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ARTICLE

**Reemergence of Guadalupe fur seals in the U.S. Pacific**

**Northwest: The epidemiology of stranding events during 2005–2016**

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**Abstract**

Guadalupe fur seals (GFS), *Arctocephalus phillippii townsendi*, an U.S. Endangered Species Act threatened pinniped, have recently reappeared in their historic range along the western seaboard of the continental United States. Starting 2005 through 2016, 169 GFSs stranded in Washington and Oregon, involving two designated unusual mortality events. The circumstances surrounding GFS strandings, mortality, and their increased presence in Oregon and Washington were analyzed during this study. Detailed necropsies, histopathology ( $n = 93$ ), and epidemiological analysis found three main causes of death (COD): emaciation (44%), trauma (29%), and infectious disease (19%) and the factors associated with overall strandings and emaciation. Trauma included many cases found associated with fisheries interactions and clustered near the mouth of the Columbia River, where high levels of commercial fishing occur. The most common pathogens found associated with disease were *Toxoplasma gondii*, *Sarcocystis neurona*, and gastrointestinal helminths. Seasonality and upwelling were associated with higher stranding numbers regardless of COD. Seasonal migration into the region,

coinciding with postweaning, suggests young GFSs are in search of prey and habitat resources. Re-emergence of GFSs is likely due to conservation efforts, which have been critical for species recovery in the region. Continued monitoring is needed as this vulnerable species continues to rebound.

**KEYWORDS**

conservation, epidemiology, Guadalupe fur seal, mortality, pinniped, population, recovery, stranding

## 1 | INTRODUCTION

Guadalupe fur seals (GFS), *Arctocephalus phillippii townsendi* (Merriam, 1897), are an otariid species native to the Eastern Pacific Ocean. These seals breed primarily in Mexico on the Isla Guadalupe and Isla Benito del Este; however, other small rookery sites have been found scattered on islands as far north as San Miguel Island, California, where small numbers of pups have been born annually since 1999 (Melin & DeLong, 1999). Due to their preference for open ocean prey such as squid species, GFSs spend significant foraging time well off the coast in pelagic environments (Gallo-Reynoso & Esperón-Rodríguez, 2013). Historically, GFS, like other species of fur seals, were hunted for their fur leading to their presumed extinction in the 1800s (Rick et al., 2009; Townsend, 1931). The first sighting, since the presumed extinction status, was of two males at Isla Guadalupe in 1928 (Townsend, 1931; Wedgeforth, 1928). None were reported again until 1949 when a single male was seen, and then in 1954 a small breeding group was identified (Bartholomew, 1950; Hubbs, 1956; Rick et al., 2009).

Since then, the GFS population has increased by an

estimated rate of 14% annually, with an estimated 20,000 animals in the population currently (Carretta et al., 2017). In 1986 the status of their population was officially classified as "threatened" under the Endangered Species Act, their population has also been protected under Mexican law, which currently lists this species as "endangered" (Carretta et al., 2010; Gallo-Reynoso, 1994; Rick et al., 2009). Their continued population growth is a promising trend for this marine predator and a testament to conservation efforts in the United States under the Marine Mammal Protection Act and the Endangered Species Act, and Mexican law. The recovery of this species is also characterized by its reappearance in their historic migration range, which appeared to cover much of the west coast from Mexico, north to Vancouver, British Columbia, Canada (Etnier, 2002; Gallo-Reynoso & Esperón-Rodríguez, 2013; Villegas-Zurita, Castillejos-Moguel, & Elorriaga-Verplancken, 2015). Movements north in Mexico and California have been associated with prey movements related to El Niño events, however, their movement further north into Oregon and Washington has not been explored as fully (Elorriaga-Verplancken, Sierra-

Rodriguez, Rosales-Nanduca, Acevedo-Whitehouse, & Sandoval-Sierra, 2016).

The extent of the historic GFS northern range was discovered through archeological research on GFS remains aged at between 1500 and 1700 A.D. in Northern Washington state; however, there were no live animals observed in the region until recently, and no census or haul-out data currently exists for this northern most region (Etnier, 2002). Since 2005, GFSs have consistently stranded and been sighted in small numbers on the coasts of Washington and Oregon (Lambourn et al., 2012). Data collected from strandings has informed the context of this reemerging species distribution in the Pacific Northwest (PNW) of the United States. Two unusual mortality events (UMEs) in the United States have been declared in the last decade by the Working Group on Marine Mammal Unusual Mortality Events (WGUMME), which is concerning for this vulnerable species (Lambourn et al., 2012). Their reemergence in the PNW may be a promising sign of recovery, but environmental, pathogenic, and demographic factors related to strandings, mortality, and their recent occurrence in the region are not well characterized or

explained.

It was hypothesized that GFSs are migrating into the PNW and may be stranding due to factors they may not encounter to the same extent elsewhere in their range. These risk factors may include varied pathogens, different predation pressures, increased human interaction, different ocean conditions, and prey availability, as well as prey quality. The population of GFSs migrating to the PNW as a naïve population to the region may be more vulnerable to the types of endemic diseases and fishery interactions that occur in the waters off the coasts of Washington and Oregon than resident marine mammals. Due to hunting, GFSs experienced a severe genetic bottleneck, which has left the population potentially vulnerable to disease outbreaks and other environmental stressors that may require more genetic diversity to overcome (Weber, 2004). As a threatened species recovering from near extinction that relies on one main rookery island, GFSs could be especially vulnerable to environmental factors such as El Nino events, and the far-reaching effects of ongoing climate change (Gallo-Reynoso, 1994; Hernández-Camacho & W. Trites, 2018; Weber, Stewart, & Lehman, 2004). To date, there



have been few published epidemiological studies which assess health trends and risk factors associated with stranding causality and disease in this species, and none in the Pacific Northwest of the United States (Hanni, Long, Jones, Pyle, & Morgan, 1997; Lambourn et al., 2012; Ziehl-Quiros, Garcia-Aguilar, & Mellink, 2017). In this study, trends in stranding/mortality and environmental factors associated with the apparent return of GFSs to the PNW are described by analyzing the strandings in Oregon and Washington since 2005 and evaluating which risk factors are associated with GFS mortality in this geographic region.

## **2 | METHODS**

### **2.1 | Demographic and stranding data**

Stranding information including data on morphometric signalment, demographics, and health were compiled for GFS in Oregon and Washington at the time of the stranding by the marine mammal stranding networks along the coasts. Collected data included stranding location, time/date, status of the animal (live/dead), stranding response notes related to any behaviors and/or clinical symptoms seen prior to and at response or if there were

human interactions observed on the beach when the animal was alive, and if dead, carcass condition was noted. These general stranding data were used for analysis of overall stranding trends in the region. Demographic data collected included: sex of the animal, age class determined by body size (a combination of length and weight as well as time of year stranding occurred) and dentition, photo-documentation, and preliminary morphometrics (Fleischer, 1978; Gallo-Reynoso & Figueroa-Carranza, 2010). Age classes were defined as: (1) WP - weaned pup, determined as distinct from yearling if stranding before June; (2) Y - yearling, assigned based on stranding after June 1, length, weight, and having fully erupted dentition, including the presence of the permanent canines, that also reflected lack of wear on teeth seen in older animals; (3) SA - subadults based on a size larger than typical juvenile animals, smaller than adult females, and their dentition staining and wear reflecting an animal having foraged for multiple years; (4) AD - adult were based on length (>130 cm), weight (>20 kg), and state of dentition evident by increased staining and wear on the teeth; and (5) UNK - unknown, if the age of a seal was impossible to

determine. Animals included in the data set were GFSSs that stranded live or dead between the beginning of 2005 and the end of 2016.

