Island Spotted Skunk Conservation Plan 2021



Photo by Juliann Schamel

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1. The Island Spotted Skunk Working Group

Section Leads: Christina Boser, Laura Shaskey, Kevin Crooks

During a meeting in Ventura, California in January 2020, the Island Spotted Skunk Working Group (ISSWG) was created as a forum for researchers, biologists, and managers to exchange ideas, share research, collaborate on projects, and discuss relevant issues regarding the island spotted skunk (*Spilogale gracilis amphiala*). The ISSWG was patterned off the Eastern Spotted Skunk Cooperative Study Group (ESSCSG 2019). The goals of the ISSWG are to: (1) enhance communication about the island spotted skunk; (2) identify management, research, and resource priorities; and (3) facilitate collaborative planning, funding, outreach, monitoring, and research opportunities. As of April 2021, the ISSWG was composed of 44 members representing 10 universities, 4 federal agencies, and 6 NGOs (Appendix 1).

2. Purpose of this Document

Section Leads: Christina Boser, Laura Shaskey, Kevin Crooks

This conservation plan was constructed following discussion by members of the nascent ISSWG at a meeting in Ventura, California January 16-17 2020. This document is adapted from a similar plan developed by the Eastern Spotted Skunk Cooperative Study Group (ESSCSG 2019). Primary goals of the document include: 1) summarize what is known about the island spotted skunk and its current status; and 2) identify the knowledge gaps that should be the target of future research, monitoring, and management actions to advance island spotted skunk conservation. The document will be updated annually, with each successive annual report posted on-line for the public.

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3. Distribution and Taxonomy

Section Leads: Kevin Crooks, Paul Collins

The island spotted skunk is endemic to San Miguel, Santa Rosa, and Santa Cruz Islands of the Northern California Channel Islands. The purported collection of a spotted skunk from San Miguel Island in the 1870s (Henshaw 1876), coupled with the occurrence of spotted skunk remains at Daisy Cave, a late Holocene site (Walker 1980), confirm both the historical and contemporary occurrence of this species on San Miguel Island. No spotted skunks were captured during intensive live-trapping conducted for island foxes on San Miguel Island since the 1980s, which suggests that spotted skunks have been extirpated from that island; more than 100 years of grazing by feral sheep and burros probably contributed to this extirpation. Today, island spotted skunks occur only on Santa Cruz and Santa Rosa Islands, where they are widely distributed. The elevational range of the island spotted skunk extends from sea level to ~600 m (1,969 ft).

Spotted skunks in western North America historically have been treated as distinct at the species level (*S. gracilis*, Grinnell 1933, Grinnell et al. 1937, Miller and Kellogg 1955, Hall and Kelson 1959, Williams 1979, Jones et al. 1992, 1997; Baker et al. 2003) or as a subspecies of the eastern

spotted skunk (*S. putorius*) (Van Gelder 1959, Hall 1981). Dragoo et al. (1993) presented genetic evidence supporting the western spotted skunk as a distinct species (*S. gracilis*) from the eastern spotted skunk (*S. putorius*). Dragoo and Honeycutt (1997) used mitochondrial DNA evidence to move three skunk genera (*Spilogale*, *Mephitis*, and *Conepatus*) from the family Mustelidae into the family Mephitidae.

Today the island spotted skunk is classified as one of 15 described subspecies of western spotted skunk (*S. gracilis*), a species found throughout much of western North America, including Baja California, and the plateau area of northern Mexico (Wilson and Reeder 2005). The island spotted skunk was first described by Dickey (1929) as an insular endemic restricted to Santa Cruz and Santa Rosa Islands and was assigned the scientific name *Spilogale phenax amphialus*. This subspecies name for the island spotted skunk was used by mammalogists (e.g., Grinnell 1933, Miller and Kellogg 1955, Hall and Kelson 1959) until van Gelder (1959) changed it to *amphiala*. Since this name change, *amphiala* has been used as the accepted subspecies name for the island spotted skunk (Hall 1981).

Using microsatellite loci from DNA, Floyd et al. (2011) found strong genetic differentiation between each of the island spotted skunk populations and mainland spotted skunks and suggested that each of the island populations may constitute evolutionarily significant units worthy of conservation (see Genetics Section 8d). The level of genetic divergence observed among island skunk populations may support the elevation of each island population to separate subspecies or possibly even full species status (Floyd et al. 2011). Further, recent analysis of the mitochondrial genome demonstrated that the two island spotted skunk populations diverged from one another and the mainland during the Holocene (Bolas et al. *in review B*). This pattern further supports elevation of island spotted skunks to species designation by island, as they have been separated from one another and the mainland for thousands of years (see Genetics Section for more detail).

4. Conservation Status

Section Leads: Kevin Crooks, Paul Collins

The island spotted skunk is listed as a Subspecies of Special Concern by the State of California. It is not listed as a threatened or endangered species under the California Endangered Species Act. Neither the island spotted skunk, nor the mainland western spotted skunk, is listed federally under the U.S. Endangered Species Act.

Crooks and Van Vuren (1994, 2000) recommended that island spotted skunks be listed as a Threatened subspecies rather than a subspecies of Special Concern by the State of California. Recent genetic evidence supports elevation of island spotted skunks to species designation by island, as they have been separated from one another and the mainland for thousands of years (Bolas et al. *in review B*). Moreover, the global distribution of the island spotted skunk is limited to two islands with a total population size likely less than 1000 individuals., They were extirpated from a third island (San Miguel) in contemporary times. Their restricted distribution and small population size make the island spotted skunk among one of the rarest mammalian carnivores globally.

5. Natural History

Section Leads: Kevin Crooks, Paul Collins

The island spotted skunk is a medium-sized skunk with a complex pattern of white stripes and spots (Van Gelder 1959, Verts et al. 2001). It differs from other subspecies of the western spotted skunk by its shorter tail (95-175 mm) with less white ventral coloration (45% white compared to 55% white in mainland western spotted skunks), slightly larger size (222-317 mm body length), broader skull (38 mm facial breadth), and proportionately less white and more black in overall pelage color (Dickey 1929, Van Gelder 1959, 1965, von Bloeker 1967). The two island populations are morphologically indistinguishable (Van Gelder 1959), although genetic evidence suggests spotted skunks on Santa Cruz and Santa Rosa islands are genetically distinct from each other and their mainland relatives (Floyd et al. 2011). Like mainland subspecies, island spotted skunks exhibit sexual dimorphism, with males averaging ~550-650 g and females averaging ~400-500 g (Van Gelder 1959, Crooks 1994a; Jones et al. 2008). Spotted skunks are considerably smaller (355-466 mm total length) than striped skunks (*Mephitis mephitis;* 575-800 mm total length) on the mainland and have softer, glossier pelage (Van Gelder 1959).

The breeding season for spotted skunks on the islands is unknown but probably similar to that of western spotted skunks on the mainland. Western spotted skunks breed in late September and early October. Total gestation lasts 230-250 days and consists of a 200-220 day period of delayed implantation (Mead 1968, Foresman and Mead 1973), after which embryos (blastocysts) reactivate and implant during March and April. After 2 weeks of development, two to five young (average 3.8) are born from April to May (Mead 1968, Verts et al. 2001). Sex ratio at birth for 9 litters of mainland western spotted skunks was 2.3:1 in favor of males (Mead 1968). Three and five uterine scars have been recorded from two skunks collected on Santa Cruz Island in September (Pearson 1948 unpublished field notes, Van Gelder 1959).

Western spotted skunks are omnivores, feeding largely on insects and small mammals and occasionally on carrion, berries, and fruits (Verts et al. 2001; see Diet Section 8f). Analysis of scat contents from island spotted skunks on Santa Cruz Island during a period of low population densities in 1991-1992 showed they consumed primarily deer mice (*Peromyscus maniculatus*) and insects along with occasional lizards and birds (Crooks and Van Vuren 1995). Jerusalem crickets (*Stenopelmatus fuscus*) were the most frequent prey, but other prey included grasshoppers, crickets, beetles (scarab, darkling, and long-horned beetles), caterpillars, European earwigs (*Forficula auricularia*), and ants. Fruits and berries were absent from this sample of 41 scats (Crooks and Van Vuren 1995). Subsequent scat analyses in 2003-2004 (Jones et al. 2008), during a period of higher skunk density, found a different and broader range of foods, including a high occurrence of invertebrates, a moderate occurrence of vertebrates, and a low occurrence of plants (fruits and berries), particularly in the dry season.

