non-mussel bivalves was recorded, and species were noted where possible. The presence of live sea otters, manmade structures (e.g., houses, active harbors, old pilings, docks) and visible freshwater inlets was recorded and the distance to each was estimated. Visible organic matter (e.g., marine snow) or surface sheens (e.g., oil slick) were also noted. Tide level was estimated after the fact using the time of sampling and online tide charts from the National Oceanic and Atmospheric Association (NOAA 2017). Eleven additional attributes were assigned using Alaska ShoreZone, a coastal mapping and imagery database funded by NOAA that provides fine scale geomorphic and biological coastline data (ShoreZone 2021). Environmental attributes obtained from ShoreZone included presence of green algae, red algae, seagrass, brown kelp, eelgrass, bull kelp, soft brown kelp, dune grass, rockweed, biological wave exposure and habitat class (Table 1). Distance to the nearest waterway was determined using ArcGIS and was defined as ‘major’ if the nearest waterway was well-defined, estimated to exist year-round (as water or ice) and resulted from the confluence of two or more upland waterways. All other waterways were considered ‘minor’.

Sample Processing

All 30 mussels from each site were thawed, shucked, pooled, combined with peptone water (1:1 weight to volume) and macerated with a hand blender for 120 sec. Homogenate (2 mL) was removed, centrifuged (7500 rpm for 10 min) and DNA extracted from the pellet using a Qiagen DNeasy Blood and Tissue DNA extraction kit (Hilden, Germany) according to the

manufacturer's recommendation for Gram positive bacteria. Seawater filters were combined with a lysozyme pretreatment buffer, cut up using sterile scissors, vortexed for 4 min in a 0.70 mm garnet bead tube (MOBIO, Germantown, MD) and then DNA extracted as described above.

Presence or absence of SBEC and *S. phocae* was determined using PCR. Published primers targeting a portion of the superoxide dismutase (*sodA)* gene were used to detect *S. phocae* (Alber et al. 2004) and SBEC (Rouse et al. 2021). Reactions were prepared using 10-12.5 µL of master mix (5Prime Hot Master Mix or OneTaq Hot Start2X), 1 µL forward primer, 1 µL reverse primer, template DNA from bay mussels (500-1000 µg) or positive control bacteria (250-350 µg) and the final reaction volume brought to 25 µL with PCR-grade water. Each PCR run contained a known positive control and a negative (no template) control. Cycling parameters for SBEC were: 94˚C for 2 min, 30 cycles of 94˚C for 30 sec, 57˚C for 30 sec, 72˚C for 30 sec, and a final elongation step at 72˚C for 5 min (Rouse et al. 2021). For *S. phocae*, cycling parameters were 94˚C for 3 min, 30 cycles of 94˚C for 30 sec, 60˚C for 40 sec, 72˚C for 45 sec and a final elongation step of 72˚C for 5 min (Alber et al. 2004). Products were analyzed using gel electrophoresis and visualized under UV light. PCR products from three selected mussel samples per bacterial species were purified using a Qiaquik PCR Purification Kit (Qiagen Hilden, Germany) and confirmed using Sanger sequencing (UC DNA Sequencing Facility, Davis, CA).

Data Analysis

In order to determine relationships among habitat attributes, continuous components (e.g., water temperature and distance measurements) and bacterial presence/absence, 2-sample t-tests and Pearson's chi-squared analysis were completed in R (version 3.4.1). Results were considered significant when $p<0.05$. Logistic regression models were run using R with combinations of the 8 most significant attributes identified in the chi-squared tests. Model results were compared using Akaike's Information Criteria corrected for small sample bias (AICc; Akaike 1973). PCR results were analyzed for geographical clustering using SaTScan software using a Bernoulli model, selecting for high and low occurrence penalty. Results were considered significant when $p<0.10$.