## **2.2 | Necropsy and histopathology**

Necropsies were performed by trained biologists, veterinarians, or veterinary pathologists associated with the responding stranding network using standardized protocols (Pugliares et al., 2007). Complete necropsies were conducted only on freshly dead or moderately decomposed carcasses. Using the condition codes established by Geraci and Lounsbury (1993), carcasses were deemed fresh if tissues were intact, with fresh smell, and little to no gas in gut; this often indicated the animal was found less than 24-48 hr postmortem. These carcasses were collected for a complete necropsy, including sample collection for further laboratory diagnostics. Carcasses that were decomposed with organs intact, but with bloat in gut present were considered as moderately decomposed, taking into account ambient temperature and immersion in water, were deemed to be carcasses that were 3-5 days postmortem. With moderately decomposed carcasses, limited or complete necropsies were

performed depending on the extent of autolysis and scavenging, with more limited samples harvested for follow up diagnostic testing. In some cases, scavenging and exteriorization of viscera hampered complete postmortem examinations, while in other cases, carcasses were reported and documented, but not necropsied due to subsequent loss from tidal exchanges or inaccessibility for responders.

During GFS necropsy, stomach and intestinal contents were collected; hard parts of prey were counted at the time of necropsy and submitted to a reference laboratory for further analysis. Representative samples of parasites found at necropsy were collected manually, identified by morphological criteria to family level by a trained biologist, and preserved in 70% ethanol for further identification by a parasitologist. Signs of trauma were assessed upon gross necropsy examination and included external wounds associated with hemorrhage, entanglement with fishing gear, and hemorrhage associated with blunt force injuries. For GFSs with complete and partial necropsies, samples for histopathological analysis and ancillary diagnostics included tissues and lesions for isolation of

pathogens via culture and molecular methodologies.

Histopathology was carried out at one of the following pathology laboratories: Animal Health Center, OSU Veterinary Diagnostic Laboratory, Colorado State University Veterinary Diagnostic Laboratories, or Northwest ZooPath. Representative samples from each of the major organs were collected. Tissues were fixed immediately in 10% buffered formalin for histological examination and additional samples were frozen for further analysis. In some instances, decomposition led to the tissues being autolyzed and therefore they were not useful for obtaining histopathology results. If histopathology suggested a need for further testing, molecular and ancillary diagnostic testing was done. In select cases, more targeted diagnostic studies were pursued to confirm a specific etiology. For ancillary diagnostic studies, sampled lesions were frozen at -20°C until analysis.

### **2.3 | Molecular testing and ancillary diagnostics**

Histopathology or premortem clinical signs consistent with a coccidian parasite infection, including protozoal encephalitis, was identified in 30 GFSSs, and further diagnostic studies and molecular characterization were pursued. Brain, lymph nodes,

liver, heart muscle, skeletal muscle, and lung samples were sent to the National Institutes of Health Laboratory of Parasitic Diseases in Bethesda, Maryland, to screen for *Toxoplasma gondii*, *Sarcocystis neurona*, and *Neospora caninum*. To confirm the presence and determine the species of coccidian parasites, multiple laboratory modalities were used. Indirect fluorescent antibody test (IFAT; Miller et al., 2001) was used when animals live stranded or fresh serum was prepared from postmortem heart blood, PCR testing and immunohistochemistry was performed on suspect cases based on necropsy or histopathology as described in Gibson et al. (2011). Primary antibodies included rabbit polyclonal for *T gondii* (Biogenix, San Ramon, CA), rabbit polyclonal for *S. neurona* (Virginia-Maryland Regional College of Veterinary Medicine, Blackburg, VI) and mouse monoclonal antibody for *N. caninum* (VMRD, Pullman, WA).

Frozen tissues for a subset ( $n = 36$ ) of necropsied seals were sent to the Animal Health Center, Abbotsford, British Columbia, to be tested for bacteriology and virology. These included lung, spleen, lymph nodes, brain, and intestine, which were collected for conventional aerobic culture. Intestine

samples ( $n = 29$ ) were inoculated into selective media for attempted *Salmonella* spp. isolation (Quinn et al., 2011). Lymph node, lung, brain, spleen, and when available, thymus and tonsils were screened by polymerase chain reaction (PCR) for *Brucella* spp. ( $n = 9$ ) and canine distemper, and if indicated at necropsy, kidney was collected and screened for *Leptospira* spp. ( $n = 8$ ). In addition, two live seals had serological tests for both diseases (Cameron et al., 2008; Lambourn et al., 2013). The viruses tested for via PCR based on histopathology findings included influenza sp. ( $n = 20$ ), canine distemper ( $n = 32$ ), and herpesvirus ( $n = 1$ ) (Barrett, Shrimpton, & Russell, 1985; Sierra et al., 2014; Spackman et al., 2002; Wu, McFee, Goldstein, Tiller, & Schwacke, 2014).

Biotoxin exposure was determined through testing for agents such as domoic acid or saxitoxin in stomach contents, urine, feces, and/or blood. More specifically, samples from a subset of GFSSs ( $n = 33$ ) that had stomach contents, urine, and/or feces available during necropsy were screened for domoic acid and saxitoxin using high performance liquid chromatography with mass spectroscopy (HPLC-MS), receptor-binding assay, and high-

performance liquid chromatography with standard ultraviolet absorbance (HPLC-UV), respectively (Lefebvre et al., 2010). Screenings were done by the Wildlife Algal-toxin Research and Response Network for the U.S. West Coast (WARRN-West), at the Northwest Fisheries Science Center, National Marine Fisheries Service or the Florida Institute of Technology, Ocean Engineering and Marine Science Department.