At lower skunk population densities in 1992, mean home range size (Minimum Convex Polygon, MCP) for spotted skunks on Santa Cruz Island was 23.2 ha (n=7) during the wet season and 40.0 ha (n=1) during the dry season (Crooks and Van Vuren 1995). At high population densities in

2003-2004, mean annual MCP home range size was 46.9 ha (n=26), and mean seasonal home range size was 29.4 ha (n=33) (Jones et al. 2008). Thus, home range size did not decrease with increasing skunk density as might be expected. Males have larger seasonal home ranges than females (Jones et al. 2008). Home ranges of adults were largely non-overlapping, consistent with expectations for a solitary species (Jones et al. 2013). Contrary to expectations, Jones et al. (2013) found no evidence of matrilineal spatial structure. Relatedness of adult females was not negatively correlated with geographic distance, nor was it positively correlated with overlap of home ranges, possibly because of excessive dispersal caused by high densities in 2003-2004. Jones et al. (2013) did find evidence of a patrilineal structure at one study site, where relatedness among males was negatively correlated with distance. The explanation for differences between males and females in genetic structure is uncertain, but it may have reflected the differential influence of high density on dispersal of males and females.

Island spotted skunks show habitat preferences similar to those reported for the mainland subspecies (Grinnell et al. 1937, Zeiner et al. 1990; Verts et al. 2001; see Habitat Use Section 8h). Based on a radio-telemetry study in 1992, skunks on Santa Cruz Island showed a preference for ravines dominated by coastal sage scrub (Crooks and Van Vuren 1994, 1995). Radio-collared skunks in 2003-2004 used a wide variety of habitat types, although they tended to avoid open habitats compared to more heavily vegetated sites (Jones et al. 2008). High skunk densities during this period may have forced them to use a broader array of habitats than they did in 1992. On Santa Rosa Island, spotted skunks were associated with rocky canyon slopes, cactus patches (Sheldon 1927), chaparral, coastal sage scrub, open woodland, other scrub-grassland communities, and riparian habitat. On both islands, the species also has been recorded in or under human dwellings and ranch outbuildings (von Bloeker 1967, Laughrin 1982; Crooks 1994b). A study of microhabitat associations of skunks using detections from cameras and traps in 2015-2017 found that skunks on both islands had positive associations with ground-level cover provided by rugged topography or woody vegetation such as low-growing shrubs or trees and logs (Bolas et al. *in review a*).

On Santa Cruz Island in 1992, spotted skunks denned under shrubs (29.8%), in open grassland (21.3%), in cavities in rocks (21.3%), in road cuts (10.6%), under human structures (10.6%), and in cavities in roots and trunks of trees (6.4%, Crooks 1994b). Individuals used several dens distributed throughout their home range; some dens were used by two or more individuals either sequentially or, for females, simultaneously (Crooks 1994b). In 2003-2004, island spotted skunks were documented occasionally using unprotected dens and appeared to share dens less commonly than recorded in 1992 (Jones et al. 2008).

Golden eagles (*Aquila chrysaetos*) were known predators of island spotted skunks (Collins and Latta 2009, Collins et al. 2009) during a period when golden eagles were extensively nesting and foraging on the islands. Golden eagles have been rarely observed on the islands for the last decade, since the removal of feral pigs and grazing animals, reintroduction of bald eagles, and the recovery of island foxes. Other avian species that may prey on or scavenge occasionally on spotted skunks include red-tailed hawk (*Buteo jamaicensis*), bald eagle (*Haliaeetus leucocephalus*), and common raven (*Corvus corax*). However, these avian predators are active during the day when skunks are unlikely to be active outside their dens. On the other hand, island foxes are both diurnal and nocturnal and fox predation on island spotted skunks has also

been previously documented at low rates (Cypher at al., 2014; and see Interaction with Foxes Section 8c).

Island spotted skunks are primarily nocturnal and highly secretive, with activity beginning around dusk, peaking during the early evening, and continuing on and off until around dawn (Crooks and Van Vuren 1995, Jones et al. 2008). Females tend to be more active than males, particularly at dusk and during the day, although some daytime activity may be restricted to within dens (Jones et al. 2008). Temporal separation of island spotted skunks (nocturnally active) and island foxes (active throughout the day and night) may moderate resource and interference competition between them (Crooks and Van Vuren 1994, 1995; See Interaction with Foxes Section 8c).

6. Population Trends

Section Leads: Kevin Crooks, Adam Dillon

Historically island spotted skunks were relatively rare (Sheldon 1927, 1928, Laughrin 1982, Crooks 1994a), at least until the decline of island foxes on the northern Channel Islands during the 1990s due to predation by golden eagles (Roemer et al. 2001, 2002). Capture success for skunks (skunk captures per 100 trap nights) increased dramatically on Santa Cruz Island from 0.5% in 1992 (Crooks 1994a), to 3.8%-10.3% in 1998 (Crooks and Van Vuren 2000; Roemer et al. 2002), to 13.9% in 1999 (Roemer 2002), to 38.1% in 2003-2004 (Jones et al. 2008), suggesting a population increase (Dillon et al. in prep; Figure 1). Estimated density of skunks in 2003-2004 was exceptionally high (~9-19 skunks/km², Jones et al. 2008). NPS documented a similar increase in spotted skunks on Santa Rosa Island with trap success increasing from 5.8% in 1998 (Roemer 1999) to 57.9% in 2006 (Dillon et al. in prep: Figure 1). Skunk densities remained high on Santa Rosa Island through the mid-2010s (~8-16 skunks/km² in 2009-2014 (Dillon et al. in prep; Figure 1).

The increase in skunk numbers was likely due in part to competitive release due to the decline of island foxes (Crooks and Van Vuren 2000; Jones et al. 2008; Dillon et al. in prep; see Interaction with Foxes Section 8c). Consequently, as fox populations recovered following the removal of golden eagles from the Channel Islands, skunk populations declined, likely as a result of renewed exploitative and/or interference competition (including predation) with island foxes (Dillon et al. in prep). On Santa Cruz Island, the numbers of skunk captures on standardized fox trapping grids has dropped by an order of magnitude since 2009, with fewer than 10 captures annually from 2015 through 2019. On Santa Rosa Island, the skunk population decline occurred later, with declines of skunk captures on fox trapping grids evident since 2013 and a mean of 10 captures annually from 2017 through 2019. Although capture numbers for skunks are based on a trapping protocol designed for island foxes, cameras superimposed on trap grids on Santa Cruz Island in 2016 and both islands in 2017 supported the assumption that capture success is an accurate reflection of trends in skunk abundance: during the trapping season, detection rates of skunks using both traps (0.92/100 operating nights) and cameras (0.72/100 operating nights) were quite low (Bolas et al. 2020). The future trajectory of island spotted skunk populations, and potential equilibrium numbers of skunks, is currently unknown.

Figure 1. Island spotted skunk trap success and density with 80% confidence intervals on (A) SCI and (B) SRI. Grey boxes indicate when fox captive breeding occurred. Reprinted from Dillon et al. In Prep.



7. Possible Threats

Section Lead: Kevin Crooks, Paul Collins

Possible threats to island spotted skunk persistence include rarity, island foxes, disease, vegetation changes, and low genetic variability. Overall, the status of island spotted skunks is of concern due to the low numbers of island skunks trapped, captured on wildlife camera images, and observed in the field over the past 5-7 years, its restricted distribution, relatively specialized resource use, and sensitivity to environmental perturbations (Crooks and Van Vuren 1994, 2000; Jones et al. 2008; Bolas et al.in prep; Dillon et al. in prep; Gagorik et al unpublished data, Shaskey et al unpublished data). The rarity of island spotted skunks, likely numbering less than 1000 individuals total, increases the risk that populations on the Santa Rosa and Santa Cruz Islands could be extirpated, or that both populations could be lost and the subspecies driven extinct.