Results

Identification of SBEC and S. phocae *in Samples*

Mussel and water samples were collected from Kachemak (n=107 and 19, respectively) and Resurrection (n=19 and 12, respectively) Bays. SBEC were identified in mussels or water in

30% (38/126) of sites sampled in Kachemak Bay and 29% (9/31) of sites sampled in Resurrection Bay. *Streptococcus phocae* was identified in mussels or water from 27% (34/126) of sites in Kachemak Bay and 35% (11/31) in Resurrection Bay (Figure 3). Both SBEC and *S. phocae* were identified in samples from 18% (23/126) of sites in Kachemak Bay and 19% (6/31) of sites in Resurrection Bay; all detections of both were in mussels only. Of the positive detections, 98% were from mussel samples and 2% were from water filter samples. When mussel and water samples were analyzed separately, SBEC was detected in 35% (37/107) of mussels and 5% (1/19) of water samples collected in Kachemak Bay and 47% (9/19) of mussels and 0% (0/12) of water samples collected in Resurrection Bay. *Streptococcus phocae* was detected in 34% (36/107) of mussels and 5% (1/19) of water samples collected in Kachemak Bay and 58% (11/19) of mussels and 0% (0/12) of water samples collected from Resurrection Bay (Table 2).

Spatial Analysis

Spatial cluster analysis using SaTScan revealed one statistically significant cluster of low occurrence (p=0.02; Figure 4C) for both bacterial species combined on the southeast side of Kachemak Bay. Spatial analysis of SBEC and *S. phocae* individually revealed a similar trend, with one statistically significant (p=0.06) cluster of low occurrence of SBEC and one statistically significant (p=0.04) cluster of low occurrence of *S. phocae* in Kachemak Bay, both centered within 2 km of the cluster found when analyzing both bacteria together (Figure 4A and 4B). One statistically significant cluster of high occurrence (p=0.01) for *S. phocae* in Kachemak Bay was also found (Figure 4B). No statistically significant clusters of high or low occurrence were detected in Resurrection Bay.

Relationship with Habitat Type

Habitat attributes recorded in the field are summarized in Table 3. There were no visible organic matter or surface sheens observed at the sampling sites; therefore, these attributes were not included in the analysis. No significant relationships were identified between the presence of SBEC or *S. phocae* and continuous habitat variables (water temperature, tide level at collection, distance to live sea otters during sample collection, distance to manmade structure(s), distance to visible inlet, distance to major freshwater inlet (determined by GIS) or distance to minor freshwater inlet (as determined by GIS)). However, statistically significant relationships were identified between the target bacteria and non-continuous habitat variables (Table 4). In Kachemak Bay, SBEC was associated with eel grass (p=0.04) and rockweed (p=0.004), and *S.*

phocae was associated with boulder substrate (p=0.02). In Resurrection Bay, SBEC presence was related with habitat class (p=0.04) and rock substrate (p=0.01) and *S. phocae* was associated with rock substrate (p=0.03). When results from both bays were combined, significant relationships were found between SBEC and rockweed (p=0.007), and *S. phocae* and soft brown kelp (p=0.04).

Logistic regression modeling of all bacterial detections suggested that the target bacteria were most strongly correlated with bay and decreasing substrate size based on AICc rankings (Table 5). Parameter estimates from the best ranked model showed that the bacteria were positively correlated with Resurrection Bay (0.97 ± 0.54) but negatively correlated with substrate size (-0.32 ± 0.27).

Discussion

Analysis of mussel and water samples revealed a significant presence of SBEC and *S. phocae* in both Resurrection and Kachemak Bays with approximately 30% of sites testing positive for one and 20% of sites testing positive for both. Both SBEC and *S. phocae*were also positively correlated with Resurrection Bay. This was unexpected given that 70% of sea otter deaths from strep syndrome have occurred in Kachemak Bay (Burek-Huntington et al. 2021). However, differences in death rates between the two bays may be reflective of two factors. Kachemak Bay has a larger human population that likely results in more carcasses being reported and necropsied than in Resurrection Bay. Additionally, the population of sea otters in Resurrection Bay is smaller compared to Kachemak Bay. Census data indicated a density of 6.37 sea otters per km^2 in Kachemak Bay (Garlich-Miller et al. 2018) versus 1 sea otter per km^2 in Kenai Fjords National Park, which borders Resurrection Bay and presumably has a similar density (Coletti et al. 2016). Therefore, while the bacteria are present in both bays, the more remote nature of Resurrection Bay and smaller sea otter population may result in lower detection of strep syndrome than in Kachemak Bay.