#### **2.4 | Causes of death (COD)**

Based on the combination of gross necropsy, histopathology, and further diagnostic testing, one or more contributing causes of death were determined by the pathologist and/or the attending biologist who performed the necropsy. Causes of death were condensed into five categories for analysis: 1 - emaciation, 2 - infectious/inflammatory disease, 3 - trauma, 4 - biotoxin exposure, and 5 - other causes. A primary COD was identified, and secondary and tertiary contributing causes were also determined for multi-factorial necropsy cases. If upon necropsy the animal was markedly emaciated with little to no blubber layer, they lacked gastro-intestinal contents, and their fat levels in tissues was markedly low as determined by the



attending pathologist, emaciation was inferred to have at minimum been a contributing factor in mortality. If no signs of trauma and no other additional tests were warranted based on histopathology and necropsy, emaciation was determined by the attending pathologist as the primary COD. An infectious/inflammatory disease determination included gross or microscopic signs of inflammatory response to an infectious agent, isolation/detection of pathogens from lesions, and severity of infection indicating a primary COD above other contributing causes. Seals with evidence of fisheries interaction, entanglement, penetrating wounds, and broad hemorrhage indicative of blunt force injury were all considered to have had trauma as a contributing factor to mortality, if not as the primary cause of death. The more specific diseases, trauma, and other causes were determined upon gross and microscopic analysis and are presented in the Results; however, they were not used in statistical analysis due to the limited sample size for each. Primary, secondary, and tertiary CODs were assigned when possible for necropsied GFSSs and all three were considered contributing CODs for downstream analysis. In some

cases, contributing causes of death were evident, however, a definitive primary COD was unable to be determined due to scavenging and/or decomposition. If the animal did not clearly fit into one of the leading causes of death listed, and primary COD was determined, then they were listed as "Other" and are described further in the results section.

## **2.5 | Statistical analysis**

All statistical analysis included demographic and cause of death data collected from each level of examination discussed above, along with the environmental risk factors such as ocean conditions. All data were analyzed using the software R version 3.3.2 and SaTScan. Location, month and year of stranding, age class, sex, and ocean condition factors were used as stranding risk factors for all GFSS. Monthly oceanographic data and ecological integrity data were collected from the Pacific Fisheries Environmental Laboratory Live-Access Server (<http://www.pfeg.noaa.gov>), as well as from the National Data Buoy Center for Station 46050, the buoy at 44.6°N and 124.5°W, Stonewall Bank (20 nmi west of Newport, Oregon), and used as independent variables in the logistic regression analysis. These

factors included: the Pacific Decadal Oscillation index (PDO), which is a monthly value (range -3 to 3) calculated from the spatial average of sea surface temperature in the Pacific Ocean; upwelling index, which is measured in  $\text{m}^3/\text{s}/100$  m coastline and indicates amount of upwelling occurring from deep nutrient rich water to the surface; monthly average sea surface temperature at the buoy (SST) measured in Celsius degrees ( $^{\circ}\text{C}$ ); multivariate El Niño/Southern Oscillation index (MEI), a bimonthly time series index calculated from SST, sea level pressure, zonal and meridional components of the surface wind, and outgoing longwave radiation over the tropical Pacific basin; monthly northern copepod biomass anomaly, which is a measure of biomass above or below the average set at 0 with a range of -2 to 2; the monthly southern copepod biomass anomaly with a measure above or below the average set at 0 with a range from -2 to 2; and monthly copepod community richness, which is a measure above or below the average set as zero with a range of -10 to 10. The stranding risk factors, and ocean condition risk factors were used in regression analysis to determine their association or potential effect on the number of strandings, as well as for each leading

cause of death as described below.

The sample size of fur seal strandings did not allow for use of all the variables without overparameterization, therefore a reduction of the collinear factors was necessary. Kendall rank correlation analysis was used to determine correlations between ocean condition factors (SST, northern copepod biomass index, southern copepod biomass index, copepod richness, PDO, upwelling, MEI) and factors that were correlated with five or more other factors were not included in the analysis. Bivariate regression analysis was done with the noncolinear factors to determine significance and effect of the individual variables on the counts of strandings. Factors with  $p < .25$  were included in the multiple regression analysis. This less strict  $p$ -value was required at this early step to accommodate the amount of collinearity of the multiple ocean condition factors interested, and the low sample size during the initial bivariate regression analysis. Interactions of the independent variables were included in the model analysis as well. Forward model selection was used to find the best fit model by use of the log likelihood ratio test, this model selection was based on  $p = .05$  as is

standard for analyses.

A zero-inflated negative binomial model (ZINB) was used for the regression analysis of the number of strandings per month over the entire time frame. Residuals of a Poisson regression revealed the data were zero-inflated and overdispersed. Therefore, the Vuong test was used to compare nonnested models for fitness to the data, and revealed the ZINB model was most appropriate (Burger, Van Oort, & Linders, 2009; Greene, 1994). The ZINB regression analysis employs a two-part model with a count model and zero-inflated logit model that discerns the processes with which the zero outcomes are associated, such as, seasons or months with few to no strandings in any year. As seasonality of strandings is well known, descriptive statistics on the role of seasonality on stranding demographics was conducted, which led to seasonality being used for the zero-inflated logit linked portion of the model, winter was used as the reference variable (December-February), while the count portion of the model employed the ocean condition factors as well as year and month. The seasons broken down by month are as follows: Spring, March-May; Summer, June-August; Fall,

September–November. The best fit model was selected through forward model selection based on use of the log likelihood ratio test, and the final model was validated using a randomly selected subset of the data.

The main causes of death were analyzed for spatio-temporal clustering using the freeware SaTScan. The Bernoulli model was used for each leading COD separately to find spatio-temporal clusters unique to each leading COD (Kulldorff, 1997). In addition, a multinomial clustering approach was used to determine concurrent clusters of the categorical contributing causes of death according to time and space, and whether certain CODs occurred together in areas and time (Jung, Kulldorff, & Richard, 2010). Logistic regression analysis was conducted to determine risk factors related to each of the leading cause of death categories. Each primary cause of death category had a separate regression analysis, where the outcome variable was death due to the specific category compared to those with other causes of death. Therefore, this analysis only included seals with determined CODs ( $n = 93$ ). The environmental risk factors that could be related to the COD were examined through bivariate

logistic regression analysis for each primary and contributing cause of death. The multiple regression analysis was only carried out for the COD for which significance ( $p < .05$ ) was observed during the bivariate analysis that indicated a relationship between ocean condition and the COD. The best fit multiple logistic model for the leading COD was then determined by the smallest Akaike information criterion (AIC) value during a backwards stepwise regression analysis.

### 3 | RESULTS

#### 3.1 | Demographic and stranding range

Strandings of GFSs in the PNW were first reported in 1992 ( $n = 2$ ) and have been increasing with regularity since 2005, ranging from one seal in 2005 to a high of 56 seals in 2012, with fluctuations around a mean of 14.1 per year and a median of 8.5 (Figure 1). Of the 169 GFSs stranded, 21 were live at response and 148 were dead; of the 21 live stranded GFSs, 7 subsequently died, therefore 155 seals were dead at final disposition.

Throughout the study more juveniles stranded than any other age class, with a total of 139 yearlings, 20 weaned pups, 4 subadults, 5 adults, and 1 unknown age class. Of the stranded

GFS there were 53 confirmed females 55 males, 61 of unknown sex. Regardless of age class and sex, the majority of strandings occurred between May and July ( $n = 152$ , 89.9%) with June as the peak month of stranding every year since 2005 (Figure 2). Strandings ranged along the entire Oregon and Washington Pacific coasts (Figure 3).