Historically, island spotted skunks were adversely affected by habitat degradation from feral and domestic herbivore grazing (sheep, cattle, horses, goats, deer, and elk) and feral pig rooting on the islands (Crooks and Van Vuren 1994, 2000; Jones et al. 2008). During the past 25 years, feral herbivores have been removed from both Santa Cruz and Santa Rosa islands, resulting in vegetative recovery that likely improved habitat quality for skunks. However, lack of vegetation such as low shrubs or trees may have reduced microhabitats that skunks use as refugia for protection from predators or competitors (Bolas et al. *in review a*). Hence spotted skunks may continue to benefit from ongoing recovery of vegetation following feral herbivore removal. Island terrestrial ecosystems are likely to continue to transform due to climate change, although the impacts on spotted skunks populations are unclear. Prolonged drought, and hence decline of habitat productivity and potentially key prey populations (e.g., deer mice, see Prey Population Dynamics Section 8f) is a potential driver of skunk population decline. In addition, increased risk of wildfire as a result of climate change is another potential threat; widespread fires on either island could negatively and perhaps dramatically impact habitat availability and suitability for skunks.

Island spotted skunks are competitively inferior to island foxes, at least in habitats where their interactions have been observed, and may be impacted by both exploitative and interference competition (including predation) with foxes (see Interaction with Foxes Section 8c). Thus, the recent recovery of island fox populations is likely contributing to marked population declines in island spotted skunks over the past decade (Dillon et al. in prep). How island foxes may impact long-term population viability of island spotted skunks is unclear. On the other hand, it is believed that island spotted skunk populations have coexisted with island fox populations on Santa Rosa and Santa Cruz Islands for at least 7,000 years (Goddard et al. 2015, Hofman et al. 2015). Likewise, it is believed that an island spotted skunk population coexisted with an island fox population on San Miguel Island for several thousand years until island spotted skunks were extirpated from that island sometime after 1870 (Henshaw 1876, Walker 1980).

Insular endemic species like the island spotted skunk evolve in isolation and often lack natural defenses to predators and novel pathogens, both of which have the potential to elevate extinction risk for this island endemic carnivore (see Disease Section 8e). Spotted skunks on Santa Cruz Island have tested positive for canine heartworm and canine parvovirus but showed no previous

exposure to canine adenovirus, canine distemper virus, canine herpes virus, three serovars of *Leptospira* bacteria (*pomona, canicola,* and *icterohaemorrhagiae*), and pseudorabies (Bakker et al. 2006). In recent years, carnivore amdoparvovirus (i.e., Aleutian disease) has been circulating in California mainland skunks and causing high mortality rates in some local populations, however island spotted skunks have not yet been screened for the virus. In addition, Harris et al. (2021) reported 50% CDV-related mortality in their collared eastern spotted skunks (*S. putorius*) in DuPont State Recreational Forest, NC. The possible introduction of novel diseases from domestic cats and dogs brought to the islands as pets also poses a threat to island spotted skunks; and more recently, given the susceptibility of farmed mink, the threat of human-skunk transmission of SARS-CoV-2 should be considered (Michelitsch et al. 2021; https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html).

It is also unclear the degree to which lack of genetic diversity in these rare, isolated insular populations represents a threat to skunks either in the short-term (e.g., reduced fitness through inbreeding depression) or the long-term (e.g., reduced adaptive potential to environmental disturbances). Floyd et al. (2011) assessed genetic variation using microsatellites at eight loci in 208 spotted skunk and found diversity in island spotted skunk populations (observed heterozygosity [Ho] = 0.45, expected heterozygosity [He] = 0.58, average number of alleles [A] = 3.17) was roughly 30% lower than on in mainland populations (Ho = 0.65, He = 0.78, A = 4.93). An additional study of 115 skunks on Santa Cruz Island using microsatellites at seven loci found mean heterozygosity was 0.556 (Jones et al. 2013). Further research is warranted on population genomics of island spotted skunk and potential genetic threats to their persistence (see Genetics Section 8d).

8. Key Research Areas and Knowledge Gaps

a. Population Monitoring Methods

Section Leads: Kevin Crooks, Ellie Bolas, Adam Dillon, Calypso Gagorik, Tad Theimer

Spotted skunks have been largely overlooked by scientists for decades, thus developing effective population monitoring methods is critical for understanding these elusive animals. Monitoring efforts yield not only baseline occurrence, distribution, and demographic data, but can also supply general information about habitat selection, behavior, and interspecific interactions useful for guiding research and management efforts. Unfortunately, monitoring for carnivores can prove quite challenging owing to their generally secretive nature, low population densities, and large home ranges (Gompper et al. 2006). To date, approaches to monitor island spotted skunks have included live-trapping, remote cameras, and VHF and GPS telemetry, reviewed in sequence below.

Live-trapping

The primary monitoring technique for island spotted skunks has been live-trapping skunks coincident with ongoing road transects and trapping grids targeted towards island foxes. Island spotted skunk capture data from these trapping grids and transects on Santa Cruz Island are available for a period of nearly two decades (2000's & 2010's). Additional trapping data from the 1990s on both islands are also available (Crooks 1994a,b; Crooks and Van Vuren 1994, 1995, 2000; Roemer et al. 2002; Dillon et al. in prep). The National Park Service (NPS) and TNC have maintained standardized fox trapping grids on both islands from 2009 to present. These data suggest that island spotted skunk numbers were low during the early 1990s, climbed rapidly in the late 1990s/early 2000s, remained high through much of the 2000s, and since the early 2010s have decreased to lows similar to those of the early 1990s (see Population Trends Section 6). The sharp increase in the 1990s and high level of island skunk captures through the 2000s is coincident with a sharp decrease in island fox numbers in the 1990s, low fox numbers through the early-to-mid 2000s, a sharp rise in island fox numbers in the late 2000s, and high numbers of island foxes over the past five years or more (Dillon et al. in prep). However, fox trapping grids are not targeted towards skunks, and could likely be modified to improve skunk capture success. Notably, most fox monitoring has occurred between June-October and therefore traps on grids were not open during time periods when skunks are most active (Crooks and Van Vuren 1995; Jones et al. 2008; Bolas et al. 2020). Further, given skunks are primarily nocturnal, trapping methods could be modified so that traps are closed during the day and opened at dusk to prevent foxes from filling them during the day. Whether closing traps during the day to increase capture probability of skunks is worth the increased time and effort is yet to be determined.

Remote cameras

Remote camera surveys are a relatively inexpensive and low-effort approach to survey secretive carnivores, including spotted skunks. Remote wildlife cameras, stratified by habitat type and operated continuously, can yield valuable information on: 1) **occupancy**, an increasingly popular

tool to monitor distribution of rare or cryptic species (e.g., Shannon et al. 2014); 2) **habitat use**, via occupancy models, mixed-models, and relative activity indices (e.g., Lewis et al. 2015a, Bolas et al. *in review a*); 3) **extinction/colonization dynamics**, via dynamic multi-season occupancy models (e.g., Schuette et al. 2013); 4) **spatio-temporal interactions with foxes**, via multi-species occupancy or co-detection models (e.g., Lewis et al. 2015b, Bolas et al. *in review a*); 5) **daily activity patterns**, via time stamps on photographs (e.g., Lendrum et al. 2017, Bolas et al. *in review a*); 6) **relative abundance**, via detection rates (e.g., Bolas et al. 2020); 7) **population size**, if skunks are individually identifiable on camera (see below).