Mussels were preferentially collected for assays because, as filter-feeders, they concentrate pathogens in the environment. However, mussels were not available at all sites and, therefore, water was collected for analysis. One liter of seawater was collected and filtered, which is in the range of the most commonly reported water volumes (0.5 – 2L) used for eDNA analysis (Farrell et al. 2021). Detection of SBEC and *S. phocae* in water samples was low, which could reflect differences in DNA extraction efficiency between mussel and water samples or a

lack of bacterial presence at sites where water was sampled. Unfortunately, both mussels and water were not collected at any sites to compare detection levels. Nevertheless, water samples have been successfully used in other studies to detect pathogenic fungi, viruses and bacteria in aquatic environments (Amini and Kraatz 2015; Farrell et al. 2021). While mussels are capable of filtering much larger volumes of water from their surrounding environment than 1L, detection of eDNA in water has been shown to be sensitive and a beneficial tool for monitoring environments for pathogens (Amini and Kraatz 2015; Farrell et al. 2021).

No significant relationships were found between the target bacteria and continuous habitat variables suggesting these parameters do not directly affect their presence in the marine environment. An anthropogenic source of exposure was suspected because SBEC have been associated with human disease, and urbanization and freshwater runoff have been implicated as risk factors for enteric bacterial pathogen exposure in southern sea otters (Miller et al. 2010). However, the results did not show an association with freshwater inlets or manmade structures suggesting these are not risk factors for strep syndrome. Additionally, a relationship between water temperature and target bacterial presence was expected. Like other *Streptococcus spp.*, *S. phocae* and the members of SBEC are mesophiles that are able to grow at low temperatures, but grow faster as temperatures increase (Reuter 1992; Yañez et al. 2013). Temperature measurements in the field confirmed a warmer average temperature in Kachemak Bay compared to Resurrection Bay (Table 3). However, this temperature difference was likely not sufficient to affect bacterial presence. Seasonal fluctuations in temperature would be more drastic and may influence presence of SBEC and *S. phocae*. Other pathogens, such as *Vibrio* spp., prefer warmer waters and are isolated more frequently during the summer months in northern latitudes (Boer et al. 2013). Additional research is needed to investigate seasonality of strep syndrome bacteria.

In Kachemak Bay, SBEC was associated with eel grass and rockweed, and *S. phocae* was associated with boulder substrate. In Resurrection Bay, presence of SBEC was related to habitat class and rock substrate, and *S. phocae* presence was related to rock substrate. These differences may stem from intrinsic differences in the bays. Kachemak Bay is in Cook Inlet, a shallow ocean basin (45 fathoms or less) with a large 4.93 m average tidal flux (NOAA 2017), whereas Resurrection Bay is a deep ice-free fjord with a 2.54m average tidal flux (NOAA 2017). Differences in depth, particularly in the nearshore areas, contribute to different temperature regimes and primary productivity. The presence of aquatic vegetation may provide a suitable

habitat for the persistence of SBEC and *S. phocae*. For example, enterococci are able to survive in the environment longer in submerged aquatic vegetation than in sediment or water (Badgley et al. 2010), presumably because vegetation may provide a substrate for bacterial adherence. Another factor influencing SBEC and *S. phocae* persistence may be the marine microbial community. Indigenous microbiota have been shown to inhibit *Escherichia coli* survival, possibly through competition for nutrients or predation (Korajkic et al. 2013). Variations in the marine microbiome throughout Kachemak and Resurrection Bays may influence where SBEC and *S. phocae* are best able to persist. Habitat class assignments in ShoreZone take into account wave exposure, geomorphology and biota, and the association of SBEC with habitat class in Resurrection Bay suggests multiple habitat attributes are necessary for their environmental persistence. This is understandable considering habitat attributes are often dependent on one another. For example, a mobile substrate, such as sand, has limited macrobiota (ShoreZone 2021). Overall, these results suggest a complex relationship between SBEC, *S. phocae* and their habitat requirements in the marine environment.