### 3.2 | GFS stranding numbers regression analysis

The log likelihood test revealed that the best fit model including season as the logit portion only included the covariate of upwelling as a variable explaining increased stranding (Table 1). Correlation analysis revealed only five variables to be used in the bivariate analysis to avoid collinearity: year, month, upwelling, southern copepod biomass anomaly index, and copepod richness. MEI was significantly correlated with eight variables so was not included in the multiple regression analysis. However, as MEI has been related to GFS movements previously, a bivariate ZINB regression analysis was done using MEI as the independent variable, and revealed it was not significantly related to strandings of GFS in the PNW ( $p > .06$ ). The bivariate ZINB regression analysis of



the five noncollinear factors kept year, month, and upwelling for further multiple variable analysis. These variables were then included in a multivariable ZINB regression analysis and the best fit model with upwelling and season included was determined by use of the log likelihood test. The model was validated by applying a randomly selected subset of the data to ensure no assumptions were violated. As seasonality was an important consideration during the analysis, the demographic and cause of death numbers by season showed that most seals stranding in the summer were juvenile animals (97%,  $n = 129/133$ ), while only five animals stranded during winter months, four of which died (Table 2).

### **3.3 | Necropsy and histopathology results**

The three major causes of death categories found for the 93 necropsied GFSS were emaciation, trauma, and infectious disease. Small sample sizes precluded analysis of beyond two over-arching categories of infectious disease and trauma (Table 3).

Emaciation and infectious disease occurred concurrently in nearly half of examined GFSS ( $n = 41$ ). Emaciation was the most common contributing COD ( $n = 66$ ) and was determined to be the

primary COD in 41 cases (44.1%, Table 3). Infectious or inflammatory disease was the second most common contributing cause ( $n = 49$ ) and was the primary COD in 18 cases (19.4%). Trauma of various origins contributed to the mortality of 36 GFSs and was the second most common primary COD ( $n = 27$ , 29.0%), the types of trauma included, entanglement or fishery related trauma ( $n = 13$ ), blunt force trauma ( $n = 11$ ), bullet wounds ( $n = 2$ ), and shark attack ( $n = 1$ ) Table 4). In GFSs examined in this study, 13 seals died due to entanglement or fisheries interactions, either suspected or confirmed by gear entanglement or bycatch, these cases accounted for 48.1% of the trauma cases and the most common cause of trauma, followed by blunt force trauma (40.7%,  $n = 11$ ; seen by extensive internal hemorrhage). Of the seals necropsied, 19 (20.4%) had indications that human interaction was related to death via trauma either as the primary or secondary COD, while the majority ( $n = 61$ ) were cases in which human interaction could not be determined, and the remaining cases were determined to not be related to human interaction.

### **3.4 | Ancillary diagnostics results**

Upon necropsy, 85 GFSSs' stomach and/or scat contents were analyzed, 50 GFSSs had no food contents in their stomachs, 9 seals were either too decomposed or had a scavenged stomach at necropsy, 2 had plastic or Styrofoam in the stomach, 26 had minimal food contents found somewhere in their gastrointestinal tract, and one had a stomach with approximately 1,400 ml of fish remains including five partially digested sculpins. Seals with food contents had the following prey items found in their stomachs: squid beaks ( $n = 9$ ), some of which were identified as boreal clubhook squid (*Onychoteuthis borealijaponica*) ( $n = 4$ ) boreopacific armhook squid (*Gonatopsis borealis*) ( $n = 1$ ), and clawed armhook squid (*Gonatus onyx*) ( $n = 1$ ), fish parts including from smelt (*Osmeridae* spp.), herring (*Clupea* spp.), a sanddab (*Citharichthys* sp.), a North Pacific lanternfish (*Tarletonbeania taylori*), lamprey (*Petromyzontiformes* spp.), greenling (*Hexagrammidae* spp.), and skate spp. ( $n = 12$ ), shrimp ( $n = 5$ ), and crab/crustacean parts ( $n = 2$ ).

Necropsy and histopathology results in many cases led to bacteriology, parasitology, toxicology, and viral detection, the results of which were taken into account when determining

primary and contributing COD. The number of animals tested for each were as follows: protozoal parasitology  $n = 30$ , helminthic parasitology  $n = 21$ , bacteriology  $n = 36$ , toxicology  $n = 33$ , and viral detection  $n = 28$ . Seals with clinical signs upon necropsy of coccidian parasite infection ( $n = 30$ ) were tested via polymerase chain reaction (PCR) and immunohistochemistry (IHC) for coccidian parasitic infections. Of the 30 suspect positive seals, 22 were found to be positive for at least one coccidian parasite (Table S1). Eighteen had associated disease processes and pathology, including inflammation present in tissues and evidence of meningoencephalitis, which were deemed by the attending pathologist to be either a contributing or the primary cause of death. Fifteen seals tested positive for *T. gondii* via immunohistochemistry in lymph nodes and/or PCR from brain, muscle, lymph node, and/or heart. Eleven GFSs were PCR-positive for *S. neurona*, including one adult GFS which also tested positive via IFAT screening while alive then subsequently died, showing pathology in the brain consistent with an *S. neurona* infection. Three tested positive via PCR for *N. caninum*. One of the *N. caninum* positive GFSs was also infected with *S. neurona*,

the other two were infected with both *T. gondii* and *S. neurona*. The details associated with the protozoan parasite testing results are found in Table S1.

Pathogens detected with lower frequency included gastrointestinal helminthic parasites and bacterial pathogens. The gastrointestinal helminths observed in 21 GFS during necropsy are detailed in Table S2. In general, the level of infection with gastrointestinal helminths was low in necropsied GFSs. A subset of 36 seals with suspect pathology and/or available frozen tissues were analyzed for bacterial pathogens via bacterial isolation methods; 25 tested positive upon culture or PCR for one or more gram-negative and gram-positive bacteria. Most were found in the intestinal tract; however, many were also found in spleen, lung, or lymph nodes, with occasional culturing of bacteria from the brain. Bacteria of note that were cultured and detected via PCR from these 25 GFSs are detailed in Table S3.

The additional diagnostic testing included tests for biotoxins and viruses when warranted based on clinical signs upon histopathology or a foreseen risk based on biotoxin

presence in conspecifics in the region at the time of stranding. Cases with domoic acid toxin were detected, but no positive cases of saxitoxin, nor any positive cases of viral infections for any of the target viruses (influenza sp. and canine distemper). Of the 33 GFSs with urine, feces, or serum tested for biotoxins, six tested positive for detection of domoic acid by Biosence ELISA. Only one of the six positive cases showed signs of pathology associated with domoic acid toxicity. As there was only one case of domoic acid toxicity, this contributing COD is listed as "other" for the remaining analyses. Based on histopathology no other diagnostic tests were required.