Remote cameras have been used to identify individual striped skunks using pelage patterns in photographs (Theimer et al. 2017). The feasibility of individual identification using pelage patterns of photographed spotted skunks is still unclear. An ongoing project in Oregon is using Artificial Intelligence (in collaboration with WildMe) in an attempt to individually identify mainland western spotted skunks (Marie Tosa, personal communication). If spotted skunks are individually identifiable by pelage patterns, this would allow a photographic mark-recapture approach to estimate population size, density, and apparent survival (e.g., pelt patterns have been used for population estimation of bobcats in mainland southern California; Alonso et al. 2015). Another more resource intensive option would be to trap and physically mark skunks (e.g., with collars or fur dye) in select camera grids, perhaps associated with nearby fox trapping grids. If the physical marks allow individual identification, this would enable a mark-resight approach to estimate population size and density (e.g., Alonso et al. 2015). Even if individually identifiable marks are not feasible (e.g., due to logistics or small size of mark), the use of "batch marks" (i.e., where animals are simply considered marked or not), can still allow for population estimation. An added benefit of remote camera surveys for island spotted skunks is that the same camera population estimation procedure could be employed for island foxes concurrently, allowing for enhanced monitoring of distribution and activity foxes as well as their interactions with skunks.

To evaluate the extent to which remote cameras might provide an additional metric of island spotted skunk populations, Bolas et al. (2020) overlayed wildlife cameras at island fox trapping grids on Santa Cruz and Santa Rosa Islands in 2016 and 2017. On both islands, detection rates of skunks with cameras (0.72/100 operating nights) did not differ significantly from that with fox traps (0.92/100 operating nights) during the summer, suggesting similar reliability of the two methods for detecting skunks. Low detection rates of island spotted skunks were comparable to those of other spotted skunk populations that are considered rare and of conservation concern. Bolas et al. (2020), however, suggested that cameras may be more efficient than traps for monitoring skunks, as the rugged terrain of both islands poses logistical challenges for trapping, especially during the wet season. Further, remote cameras may be stationed over larger areas or longer durations with much less effort than traps.

In addition, Bolas et al. (2020) found that skunk detections with cameras increased from summer to early winter in 2017, which suggests that winter monitoring may be preferable. Similar seasonal variation in detection has been reported for other carnivores (Lesmeister et al. 2015, Stetz et al. 2019), including the eastern spotted skunk (Hackett et al. 2007) and mainland western spotted skunk (Marie Tosa, personal communication). Eastern spotted skunks in Missouri and Arkansas were nearly undetectable late spring through summer, but the causes were unclear (Hackett et al. 2007). For island spotted skunks, rainfall might influence seasonal variation in detectability. Bolas et al. (2020) found no seasonal variation in detections in 2016, near the end

of a 5-year drought, but found a seasonal trend in 2017, following a wet season of above average rainfall. Similarly, rainfall on Santa Cruz Island in 1991-1992 was also above average, and trapping revealed seasonal variation in skunk detections, with lower detectability during summer and fall (Crooks 1994*b*). The increase in skunk detections in late fall and early winter may be the result of a change in skunk numbers and/or activity, and the influence of rainfall is uncertain. Drought may curtail reproduction, resulting in fewer juvenile skunks, or it may reduce ranging movements by males, as has been suggested for eastern spotted skunks (Lesmeister et al. 2009).

One of the first comprehensive studies evaluating monitoring methods for spotted skunks was conducted by Hackett et al. (2007) on the eastern spotted skunk. They evaluated three common techniques: live-trapping, track plate boxes, and remote camera traps. Results suggested that track plate boxes may be more effective than remote cameras or live-trapping for non-invasively surveying spotted skunks. Remote camera technology, however, has improved considerably in the decades since the publication of Hackett et al. (2007), and this improvement and the comparative logistical ease of using remote cameras instead of track plates suggests significant benefits to using this method. Currently, a majority of the known monitoring efforts for eastern spotted skunks are relying solely on remote cameras to detect the species (ESSCSG 2019).

Baited camera stations have been successfully used in several states to detect eastern spotted skunks, often where they had not previously been known to persist (Hackett et al. 2007, Lesmeister 2007, Wilson et al. 2016, Sprayberry 2016, Boulerice and Zinke 2017, Thorne et al. 2017). Eastern spotted skunks have been successfully attracted to sites using a variety of baiting methods, though canned sardines in oil is the most commonly used bait in recent years. Scent lures such as Caven's Gusto, fatty acid scent tabs, cherry oil, or other strong-smelling attractants have supplemented the bait at many of these camera stations; however, additional attraction provided by scent lures has yet to be quantified. There are also indications that camera brand may have a significant effect on detection rates (Urbanek et al., in review). Although baiting cameras may increase detection probability of island spotted skunks, continuously reapplying bait to stations to maintain consistent attraction may prove logistically challenging, thereby diminishing one of the primary benefits of non-invasive, long-term camera monitoring. In addition, baiting also attracts island foxes, potentially increasing contact and perhaps negative interactions (e.g., predation) with skunks (Gagorik unpubl. data). Also, a baited camera network could influence capture patterns of foxes and skunks at existing long-term monitoring grids. Thus, baiting cameras is currently not a desirable option on the islands.

A primary monitoring recommendation generated from the January 2020 workshop was to design and implement island-wide camera grid sampling targeted towards island spotted skunks on Santa Cruz and Santa Rosa Islands (see Monitoring Recommendations Section 9 below). This would entail systematically stationing grids of cameras, stratified by habitat type, across both islands. Once stationed, if unbaited, cameras could be left unattended for months, perhaps with quarterly checks to replace batteries, SD cards, and allow for camera maintenance. Eventually, periodic camera surveys (e.g., every 2-3 years) could be a tool to monitor skunk population trends in the future.

VHF Telemetry

Several efforts to monitor local populations of spotted skunks have also been successfully completed using very high frequency (VHF) transmitters (ESSCSG 2019). Specifically, VHF telemetry has been conducted on island spotted skunks in localized study areas on Santa Cruz Island (Crooks 1994a, b; Crooks and Van Vuren 1995; Jones et al. 2008; Jones et al. 2013; Gagorik et al. unpublished data). These studies, however, were primarily to collect basic ecological data (e.g., home ranges, habitat use, den sites, mortality) and not for population estimation. VHF transmitters suitable for spotted skunks have been found to last approximately 9-12 months. Many studies on spotted skunk have reported that the fossorial nature of spotted skunks may cause the antenna of the transmitter to break off after several months of monitoring, resulting in a vastly diminished signal and difficulty maintaining frequent monitoring events.

GPS Telemetry

Because spotted skunks weigh only 200-800 grams, use of GPS transmitter technology for studying this species requires serious consideration regarding trade-offs between number of locations taken, potentially short battery life, and the relatively large size of the collar on the animal (ESSWG 2018). On Santa Cruz Island in 2018-2019, ten spotted skunks were fitted with Lotek LiteTrack20-RF Swift Fix GPS collars (Gagorik et al. unpubl. data). Based on the GPS program uploaded to the collar, the worst-case battery lifetime for a skunk collar deployed in the summer of 2018 estimated battery life ending approximately August 2019. Remote download capabilities (RF) were present on all collars. With this feature, the data would be stored on board with the ability to download by a portable receiver and a handheld yagi antenna, provided that the observer could track the animal with the VHF signal and get close enough for the receiver to connect with the collar. GPS collars were programmed to take one fix every 3 days at 2200 to inform general space usage through time. In addition, for one week during the fall (October), winter (February), spring (May) and summer (July), collars would take fixes from 1900-0700, at intervals of 1 fix every 30 minutes to inform activity, habitat selection, and seasonal movement patterns. GPS tracking proved difficult, however. Ultimately, incomplete GPS data sets from 6 skunks were downloaded, only 3 of the 10 deployed collars were recovered and personnel were unable to find remaining animals, even with extensive aerial flights, ground searches, and targeted trapping. Upon recovery of the collars, all were found to be missing antennas and no longer transmitting a VHF signal. Overall, GPS collar monitoring is currently a challenging and expensive means of monitoring skunk populations.