Areas of low occurrence of SBEC and *S. phocae* were present at the heads of Peterson and China Poot Bays within Kachemak Bay. While the clusters are large (12.26 km and 10.39 km radii, respectively), both contain extremely shallow areas (<1 fathom) that tend to partially dry out during the low tide cycle, and may negatively impact survival of the target organisms. Abundance surveys conducted in 2017 confirm that sea otters utilize these areas, although densities are low, particularly during the summer months (Garlich-Miller et al. 2018) when our samples were collected. A cluster of high occurrence of *S. phocae* was found on the northeast side of Kachemak Bay. Studies indicate more sea otters inhabit this region during the summer months when sampling occurred (Garlich-Miller et al. 2018). A high presence of harbor seals (*Phoca vitulina*), which can be infected by *S. phocae* (Skaar et al. 1994), was also noted during sampling. However, harbor seals were not sampled during this study and their infection status was unknown. The congregation of harbor seals and sea otters in this area may increase the risk of *S. phocae* infection in sea otters, but additional research is needed to evaluate this.

The relationship between the target bacteria and decreasing substrate size indicated that areas where the dominant substrate comprises smaller particles, such as sand, may have a greater potential to harbor SBEC and *S. phocae*. Other bacterial species have been associated with substrates of smaller particle size, so this finding is not surprising (Dale 1974; Mutter et al.

2016). Studies on enterococci and *Vibrio* spp. demonstrated that they persisted longer in sediment than in the water column (Badgley et al. 2010; Boer et al. 2013). Sediment may provide a substrate for adherence that allows longer retention in the aquatic environment. Further, this result agrees with spatial cluster analysis of stranded sea otters (Worman, personal communication), where it was found that strandings clustered on a sandy stretch of beach near the town of Homer, Alaska. The relationship between bacterial presence and sand may also be associated with the presence of bivalves. Clams are a preferred sea otter diet item and experiments have shown that clams can harbor the bacteria (Rouse et al. 2021). Other than in the case of substrate size and bay effect, the results of our logistic regression modeling did not reveal a relationship between SBEC and *S. phocae* and habitat variables explored. It may be that, like many environmental phenomena, several habitat attributes (including ones not examined in this study) influence patterns of SBEC and *S. phocae* persistence in the environment.

This study confirmed the presence of SBEC and *S. phocae* in the marine environment outside of a mammalian host. Relationships were identified between bacterial presence and certain habitat attributes, which may be linked to habitat requirements for the bacteria or another host, such as bivalves, that may serve as an infection source for sea otters. There was also a cluster of high *S. phocae* occurrence in Kachemak Bay where sea otters and harbor seals were observed, suggesting there could be an association between harbor seals and sea otter infections. This project has provided the initial steps in determining habitat associations and possible transmission routes of SBEC and *S. phocae* to sea otters. Additional research is needed to further investigate these findings and determine their significance to strep syndrome transmission.

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Tables

Table 1: Definition of biological wave exposure and habitat class attributes.

ATTRIBUTE	DEFINITION	CATEGORIES
Biological Wave Exposure	The presence and abundance of indicator species is used to characterize wave energy at a shoreline unit based on the tolerance of the species present	Very Protected Protected Semi-Protected Semi-Exposed Exposed Very Exposed
Habitat Class	The physical (substrate mobility) and biological (biological wave exposure and biota presence) features of a shoreline unit are considered to assign habitat class	Wave Structured Shorelines: Immobile Partially Mobile Mobile Non-Wave Structured Habitat: Riparian Current

	Glacier
	Anthropogenic
	Lagoon
	Periglacial

Table 2. Percent of mussel and water samples positive for *Streptococcus bovis/equinus* complex (SBEC)*, S. phocae*, or both in Kachemak and Resurrection Bays.

	KACHEMAK BAY		RESURRECTION BAY	
	Mussels (n=107)	Water (n=19)	Mussels (n=19)	Water (n=12)
SBEC	35%	5%	47%	0%
Streptococcus phocae	34%	5%	58%	0%
SBEC *and S. phocae*	21%	0%	32%	0%

Table 3: Habitat attributes recorded in the field.