### **3.5 | Spatio-temporal clustering**

Bernoulli spatial and temporal clustering models for each of the three main causes of GFS death revealed only one significant cluster of trauma cases; the center was located at 46.16°N, 123.97°W with a radius of 68.65 km (Figure 3). In that area, 16 of 21 (76.2%) GFSs died from various types of trauma. Other clusters for different individual CODs were found during the analysis, however, none were significant. Multinomial spatial

clustering analysis showed two significant clusters with multiple causes of death concurrent in two areas. The first significant cluster ( $p = .004$ ) was predominantly trauma cases (emaciation = 2, trauma = 13, infectious disease = 3, other = 1), and was found localized in roughly the same area as the individual Bernoulli trauma cluster; so, it was not included on the map (45.72°N, 123.94°W, radius = 78.71 km). The second significant multinomial cluster was found further north (46.95°N, 124.17°W, radius = 20.71 km; Figure 3) and included emaciation and infectious diseases cases ( $n = 15$  and 6, respectively).

### **3.5 | Factors relates to major causes of death**

The logistic regression analysis of factors such as ocean conditions relating to the leading cause of death showed that emaciation was the only major COD category for which ocean conditions were related. No factor was found to be significantly related to trauma cases or infectious disease cases during the bivariate logistic regression analysis, therefore only emaciation was used to explore the effects from ocean conditions (Table S4). From this analysis, five factors (month, PDO,

northern copepod index, southern copepod index, and copepod community richness) were initially included in the multiple regression analysis for the leading COD, emaciation. Through multiple regression analysis, the final model revealed that emaciation was most related to PDO (odds ratio: 1.713%-71.3% increased odds,  $p = .106$ ) and copepod richness (odds ratio: 0.937%-6.3% decrease in odds,  $p = .470$ ). This analysis does not account for interactions of emaciation and infectious disease, which have been observed and could contribute to mortality outcomes in many GFSs.

#### **4 | DISCUSSION**

Reemergence of GFSs in their northern historic range, and subsequent strandings, are seasonally driven and occur during times when upwelling of cold nutrient-rich deep water is highest in the region. Juvenile GFSs strand and appear to be migrating north postweaning, which is consistent with the archeological findings in northern Washington indicating a predominance of this age class historically, and a study utilizing telemetry after rehabilitation and release (Etnier, 2002; Lander, Gulland, & DeLong, 2000; Norris, DeRango, DiGiovanni, & Field, 2015).



These juvenile seals appear to be stranding in greatest numbers due to some of the most common causes of death in postweaning pinnipeds, emaciation, trauma, and infectious disease which reflect early stage postweaning seals being unsuccessful in their attempts to forage and thrive independently in this area (Huggins et al., 2013; Steiger et al., 1989; Warlick et al., 2018). Trauma cases were found clustered in northern Oregon and the southern portion of Washington, which is a known area of high productivity and commercial fishing (Wieting, 2012). A small cluster of emaciated and infectious disease cases was found further north in Washington, centered near Ocean Shores. Infectious agents such as gastrointestinal, and particularly coccidian parasites, were found more commonly than other pathogens. Ocean conditions associated with high productivity were related to strandings and the major COD, emaciation. The seasonality of GFS strandings and these ocean conditions show that juvenile GFSs are likely moving in search of prey associated with the highly productive time of year.

Seasonality is implicated as a driving force for the presence of GFSs in this area. During the summer months when

strandings were most prevalent, ocean currents bring nutrient rich, cold water from the northern seas into the PNW waters and upwelling brings up nutrient rich, deep water (Peterson et al., 2014). Confluence of currents and upwelling lead to nutrient rich and highly productive waters in this area, which are utilized by many marine animals, including large schools of migrating fish stocks exploited by a wide range of predators. It is possible that the GFS are following northward migrating prey sources such as squid, sardines, and anchovies from Mexico and California, or that they are migrating north to find seasonally abundant prey in these productive waters (Wetherall, 1991). The mere seasonal increase of GFSs, as well as an overall yearly increase in the area, as documented in this study, may be the driving factor for increased stranding events. Our results support this conclusion as seen by the relation of monthly variation of upwelling and season to increase strandings, and the significant effects of season and increased SST in initial bivariate models. Similar findings looking at SST, productivity, and changes in GFS foraging habits has been observed in Mexico related to El Niño events, which are commonly associated with a

higher SST (Elorriaga-Verplancken et al., 2016). This study did not find that increased stranding of GFS in the Pacific Northwest was related to El Niño events or any extreme warming event as documented elsewhere in their range, as neither SST nor the MEI were kept in the final most explanative model or found to be significantly related to increased stranding, as upwelling and seasonality were. There may be observation bias during the summer due to an increased presence of beachgoers to report the stranding events more frequently. However, the potential bias cannot explain the degree of seasonality witnessed in this study, as there are still surveys on many beaches conducted during the other seasons, and the results found are consistent with the life history of this species seen previously.

Juvenile pinnipeds strand more than any other age groups because they are naïve to challenges in their ecosystem (Baker, Jepson, Simpson, & Kuiken, 1998; Hanni et al., 1997; Osinga et al., 2012). Weaned pups are at the most difficult stage of their life as they quickly exhaust fat stores obtained through nursing and are learning to forage (Bowen, den Heyer, McMillan, & Iverson, 2015; Gerdts, van Drunen Littel-van den Hurk, & Potter,

2016; Harding, Fujiwara, Axberg, & Harkonen, 2005). Our findings, that young GFSs in Washington and Oregon are in suboptimal nutritional condition, are consistent with these natural trends. Increased presence of juvenile GFSs in the PNW may indicate that they are avoiding competition with adults for resources or following a relatively easy prey source to forage on. However, this puts these young GFSs at risk of trauma due to predation or fisheries interactions as well as novel pathogens. After weaning, young animals no longer benefit from continued exposure to maternal antibodies and therefore have a relatively naïve immune system to pathogens (Ross, Pohajdak, Bowen, & Addison, 1993; Ross et al., 1994; Van de Perre, 2003), which can lead to an increased risk of succumbing to infectious disease.

Trauma, emaciation, and infectious disease were found occurring in all age classes along the Washington and Oregon coasts, however, distinct clusters were identified. A cluster of trauma cases around the mouth of the Columbia River observed in our study (Figure 3) is of interest as this is a heavily used area by the fishing industries, and one of the leading causes of death due to trauma were fisheries interactions, including

entanglements (Hirose, Miller, & Hill, 1998). The role of fisheries in GFS and marine mammal deaths should be further studied and reduced where possible. Upon necropsy, often the only sign indicating acute trauma in GFSs was hemorrhage, leading to a limited determination of blunt force trauma as the likely type of trauma without a definitive source being determined, such sources could be predation or human related. Human interaction related to mortality in marine mammals is often difficult to assess adequately unless gear from fisheries is found or obvious signs of human related trauma are present, so it is possible human interaction is underrepresented in this study (Moore et al., 2013).