Non-invasive genetics

Another possible population estimation technique for spotted skunks is the non-invasive collection of DNA samples via hair snares or scat. Hair samples from western spotted skunks have been collected from both barbed wire snares (n = 2 samples) and glue strip snares (n = 1 sample) placed within enclosed sooted track plate boxes on the northern California mainland (Zielinksi et al. 2006); however, DNA extraction from these samples was unsuccessful. In addition, there has been exploratory work on eastern spotted skunks in West Virginia using baited hair snares constructed with PVC pipe and rifle cleaning brushes (Charles Wagy, http://easternspottedskunk.blogspot.com/2015/10/spotted-skunk-hair-snares.html). Remote cameras at these hair snare stations indicated spotted skunks visited the snares and hair samples were obtained on the brushes. Effective hair snare designs for spotted skunks require more research.

DNA can also be sampled from carnivore scat, and if conducted in a mark-recapture framework, can yield population estimates (e.g., for bobcats in mainland California; Ruell et al. 2009). Skunk scats are difficult to locate, but can be found near den sites, which can be most easily located via telemetered animals (Crooks 1994b; Jones et al. 2008). The use of scat detection dogs might be another possibility to find spotted skunk scats (Marie Tosa, personal communication). Scat detection dogs were able to successfully distinguish scat of San Joaquin kit foxes and striped skunks on mainland California (Smith et al. 2001), thus it is conceivable that dogs might be able to distinguish scats from island foxes and spotted skunks. However, the logistic feasibility of using scat detection dogs on the islands is unclear, and potential negative impacts of dogs on both island foxes and spotted skunks (e.g., harassment, disturbance, predation, disease transmission) would need to be seriously considered.

Key knowledge gaps

- Guidance on most effective long-term population monitoring techniques and strategies for island spotted skunks (see Monitoring Recommendations Section 9)
- Design of remote camera trapping grids to monitor island skunk populations
- Evaluation of possible techniques to generate robust estimates of population size/density:
 - Mark-recapture population estimation via individual identification from pelage patterns in remote camera photos
 - Mark-resight population estimation via individual identification from physical marks (e.g., identifiable collars) in remote camera photos
 - Non-invasive genetic sampling (e.g., via hare snares or scats)
- Island-wide population estimates for island spotted skunks on Santa Cruz and Santa Rosa Islands
- Estimation of key demographic rates, including survival and reproduction (see Population Viability Analyses Section 8b)

b. Population Viability Analyses

Section Leads: Vickie Bakker, Kevin Crooks, Adam Dillon, and Dan Doak

There is compelling evidence that island spotted skunks have declined precipitously in population size in the past decade on both Santa Cruz and Santa Rosa Islands (see Population Trends Section 6). The recovery of island foxes, their dominant competitor, are presumed to be a primary, though perhaps not the sole, driver of these declines (Dillon et al. in prep). However, the underlying mechanisms of this decline are not fully understood, such as the life stages and vital rates responsible for the negative population growth rates. Population viability analysis (PVA) could serve as a useful tool for understanding the population dynamics and current status of island spotted skunks and for evaluating future monitoring and management actions (Bakker and Doak 2009; Bakker et al. 2009). In particular, a demographic modeling approach is necessary to estimate how much changes in different vital rates contributed to changes in overall population growth.

PVAs can be divided into two general categories: count-based and demographic approaches. Count-based PVAs use the variation and trend in observed counts to estimate the mean variance in annual growth rates, and thus project future extinction risk. Demographic age- (or stage-) structured PVAs use the mean and variance of age-specific demographic rates, namely agespecific reproduction and survival, to similarly project future dynamics. Concurrent estimates of population size are beneficial to allow assessment of density effects on population dynamics. For both PVA types, time series for data should be long enough to estimate stochastic variance associated with model parameters. Parameterizing demographic PVAs requires substantially more data, but the resulting models allow more understanding of the drivers of population growth as well as more tools for managing vulnerable populations, including the sensitivity of population health to different demographic rates and ages. While count-based models allow estimates of population change and extinction risk, they do not provide an understanding of the reasons for these changes and thus have more limited conservation value. Demographic PVA's have proven instrumental in designing the recovery and delisting criteria for the island fox (Bakker et al. 2009; Bakker et al. 2009; Dillon et al. in prep).

Assuming that the ultimate benefits of a demographic PVA warrant the heavier data requirements, we review the data available to parameterize such a model, and the options for obtaining additional data needed for a robust analysis of island spotted skunk status. A demographic PVA is structured by age or stage to capture important differences in survival, reproduction, or both. Aging of skunks is difficult, and while first-year subadult juveniles can sometimes be differentiated from older skunks, it may not be possible to confidently differentiate monitored skunks by age. Demographic PVAs often model females only, which is appropriate if sex ratios are even or male-biased and thus males are not limiting. Trap captures of adults suggest continued, strong male bias, although it is unclear the degree to which this observed sex ratio is attributable to sex differences in trapability. In mainland western spotted skunks, sex ratio at birth was documented 2.3:1 in favor of males (Mead 1968). In the eastern spotted skunk, males are more easily captured than females due to larger home ranges and being less trap shy, resulting in skewed sex ratio estimates (Kinlaw et al. 1995). Reported sex ratios (Male: Female) are 2.5:1.0 in Florida (Kinlaw et al. 1995), 1.8:1.0 in Iowa (Crabb 1948), and 1.1:1.0 in Arkansas (Lesmeister et al. 2010).

Survival

There are no published survival estimates for island spotted skunks, or for conspecifics on the mainland, although ongoing work is estimating annual mortality rate of western spotted skunks in Oregon (Marie Tosa, personal communication). Only one published survival estimate exists for eastern spotted skunks (mean annual survival of 0.35, 0.34-0.37, 95% CI, 23 month study, Ouachita Mountains, Arkansas, Lesmeister et al. 2010). Some monitoring data specific to island spotted skunks are available to support survival estimation. Mark-recapture trapping on standardized grids was conducted from 2008 through 2019, and preliminary analysis of these data indicate an annual apparent survival of 0.5 with no clear trend in survival during the population decline (Vickie Bakker, unpublished data). More sophisticated analyses of these data could be achieved using a robust design, including potentially the use of spatial open capture recapture models (R package openCR). Using existing trapping data, we are unable to estimate early pup survival for the time period from birth until trapping, which via the island fox trapping grids has occurred in late summer and fall at ~6 months of age. One way of accommodating this limitation is to consider reproductive rate to be pups per female at the census (i.e., trapping) time (see below).

Telemetry data may also allow estimates of adult survival for the island spotted skunk. During the period of peak skunk abundance in the early 2000's, Jones et al. (2008) conducted VHF telemetry on 33 skunks (n = 17 females; n = 16 males) in two study areas on Santa Cruz Island. Island foxes were rare during this period, thereby reducing the influence of foxes on skunk survival. These data have not been analyzed for survival, but this is possible. Additionally, NPS has piloted VHF mortality surveys on East Santa Cruz Island in 2020 and there are plans to expand mortality monitoring to Santa Rosa Island and other areas of Santa Cruz Island in future.

Reproduction

We currently have no data on reproductive rates in island spotted skunks, and obtaining such data has proven extremely challenging. Efforts to collar skunks in recent telemetry/den site studies have resulted in a male-biased data set because of the high male sex skew in current live trapping. Thus, locating female den sites has been difficult and little data exists on what may be happening at these dens. Past attempts to place cameras at natal dens have met with limited success (Gagorik unpubl data). An estimate of first year subadults per female at trapping time would be adequate to parameterize population viability models. At the time of first trapping, subadults sometimes can be distinguished from adults by tooth wear, body mass, and sex.

One option for obtaining reproductive data at the time of first trapping is analyzing existing mark-recapture trapping data using open population reverse-time models (e.g., Pradel 1996, Nichols 2016). By reversing time in the traditional open population survival model structure, these models estimate the probability that an individual present at time t was present at time t-1, thereby allowing estimation of the probability that an individual is a new pup. Additionally, during the period of skunk abundance in the early 2000's, Krista Jones noted subadult skunks, as well as body mass, during targeted trapping August 2003-February 2004 and August-September 2004; these data weren't published in Jones et al. (2008), but might be available to provide an estimate of recruitment. It might also be possible to look at the timing of subadult captures to infer the season of reproduction, given currently little is known about island spotted skunk reproduction.