ATTRIBUTES	KACHEMAK BAY	RESURRECTION BAY
Substrate boulder	37.10%	28.13%
Substrate rock	71.77%	71.88%
Substrate sand	41.13%	28.13%
Substrate manmade	0.81%	0.00%
Average surface temperature (°C)†	12.49 ± 2.56 (n=96)	10.08 ± 2.39 (n=30)
Sites where live sea otters were observed	19.05%	9.38%
Average distance to sea otters (m)†	152.08 ± 137.11 (n=31)	150.00 ± 57.74 (n=3)
Within sight of manmade structure	68.25%	59.38%
Average distance to manmade structure (m)†,‡	376.27 ± 275 (n=48)	656.25 ± 787 (n=4)

†± standard deviation

‡Distances over 1,000 m could not be accurately measured and were eliminated from calculations

Table 4: Chi-square analysis of habitat attributes and bacterial presence.

	KACHEMAK BAY		RESURRECTION BAY		BOTH BAYS	
ATTRIBUTE	SBEC†	SP‡	SBEC	SP	SBEC	SP
Red algae	-	-	-	-	-	-
Green algae	-	-	-	-	-	-
Seagrass	-	-	-	-	-	-
Brown kelp	-	-	-	-	-	-
Eel grass	p=0.04	-	-	-	-	-
Soft brown kelp	-	-	-	-	-	p=0.04
Dune grass	-	-	-	-	-	-
Rockweed	p=0.004	-	-	-	p=0.007	-
Biological wave exposure	-	-	-	-	-	-
Habitat class	-	-	p=0.04	-	-	-
Substrate: rock	-	-	p=0.01	p=0.03	-	-
Substrate: boulder	-	p=0.02	-	-	-	-
Substrate: sand	-	-	-	-	-	-

†*Streptococcus bovis/equinus* complex (SBEC)

‡*Streptococcus phocae*

- indicates no significant difference

Significance $p<0.05$

Table 5: AICc results of logistic regression modeling examining effects of selected parameters on the occurrence of *Streptococcus bovis/equinus* complex (SBEC)and *Streptococcus phocae.*

MODEL PARAMETERS	K[†]	AICc[‡] SCORE	ΔAICc SCORE	AICcWt[§]
Substrate size + Bay	3	170.77	0.00	0.74
Biowave exposure + Bay	3	175.74	4.97	0.06
Habitat class + Bay	3	176.32	5.54	0.05
Rockweed + Bay	3	176.42	5.64	0.04
Eel grass + Bay	3	176.48	5.71	0.04
None	1	177.14	6.36	0.03
Rockweed + Biowave exposure + Bay	4	177.75	6.98	0.02
Bull kelp + Biowave exposure + Green algae + Habitat class + Bay	6	181.13	10.36	0
Biowave exposure + Dune grass + Eel grass + Habitat class + Rockweed + Bay	7	183.01	12.24	0
Bull kelp + Dune grass + Eel grass + Green algae + Rockweed + Bay	7	184.15	13.38	0
Biowave exposure + Bull kelp + Dune grass + Eel grass + Green algae + Habitat class + Rockweed + Soft brown kelp + Substrate size	10	184.44	13.67	0