The other cluster of CODs found near Ocean Shores, Washington, consisting of emaciation and infectious disease cases, could indicate that, when further north, reduced physical fitness is the leading risk factor to cause mortality. There could be reduced risk of trauma in this area, thus seals stranding there are dying due to overall lack of fitness rather than any acute cause. The relationship between a high PDO and a low copepod species richness with emaciation cases indicates

that in low productivity conditions there is a 71.3% increased chance of dying of emaciation. It appears that these factors together are good indicators for low prey availability and an increased risk of GFSs dying of emaciation. Their unusual mortality event in California in effect since 2015, during which many emaciated GFSs stranded along the west coast, suggests a lack of resources throughout the California current system as well (Norris et al., 2015). While emaciation is seen in wild animals frequently due to a variety of circumstances, the infectious agents putting these GFSs at risk could become more problematic as young and nutritionally challenged GFSs continue to re-establish this portion of their range.

Emaciation and nutritional stress are known to compromise immune systems, which allow a pathogen to cause disease postinfection, comparatively, an infectious agent could decrease the animal's fitness leading to emaciation from lack of adequate nutrition (Klasing, 1998). It is difficult to know if an infectious disease decreased the viability of the animal and led to emaciation or vice versa. Likely in some cases, one preceded the other, but when both occur an animal has little chance of

success in the wild environment. The concurrence of these causes further shows that the GFSSs are in a state of reduced viability further north in the PNW whether it be by pathogen load and/or lack of resources. This could be an area of increased pathogen levels or more likely, somewhere along the migration they encountered disease causing pathogens, thus reducing fitness during their movement north.

The pathogens found most commonly in stranded GFSSs during this study were coccidian and gastro-intestinal parasites. However, it is likely that the bacterial pathogens and potentially viral pathogens are underrepresented in our study as only subsets of seals were tested for such pathogens. The coccidian parasites, *Toxoplasma gondii* and *Sarcocystis neurona*, appear to have the greatest prevalence of any pathogens in GFSSs. However, these parasites are screened for more rigorously and readily than other microscopic pathogens, so the higher coccidian prevalence may be an artifact of limited testing for other pathogens. It is possible that GFSSs are exposed to these parasites anywhere along the west coast as they migrate; however, *T. gondii*, *S. neurona*, and *N. caninum* are prevalent in

marine mammals on the coast of Oregon and Washington, so the potential for their exposure in the PNW is highly plausible (Barbosa et al., 2015). Many species of marine mammals are found to be infected with parasites in this area and California, as well as many species having high mortality due to co-infection of these parasites or infection with a particularly pathogenic genotype (Barbosa et al., 2015; Gibson et al., 2011; VanWormer et al., 2014). Future work should include determining the genotypes of *T. gondii*, *S. neurona*, and *N. caninum* in their respective endemic host species in Oregon and Washington compared to southern California or Mexico to inform whether infection of GFSs is geographically restricted.

Gastrointestinal helminths were relatively common in stranded GFSs as well and are comparably easy parasites to discover upon gross examination. The level of gastrointestinal parasitic infections found in the present study did not appear to have a major direct impact on the health of the GFS population, but instead may have contributed to overall lack of physical fitness. In this study, a few specimens of anisakid nematodes; *Anisakis simplex* 1, *Contracaecum*, and *Pseudoterranova*



were found in stomachs and small intestines of GFS. *Anisakis simplex 1* is more typically found in cetaceans than pinnipeds with the capability to cause severe health problems for young pinnipeds (Smith & Wootten, 1978; Spraker, Lyons, Tolliver, & Bair, 2003). Prevalence of gastrointestinal parasites in GFSs has largely been unknown; however, prevalence of these parasites in other pinnipeds in the region has been well documented for decades (Dailey, 2005; Keyes, 1964; Kuzmina, Lisitsyna, Lyons, Spraker, & Tolliver, 2012; Kuzmina, Lyons, & Spraker, 2014; Shults, 1986). It is expected that these parasites are underrepresented in the data set, as rigorous monitoring for them was not undertaken until midway through this study, nor do we know what their prevalence is in nonstranded GFS. Thus, insufficient data are available to describe the entire parasite community of GFSs in this study. The occurrence of parasitic infections in GFS is of interest for stranding responders and rehabilitators aiming to improve response in the future.

Since 2005, a high number of stranded GFSs has occurred, while the yearly trend has increased gradually. While there were two years with higher stranding numbers than others, no ocean

condition factors were found to be directly related to these higher stranding years. In this study the two years with the highest number of strandings were 2012 which was classified as a La Niña year, and 2016 which was considered an El Niño year, so while these two years saw higher numbers the ocean conditions were very different. It is necessary to continue monitoring stranding trends in relation to ocean conditions and their population growth to determine if anomalous weather or ocean conditions are directly related to higher numbers or if it more closely related to years of high pupping numbers. Their presence and subsequent strandings will likely continue under similar circumstances, as elucidated during this study. It is presumed that the stranded GFSs are an underrepresentation of both the live and deceased seals present in the area, and it is possible that even with regular beach surveys some stranded GFSs were overlooked (Huggins et al., 2015). However, dedicated efforts by stranding networks to find stranded seals via beach surveys were conducted semiregularly in the study area as many beaches in Washington and northern Oregon can be surveyed via vehicle. This ability may add observer bias and contribute towards more

strandings being reported in northern Oregon and Washington south of the Olympic National Park as access to beaches in southern Oregon and the Olympic National Park is logistically challenging. However, the widespread strandings discovered throughout the region and the variation in major COD between clusters indicate that this bias would not likely drastically change the clustered areas if completely corrected for.

This study was the first to undertake an epidemiological study to investigate variables affecting GFSs strandings in the PNW; including the effects of ocean condition on stranding numbers and emaciation. These results indicate a need to further monitor the presence of this species and ocean condition changes related to seasonality and climate change. While no single specific disease was found to be a major cause of death, there were cases of infectious diseases documented in GFSs that are of importance to other marine mammals and humans such as *S. neurona*, *T. gondii*, and *Anisakis*. Further studies on the genetics and diseases of this species may elucidate the downstream effects of the genetic bottleneck seen in this species, which may increase risk for the entire population to

novel pathogens (Weber et al., 2004). Elevated risk of trauma around the mouth of the Columbia River, including human related trauma, indicates a need for further monitoring of the fisheries and boat activity common in that region. The findings of this research suggest that no single pathogen or source of trauma are driving deaths in GFSs or their overall stranding in the PNW.

It is our determination through this study that the increased levels of stranding are likely due to an elevated use of this habitat within their historic range and suggest a healthier population size overall, which was estimated in 2016 at approximately 20,000 individuals and similar conclusions have been suggested to explain their use of areas in central Mexico as well (Carretta et al., 2017; Ortega-Ortiz, Vargas-Bravo, Olivos-Ortiz, Zapata, & Elorriaga-Verplancken, 2019). As the climate continues to change, and anomalous ocean and weather conditions increase, the continued assessment of risks and subsequent effects in ecosystems is needed if we are to minimize damage to species like the Guadalupe fur seal, which are currently threatened (Hernández-Camacho & W. Trites, 2018). Habitat reconstruction, shifts or reclamation due to climate

change, have been documented previously for a range of marine predators, and studies done thus far suggest a continued need to assess such changes as this study has done for GFSs (Bakun et al., 2015; Hazen et al., 2013). The reemergence of this population in the PNW we found occurring is a testament to the effective implementation of the Marine Mammal Protection Act, the Endangered Species Act, and their protections under Mexican law. When increasing human impacts on ecosystems threaten many species, continued and enhanced regulation of fisheries is needed. Such actions can further reduce fisheries interactions with marine mammals, thus reducing a known and documented risk for the recovering GFS population. Additionally, as climate change continues to change ocean conditions, overall productivity, and prey availability, threatened species like the Guadalupe fur seal will likely be put at risk regardless of their population growth and the stability gained from conservation efforts.