While defining reproductive rate as pups per female at census time is adequate for population modeling and is likely the most feasible option for long-term monitoring, using this approach does not allow us to differentiate reproductive components such as probability of breeding, litter size, neonatal survival, and early pup survival, which could limit our ability to identify demographic rates that may have contributed to skunk decline. One approach to obtaining these estimates could be from locating telemetered females during the breeding and rearing seasons, although even then the difficulty of finding pups and counting offspring would present problems. However, if overall reproductive rate were identified as a key contributor to skunk decline, a targeted study could be considered to break down components of reproduction.

Population Estimation

We currently have a time series of population size estimates from island fox trapping grid data (Santa Cruz Island: 2003, 2008-2014, 2017; Santa Rosa Island: 2009-2016) using spatially explicit capture recapture methods (Jones et al. 2008, Coonan et al. 2015; Dillon et al. in prep; Figure 1). Small numbers of captures in recent years have precluded estimation of population size. Additionally, trap success data (# skunk captures/# available trap nights) are available

1992-2019 on Santa Cruz Island and 1998-2019 on Santa Rosa Island, with missing data for some years.

Key knowledge gaps

- Data from stable population
 - One limitation of any demographic estimate based on standardized grid trapping from 2008 2019 is that these data characterize the population when it was declining. These data are not likely to characterize long-term dynamics of this population.
 - One possibility is to analyze data available during the period of peak skunk abundance in the early 2000's, during a relatively stable period where the skunk population was neither rapidly increasing or declining (Jones et al. 2008)
- Survival: Options to estimate survival include:
 - Analysis of mark-recapture data from fox trapping grids using robust design. Although the existing trapping data may be sufficient to characterize age effects on survival, it is unlikely that the data are extensive enough (e.g., enough recaptures of the same individuals across years) to estimate age-dependence.
 - Analysis of existing VHF telemetry data from period of skunk abundance in early 2000's (Jones et al. 2008)
 - Ongoing VHF mortality surveys by the NPS
- Reproduction: Options to estimate reproduction/recruitment include:
 - Analysis of existing trapping data with open population reverse-time models
 - Analysis of recruitment (proportion subadults) from trapping data during period of skunk abundance in early 2000's (Jones et al. 2008)
 - Remote cameras at dens
- Estimates of population viability and extinction risk using PVA models

c. Interaction with Foxes

Section Leads: Juliann Schamel, Ellie Bolas, Kevin Crooks, Calypso Gagorik, Laura Shaskey, Tad Theimer

The island spotted skunk exists sympatrically with the island fox across the entirety of its range, on both Santa Cruz and Santa Rosa Islands. Together, these two endemic species make up the terrestrial mammalian carnivore guild. There is evidence that they compete across one or more niche dimensions, with the island fox the dominant competitor (Crooks and Van Vuren 1995, Jones et al. 2008). Both exploitative competition (e.g., competition over limited resources) and interference competition (e.g., competition through direct exclusion or aggression) are possible. This competitive relationship may influence the carrying capacity of the island spotted skunk, which may decrease as the island fox population increases (given that the range of each species is limited to the island). The precise mechanisms, and relative strength, of competition is not fully understood and is the focus of previous and much ongoing research.

Exploitative Competition:

Exploitative, or resource, competition, is a passive interaction mediated through a shared resource (e.g., habitat, time, food items), where consumption of a resource by the superior competitor decreases the resource such that it is unavailable to the inferior competitor (Schoener 1983). Exploitative competitors are likely to experience niche contractions or shifts (Case & Gilpin 1974), and tests for exploitative competition look for changes in diet, daily or seasonal activity patterns, body mass, or habitat use (Clark et al. 2005; Jones et al. 2008; Liesenjohann et al. 2011).

During the period of skunk scarcity in the early 1990s, spatial, temporal, and dietary overlap between skunks and foxes on Santa Cruz Island was substantial, suggesting the potential for exploitative competition (Crooks and Vuren 1994, 1995). Mean home range overlap between spotted skunks and island foxes on Santa Cruz Island was 0.21 (range 0-0.74) in the wet season and 0.28 (range 0-0.76) in the dry season (Crooks and Van Vuren 1995). Overlap in skunk and island fox home ranges may contribute to interspecific competition between these two sympatric carnivores (Crooks and Van Vuren 1994, 1995). Skunks exhibited a narrower niche along all three dimensions compared to foxes. Skunks were more specialized in their habitat use, primarily nocturnal, and primarily carnivorous. In contrast, foxes were habitat generalists, active both day and night, and highly omnivorous. As a result, competition was likely asymmetric, with the fox as the dominant competitor, as would be expected when the broad niche of a larger, abundant, generalist predator (i.e., foxes) overlaps the narrow niche of a smaller, rare, specialist competitor (i.e., skunks).

Dramatic population increases of skunks during fox decline in the late 1990's and early 2000's (Jones et al. 2008), and subsequent skunk declines coincident with the recovery and increase in foxes (Dillon et al. In Prep. Figure 2), provide further evidence of asymmetric competitive interactions between skunks and foxes. During the period of skunk abundance and fox rarity in 2003-2004, modest shifts in skunk habitat use, diurnal activity, and diet compared to the early 1990s provided some support for release from exploitative competition as a contributing factor for the population increase of skunk (Jones et al. 2008). However, Jones et al. (2008) suggested that much more dramatic changes in one or more niche axes would be expected if release from exploitative competition increases. Further, skunks demonstrated an increase in the use of unprotected, above-ground den sites, which may be evidence of a release from interference competition (Jones et al. 2008).

Several current research projects are exploring exploitative competition between foxes and skunks using new techniques. An ongoing project is investigating differences in spatial habitat use between skunks and foxes by using data collected from GPS and VHF collars deployed on both foxes and skunks on Santa Cruz Island in 2018-2019 (Gagorik et al. unpubl. data). Data are currently being analyzed, but initial findings suggest that skunks display seasonal habitat selection. Skunks exhibited large overall home range sizes but only used a small percentage of their entire range over the course of a week compared to foxes who moved through most of their overall home range within a week. Habitat factors such as slope and vegetation cover were also important, with skunks occupying areas of high slope and high vegetation cover compared to foxes.

Another ongoing investigation into island fox and island spotted skunk diet is using stable isotope analysis of whisker samples collected from both species across all major habitat types from 2011 to present on Santa Rosa Island (Schamel, Newsome, and Guglielmino, in prep). This study will assess dietary overlap and niche breadth across a gradient of population densities in both species, to evaluate the effect of intra- and inter-specific competition on dietary resource use within the context of drought (2011-2016) and changing population densities. This study will also look for evidence of individual dietary specialization.

Figure 2. Island fox density with 80% confidence intervals and island spotted skunk trap success on (A) SCI and (B) SRI, and island fox density with 80% confidence intervals on (C) SMI. Note: 1971-1978 are total fox density (adults and pups); 1993-2019 are adult fox density. Reprinted from Dillon et al. In Prep.



Interference Competition:

Interference competition is a direct, often aggressive interaction between two competitors for a shared resource (Case and Gilpin 1974; Schoener 1983). It can be expressed subtly, through passive avoidance of the superior competitor (a shift in spatial, temporal, or dietary resource use displayed by the inferior competitor), or aggressively through direct exclusion of the inferior competitor from the resource by the superior competitor (Berger and Gese 2007). In its most extreme form, superior competitors also demonstrate interspecific killing of the inferior competitor (Holt and Polis 1997; Linnell & Strand 2000; Hoogland & Brown 2016). Interference competitors are less likely to exhibit niche contraction than exploitation competitors, although niche partitioning is expected (Case & Gilpin 1974). Studies investigating interference competition examine mortality sources and rates, spatio-temporal habitat use, and direct interactions between the two species (Creel & Creel 1996; Clark et al. 2005).