[†]K = Number of parameters

[‡]AICc = AIC corrected for small sample bias

[§]AICcWt = AIC weight

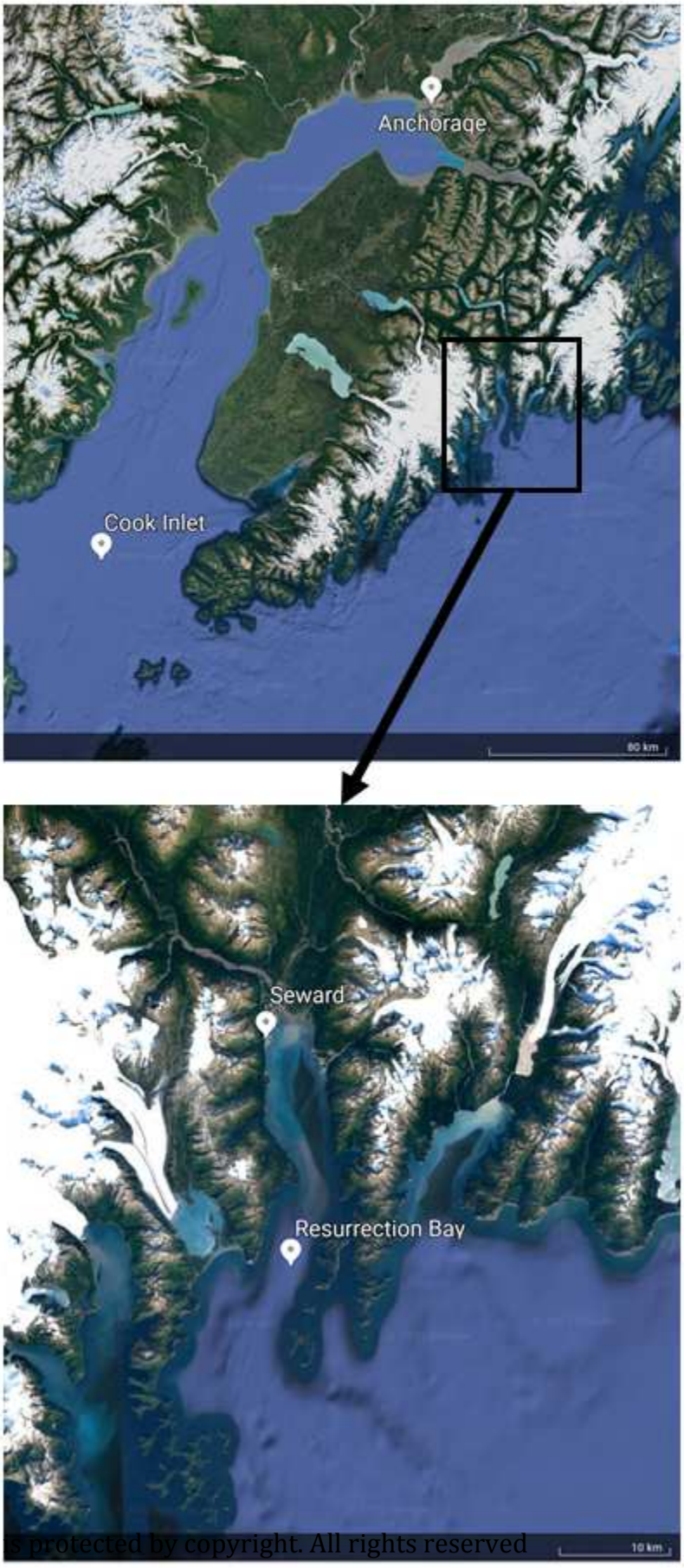
Figure Legends

Figure 1: Location of Resurrection Bay in Alaska.

Figure 2: Location of Kachemak Bay in Alaska.

Figure 3: Locations of *Streptococcus bovis/equinus* complex (SBEC) and *Streptococcus phocae* (SP) detections in Kachemak and Resurrection Bays.

Figure 4: Spatial cluster analysis of bacterial detections performed using SaTScan software for clusters of low and high occurrence analyzed for A) *Streptococcus bovis/equinus* complex (SBEC) B) *Streptococcus phocae* (SP) C) SBEC and *S. phocae.* Statistically significant clusters ($p<0.10$) are labeled. Unlabeled clusters had a p-value of greater than 0.10. Stars indicate the location of samples positive for our targets. Blue stars= SBEC, red stars= *S. phocae*, yellow stars= both SBEC and *S. phocae.*