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**TABLE 1** Multiple zero-inflated negative binomial (ZINB) regression analysis best fit model for factors associated with strandings per month in 169 stranded Guadalupe fur seals from 2005–2016 in the Pacific Northwest of the United States. Model construction used environmental variables in the negative binomial count model and seasons in the inflation model with winter as the reference variable. Best fit model selection based on log likelihood ratio test.

<b>Factors</b>	<b><i>p</i></b>	<b>Odds ratio</b>
Negative binomial count coefficients		
Upwelling	0.0137	1.01
Zero-inflation logit coefficients		
Spring	0.332	0.359
Summer	0.217	0.247
Fall	0.993	2.36e-8

**TABLE 2** Number of Guadalupe fur seal demographics stranding in Oregon and Washington during each season and the causes of mortality found during those seasons.

Season	Total	Juveniles	Adults	Male	Female	Trauma cases	Infectious disease cases	Emaciation cases
Winter	5	3	2	1	2	1	2	0
Spring	23	21	2	7	7	1	2	8 (20.5%)
Summer	133	129 (97%)	3	45	29	23 (88.5%)	14 (77.8%)	29 (74.4%)
Fall	8	6	2	1	4	1	0	2
Total	169	159	9	54	42	26	18	39

**TABLE 3** The primary and contributing causes of death reported for Guadalupe fur seals that were examined during necropsy in Oregon and Washington between 2005 and 2016, including sex and age class determined during examination. Contributing COD includes the number of primary COD cases for that category along with secondary and tertiary CODs when determined.

Cause of death (COD)	Primary COD	Contributing COD	Males	Females	Juveniles	Subadults	Adults
Emaciation	41	69	17	16	33	0	2
Infectious disease	18	49	8	8	13	1	2
Protozoal parasitic disease	4	18	1	3	3	0	1
GI parasites	1	8	1	0	1	0	0
Bacterial sepsis	2	2	0	1	0	0	1
Hepatitis	0	1	1	0	0	1	0
Unknown infectious disease process	11	20	4	5	9	0	0
Trauma	27	36	12	11	25	2	0
Predation	1	1	1	0	1	0	0
Human related trauma	17	20	7	6	15	2	0
Unknown cause of trauma	9	15	2	5	9	0	0
Other – Toxic exposure	1	2	0	0	1	0	
No primary COD identified	6						



Total seals

93

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**TABLE 4** Types of trauma found contributing to and as the primary cause of death (COD) in Guadalupe fur seals stranded in Washington and Oregon between 2005 and 2016. Contributing COD includes the number of primary COD cases for that category along with secondary and tertiary CODs when determined.

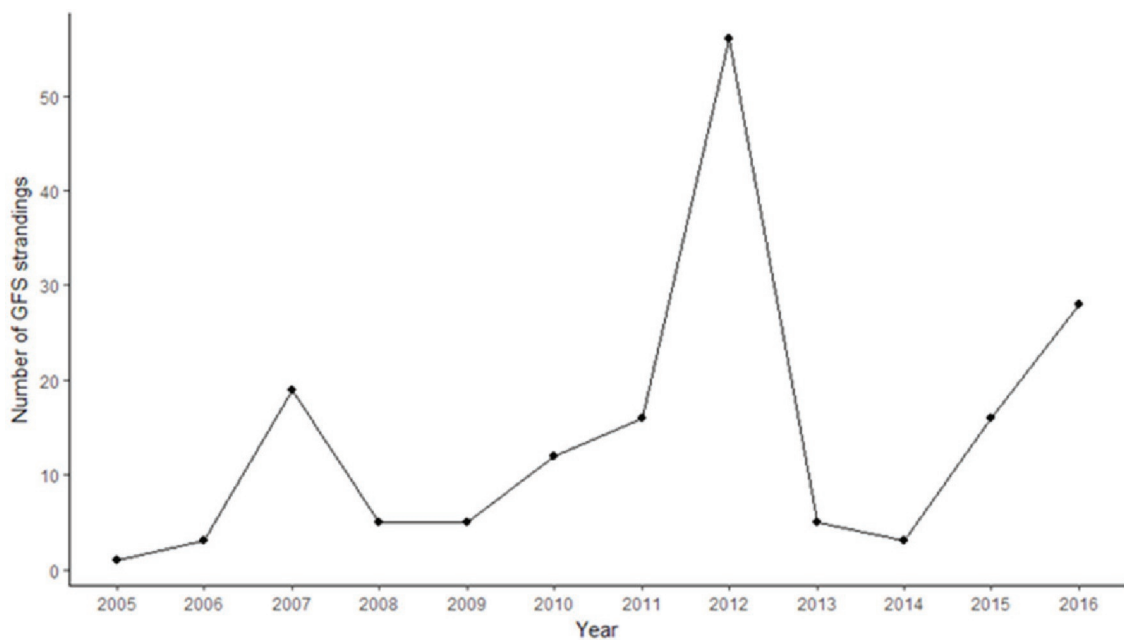
<b>Cause of trauma</b>	<b>Primary cases</b>	<b>Contributing cases</b>	<b>Males</b>	<b>Females</b>	<b>Juveniles</b>	<b>Subadults</b>
Entanglement/Fisheries related trauma	13	14	4	6	12	
Confirmed	5	6	1	3	4	
Suspected from lesions upon necropsy	8	8	3	5	8	
Blunt force trauma	11	16	5	5	9	1
Suspected human related trauma	3	3	2	1	2	
Unknown cause of trauma	8	13	3	4	7	1
Shot	2	2	1	1	1	1
Predation – shark	1	1	1	0	1	
Penetrating lacerations – unknown origin leading to bacterial sepsis	0	2				
Total cases	27	36	11	12	23 <sup>a</sup>	2 <sup>a</sup>

<sup>a</sup>There were no adult animals that died due to acute trauma, however there were two animals with unknown age classes.