Some degree of interference competition between skunks and foxes is likely. Foxes (ca. 2000g) are approximately four times larger than skunks (ca. 500g), so interference competition is likely asymmetric, with foxes the dominant competitor (Crooks and Van Vuren 1995). Compared to the period of skunk rarity and fox abundance in the early 1990s, a marked shift in den selection, toward frequent use of unprotected dens and away from multiple use, suggested that release from interference competition may have been an important factor behind the dramatic increase in skunk populations in 2003-2004 (Jones et al. 2008). Further, the recent and rapid decline of skunks, concurrent with the rise in foxes, suggests that foxes impact skunks directly.

There is anecdotal evidence of island foxes killing island spotted skunks from both Santa Cruz and Santa Rosa Islands. However, the extent and circumstances in which this occurs, and the demographic groups of skunks most impacted by this interaction, are all unknown. It is also unknown whether foxes kill and consume skunks as prey items, or if they merely kill but do not consume them in interference competition interactions. On Santa Rosa Island, at least one adult island spotted skunk was provisioned to an island fox in a Tomahawk trap (Angela Guglielmino, personal communication). Provisioning of food items to foxes in traps (especially deer mice) is common on Santa Rosa Island. On Santa Cruz Island, remote-sensing biosecurity cameras have captured photos of foxes with skunks in their mouths on a few occasions (Katrina Olthof, personal communication). At least one captive fox in a breeding facility on Santa Cruz Island was observed to have killed (but not eaten) three skunks that climbed into her enclosure (Christina Boser, pers. comm); this may have been a specialized individual behavior, as this behavior was not observed in other captive foxes living in the same facility at the same time. Island foxes and island spotted skunks have also been observed interacting on camera (stills and videos); the skunk sometimes pursues and/or performs defensive behaviors (i.e., handstands) in the direction of the fox (Crooks and Van Vuren 1995; Angela Guglielmino, Laura Shaskey, Ellie Bolas, Juliann Schamel, personal communication).

Given the potential impact that foxes have on skunks, it is surprising that the two species do not show marked evidence of partitioning habitat or diet. However, partitioning of microhabitat and temporal activity by the two species was examined using detections from traps and cameras on both Santa Cruz and Santa Rosa islands from 2015-2017. On both islands, skunks had positive associations with measures of rugged topography (terrain roughness and proximity to stream courses) and woody vegetation including low shrubs and trees and stumps (Bolas et al. *in review*)

a). Foxes also had positive associations with trees and stumps, but did not respond to shrubs and have negative associations with measures of rugged topography (Bolas et al. *in review a*). Partitioning of ground-level cover from topography may reflect skunks using these areas to avoid foxes or because foxes are not active in these microhabitats (Bolas et al. *in review a*). Additionally, the two species demonstrate some degree of temporal partitioning. On both islands, skunks were more active at night than foxes, particularly on Santa Rosa Island, and the peak in skunk activity offset that of fox activity by at least one hour (Bolas et al. *in review a*). Bolas et al. (2020) also found that seasonally, skunks appear to increase their activity in late fall and early winter as compared to the summer. In contrast, the same camera array as used for skunks showed that fox activity decreased in the fall and early winter on Santa Cruz Island, and did not change with season on Santa Rosa Island (E. Bolas, unpublished data). Habitat degradation by exotic herbivores may have reduced refugia available for skunks to avoid foxes. The combined lack of sufficient microhabitats and rapid increase in foxes may have driven the recent decline in skunks; however, skunks appear to make fine-scale adjustments in space and time that may help facilitate coexistence with foxes (Bolas et al. *in review a*).

Remote cameras placed at dens also suggest that foxes could be disturbing den sites of skunks (Calypso Gagorik, personal communication). In one instance, a series of photos from a camera placed at a skunk den in 2018 on Santa Cruz Island showed a fox digging at the den entrance while a skunk was inside (Gagorik et al. unpubl. data). In this series, a skunk enters the den in the early morning; later that day a fox arrives at the site and spends 10 minutes digging at the den entrance. After ten minutes, the fox leaves and does not return. That night, the skunk emerges, and appears unharmed. Data are currently being analyzed to assess characteristics associated with skunk dens and how often they are visited by skunks and foxes. Preliminary analysis indicates a relationship between slope and skunk visitation rates, suggesting dens at high slope are associated with greater skunk visitation rates.

Results from a multi-island, seasonal diet study of island foxes via analysis of scat collected between October 2008 and December 2009 suggest that island spotted skunks are not a significant dietary item for adult island fox (Cypher et al. 2014). This scat study was conducted at a time when island spotted skunk density was still quite high on both islands. Island spotted skunks were only detected in 3 of 180 scats from Santa Cruz Island (0.016 occurrence = 1.6% of fox scats contained island spotted skunk), and 5 of 505 scat from Santa Rosa Island (0.01 occurrence = 1% of fox scats contained island spotted skunk) (Cypher et al., 2014). These results suggest that island spotted skunks are not an important food item for island foxes, although the findings do provide evidence of some skunk consumption. However, there are other possible predatory interactions that may not have been detectable by this study. Although fox scats were collected from across as much of each island as possible, they were not collected from fox den areas. It is possible that adult island fox predate upon island spotted skunk kits and/or denning female skunks and provision these skunk kits to their own young. Evidence of such consumption would mainly show up in fox kit scat at the den. This type of intraguild predation has been observed in other sympatric carnivores (Fancourt et al., 2015) and can suppress juvenile recruitment into the population, with population-level consequences. This putative provisioning is being investigated through stable isotope analysis of fox pup whiskers collected on Santa Rosa Island during skunk abundance (Schamel, Newsome, and Guglielmino, ongoing).

If island foxes kill, but do not consume, island spotted skunks, evidence would not show up in scats or whiskers. Such intraguild interactions have been documented in other systems, including between coyotes and kit foxes in the San Joaquin Valley (Cypher and Spencer, 1998). In this case, coyotes killed, but did not usually consume, kit foxes, and were responsible for 65% of collared kit fox mortalities (Ralls and White, 1995).

Identifying Intra-Guild Competitive Interactions

In the end, pinpointing the precise interactions and parsing out the details of the dynamic relationship between island spotted skunk and island fox may be quite challenging. In interference competition in carnivores, it is not uncommon for the "winner" to switch depending on the environmental setting in which the interaction is taking place. This is part of what makes it difficult to identify, and may be the case with skunks and foxes (as evidenced in the interactions on camera described above). In addition, while the best way to identify interference competition is an actual change in abundance, these aggressive interactions may also trigger avoidance in space and time. This can make it difficult to determine whether spatio-temporal partitioning is due to exploitative competition (an indirect interaction mediated by a resource) or interference competition (a direct interaction about the resource). One option to explore such details would be to deploy GPS proximity collars on both species in the same area. These collars can reveal fine-scale movement and potential interactions, although GPS collaring skunks is challenging and thus not a priority (see Population Monitoring Methods Section 8a).

Future Monitoring Planned by NPS:

The National Park Service is planning expanded monitoring and research of island spotted skunks to fill information gaps on fox-skunk interactions and to determine whether any management actions may be necessary to prevent further skunk decline. Foxes and skunks are currently monitored as part of the park's long-term inventory and monitoring program. Traditionally this monitoring has primarily consisted of annual live-trapping at fixed grid locations, and survival monitoring of radio-collared foxes. Although skunk monitoring during summer and fall will continue through regular grid and target trapping, an expanded remote camera network will be deployed systematically across island habitats to monitor skunk and fox interactions and activity patterns year-round. Survival and mortality monitoring will be expanded to skunks for at least a 1-2 year period to evaluate skunk survival rates and determine mortality causes, including evaluating the degree that foxes contribute to skunk mortality. Telemetry will be regularly used to identify and subsequently monitor important den locations with remote cameras.