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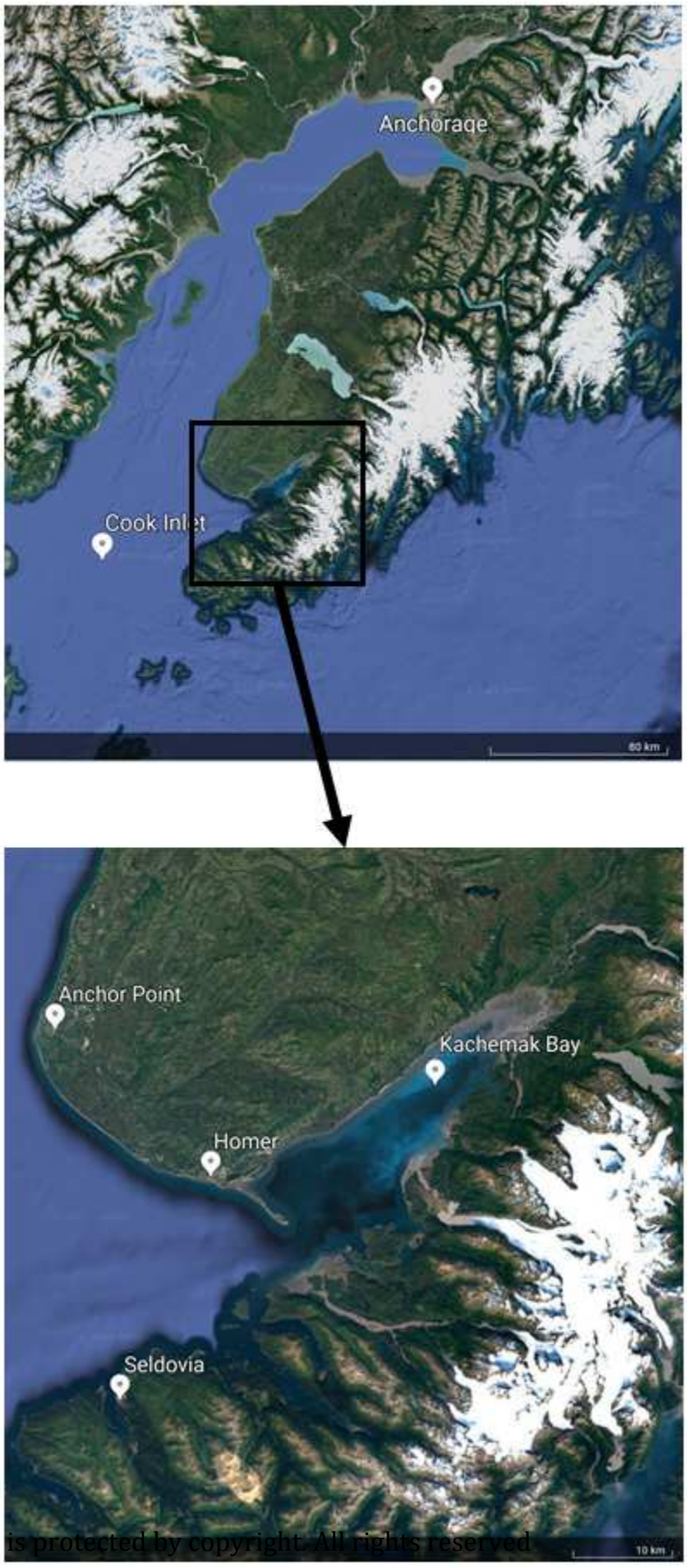

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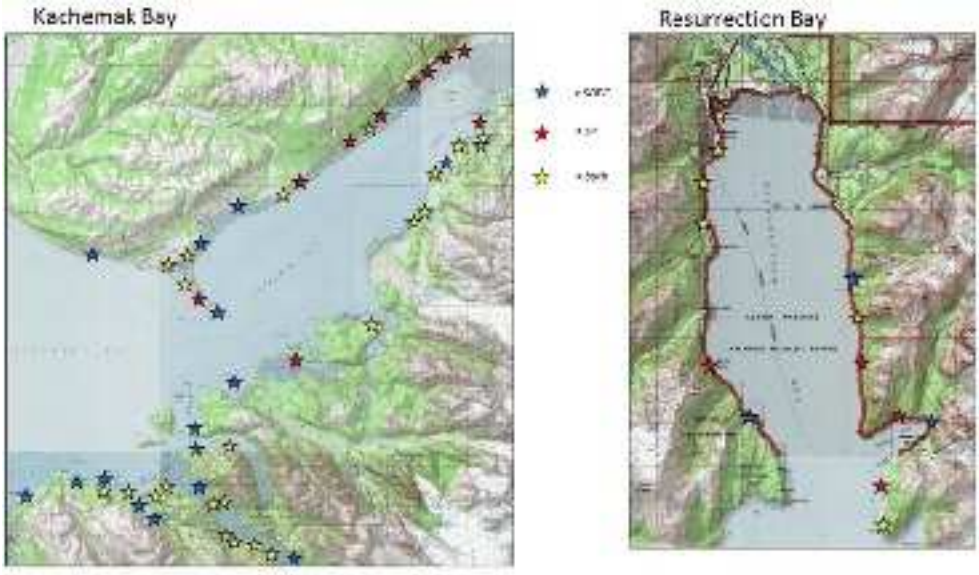