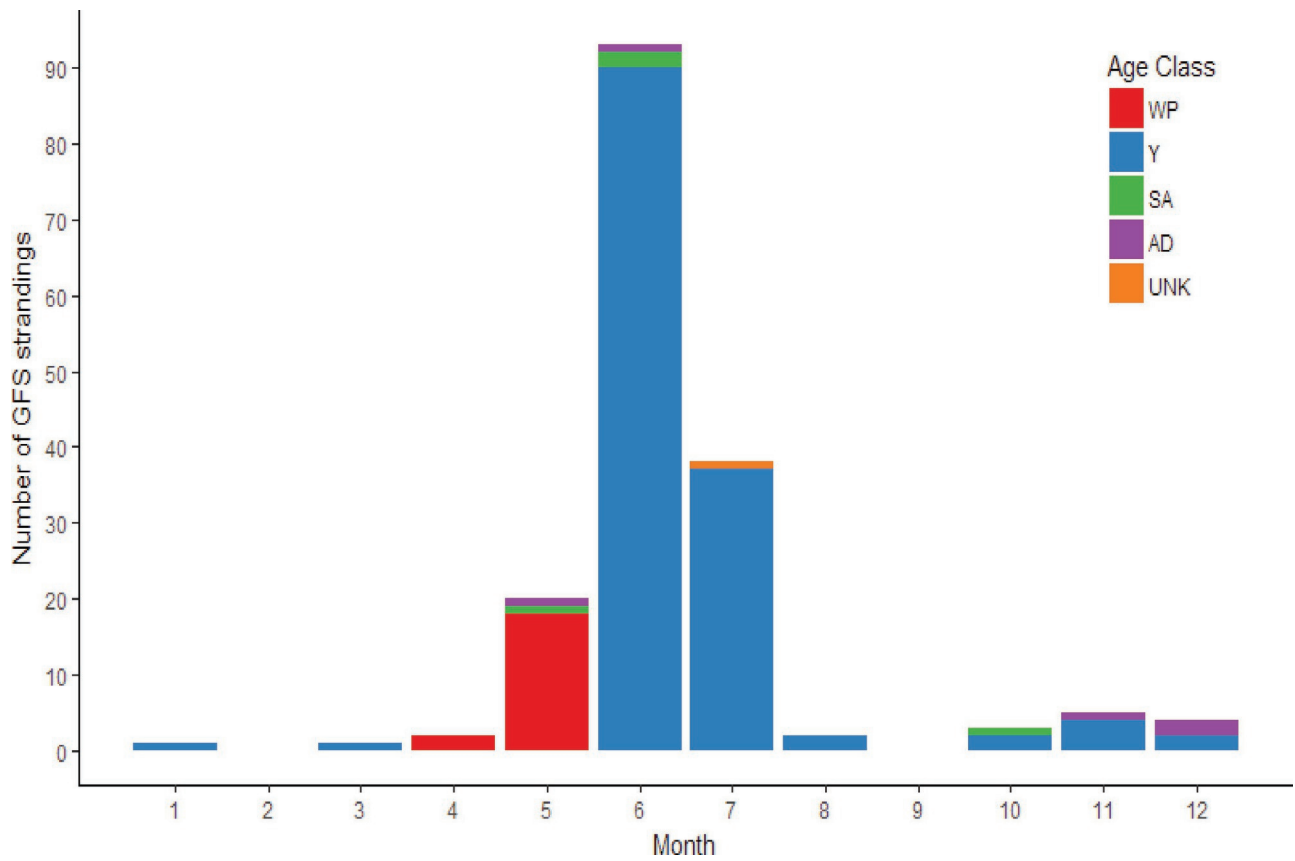
**FIGURE 1** Number of Guadalupe fur seals ( $n = 169$ ) strandings per year in Oregon and Washington from 2005 to 2016.

**FIGURE 2** Number of stranded Guadalupe fur seals in Oregon and Washington ( $n = 169$ ) of each age class for all months of the years 2005 through 2016; WP: weaned pup ( $n = 20$ ), Y: yearling ( $n = 139$ ), SA: subadult ( $n = 4$ ), AD: adult ( $n = 5$ ), UNK: unknown ( $n = 1$ ).

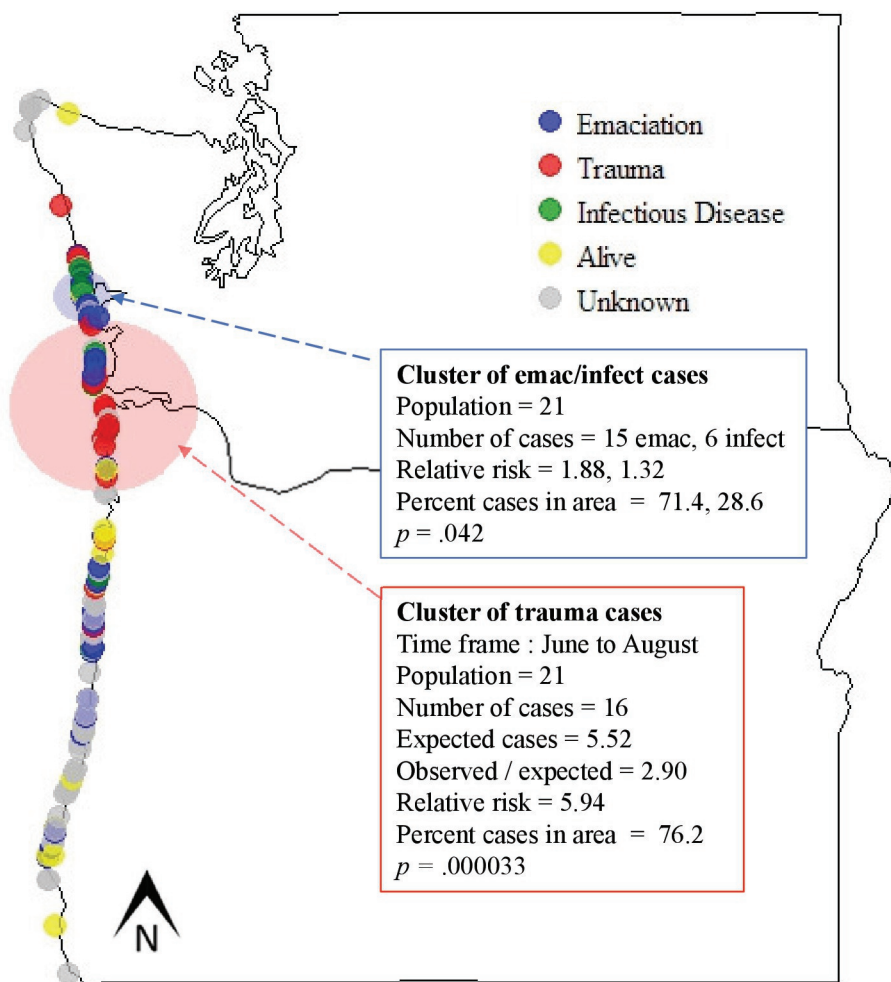
**FIGURE 3** Map of all stranded Guadalupe fur seals ( $n = 169$ ) in Oregon and Washington from 2005 to 2016 including animals with determined causes of death. Red circle indicates the significant cluster of trauma cases from the Bernoulli clustering analysis and the blue circle indicates a cluster of emaciation and infectious disease cases from the multinomial clustering analysis, included animals ( $n = 99$ ) with all having one of three primary causes of death or stranded alive.



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