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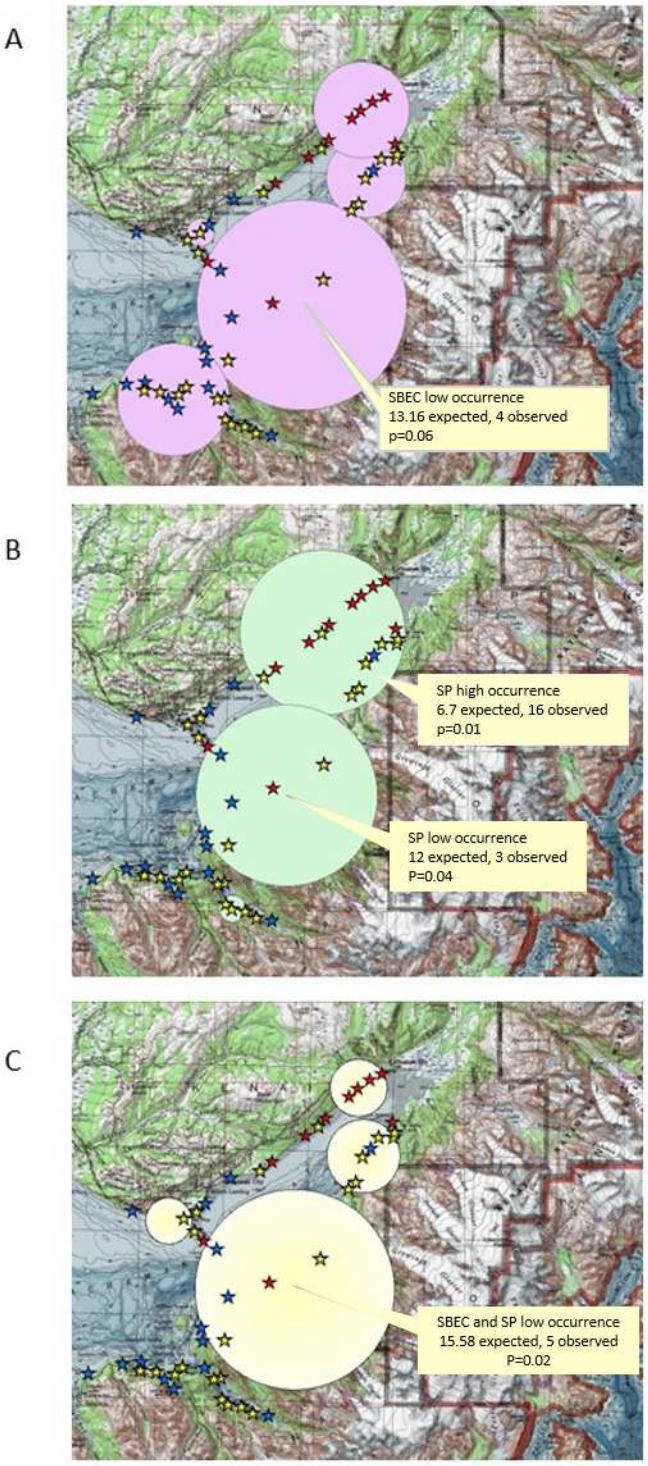

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