Key Knowledge Gaps

- Identify key aspects of fox-skunk interaction that may be drivers of skunk decline, and identify how this relationship is influenced by environmental factors
- Identify the extent to which foxes kill and/or predate upon skunks, including which life stage, and under what circumstances this occurs

- VHF mortality collars on skunks to identify possible fox predation, including necropsies of recovered skunk mortalities (initiated)
- Stable isotope analysis of fox whiskers from time of high skunk density to look for evidence of skunk as prey (underway)
- Evidence from remote-sensing cameras, camera array, and cameras at fox and skunk dens (see below)
- Fox and skunk diet analysis to identify dietary overlap and potential resource competition and predation
 - Prior studies: 1991-1992 and 2003-2004 fox and skunk scat analysis studies from Santa Cruz Island; 2008-2009 fox scat analysis study from Santa Cruz and Santa Rosa Islands
 - Stable isotope analysis of Santa Rosa Island fox and skunk whiskers from 2011present (underway)
 - Scat analysis
 - DNA metabarcoding of scat samples
- Investigate island fox seasonal activity patterns, for comparison with island spotted skunk activity patterns as identified by Bolas et al. (2020)
- Island-wide camera array to continue collecting data on fox and skunk occupancy, habitat use, colonization/extinction dynamics, spatio-temporal interactions, daily and seasonal activity patterns, and relative abundance (see Population Monitoring Methods Section 8a)
- Evaluate provisioning of skunks to fox kits at fox dens
 - Cameras stationed at fox dens. However, previous attempts to monitor provisioning and kit counts at fox dens with cameras have proven difficult due to multiple den entrances and frequent moving of den sites (David Green, Jesse Maestas, personal communication)
 - Scat collection at fox dens:
 - \circ *If* provisioning occurs, it is likely much less frequent now (2021) due to the lower density of skunks on the landscape; stable isotope research may be able to provide insight into this question from data collected during skunk abundance
- Cameras at skunk dens to identify fox disturbance of dens
 - Previous attempts to monitor skunk dens with cameras have proven difficult due to multiple den entrances and frequent moving of den sites. Also should consider potential negative impacts caused by researchers visiting skunk dens; avoid disturbing reproduction as it is critical for species persistence (Christie Boser, Calypso Gagorik, Kevin Crooks, David Jachowski, personal communication)

d. Genetics

Section leads: Ellie Bolas, Cate Quinn, and Ben Sacks

Island foxes and island deer mice have benefitted from several genetic studies, but in contrast, genetics study of island spotted skunks has been limited, and much remains unknown about their evolutionary history, taxonomic status, and population genetics.

Evolutionary History

It is suspected that skunks arrived on the northern Channel Islands either via natural dispersal on floating debris from the mainland or by Native American introduction (Floyd et al. 2011). Beginning at least 20,000 years ago, during the last glacial maximum, lowered sea levels connected the northern Channel Islands (San Miguel, Santa Rosa, Santa Cruz, and Anacapa) into one large island, Santarosae (Reeder-Myers et al. 2015). Santarosae began separating 11,000 years ago, and was fully separated by roughly 9,000 years ago. At its largest, Santarosae was approximately 7 km from the mainland and was almost 18 km from the mainland by the time all islands had separated (Reeder-Myers et al. 2015). Santa Cruz and Santa Rosa Islands, the two islands that still support island spotted skunks, are now 30-45 km from the mainland. Native Americans arrived on the islands roughly 13,000 years ago, before separation, and consistently inhabited the islands until Europeans settled and began ranching in the 1800s. If spotted skunks colonized via over-water dispersal, then the most likely timing of colonization is prior to the separation of Santarosae, when the distance between the mainland and island was at its lowest (Pergams and Ashley 1999, Floyd et al. 2011, Reeder-Myers et al. 2015). Alternatively, spotted skunks may have been valued by Native Americans and intentionally introduced to the islands (Floyd et al. 2011), or been brought by humans inadvertently sometime over the last 13,000 years.

Island and mainland spotted skunks are considered a part of the western clade of western spotted skunks. Two recent phylongenis suggest that the western clade diverged from other groups of western spotted skunks 480,000-820,000 years ago (Molly M. McDonough, Chicago State University, Chicago, IL, personal communication, October, 2020), but see Ferguson et al. (2017) for a different time frame of divergence. However, both the Ferguson et al. (2017) and McDonough phylogenies lacked the resolution or sample sizes necessary to investigate the divergence time of island spotted skunks from the nearest western spotted skunks on mainland California. To better understand the timing and mechanisms of island colonization by spotted skunks, 92 complete mitochondrial genomes (mitogenomes) of spotted skunks from Santa Cruz and Santa Rosa islands and the mainland (California and Arizona) were sequenced. Analysis of the mitochondrial DNA genome (mitogenome) is a common first step in efforts to illuminate evolutionary processes and time of genetic divergence (e.g., Goddard et al. 2015), and thus is an appropriate tool for investigating the timing of colonization by island spotted skunks. Sequencing recovered 55 complete mitogenomes, and analysis revealed that island spotted skunks grouped in a single monophyletic clade distinct from mainland spotted skunks (Fig. 3; Bolas et al. in review B). Additionally, the haplotype network demonstrated that the two haplogroups from the two islands were tightly clustered, with the most recent common ancestral sampled from Santa Rosa (Fig. 3; Bolas et al. in review B), although the two islands did not share any haplotypes. These patterns imply both island populations were colonized by a single maternal ancestor, and then they diverged shortly after establishment and have remained isolated from each other ever since (Bolas et al. in review B). The estimated range of divergence time between mainland and island spotted skunks was 1,910-11,320 years ago (Bolas et al. in review B). This time frame suggests a Holocene colonization of the Northern Channel Islands by spotted skunks that encompasses the time of the separation of Santa Cruz and Santa Rosa islands (9,400-9,700 years ago; Reeder-Myers et al. 2015), and post-dates human arrival (13,000 years ago) but pre-dates the arrival of European settlers (250 years ago, respectively; Rick et al. 2014). Further, this time frame suggests skunks may have colonized either via transport by Native Americans or rafting on floating debris, but refutes the possibility of European transport.

Of note, the timing and pattern of island spotted skunk colonization of the northern Channel Islands is very similar to island foxes. Island foxes derive from a single matrilineal founder associated with the northern islands approximately 7,300 to 19,700 years ago (Goddard et al. 2015), although it is unknown whether foxes arrived on the northern Channel Islands via rafting or human introduction. Island foxes appear to have been closely associated with Native Americans and have been found in intentional burial sites, altogether suggesting human transport is the most likely explanation (Hofman et al. 2015). In contrast, there is limited evidence of island spotted skunks in the archeological record (but see Bolas et al. *in review B* for description of the paleontological/archeological record of island spotted skunks).

Figure 3. Median-joining network of island (n = 46, *Spilogale gracilis amphiala*) and mainland California western (n = 8, *S. gracilis*) spotted skunks mitochondrial DNA, rooted to the Arizona clade (n = 2). Figure re-printed from Bolas et al. in review B



Taxonomic Status

Common tools for determining taxonomic status include use of morphometric data, analysis of genetic and genomic divergence, and investigation of evolutionary history. As discussed in Section 3 (Distribution and Taxonomy), island spotted skunks were described as a subspecies of western spotted skunks by Dickey in 1929. However, island spotted skunks were considered a "weak" subspecies because morphological differentiation from mainland populations was minimal (Van Gelder 1959). As a follow-up study, V. Bakker collected morphometric data from 48 museum specimens of island spotted skunks (16 female, 32 male). Preliminary analyses documented shorter tail length in island spotted skunks relative to mainland subspecies (*S. g. phenax*), but failed to detect clear differences in skull morphometrics (Bakker et al. 2004).

Existing genetic data provide some guidance on taxonomic status, and are consistent with a designation of island subspecies. There is a demonstration of a lack of gene flow between populations and from the mainland (Floyd et al. 2011). In particular, microsatellite analysis of eight loci from 208 spotted skunks found that island spotted skunks are genetically distinct from mainland skunks (Fst = 0.22, SE = 0.03), and between Santa Cruz and Santa Rosa Islands (Fst = 0.21), Fst values that are almost three times higher than Fst comparisons among mainland subspecies of spotted skunks (Fst = 0.08, SE = 0.03) (Floyd et al. 2011). On that basis, Floyd et al. (2011) concluded that each island population was sufficiently distinct to warrant classification as its own subspecies, although island spotted skunks continue to be classified as a single subspecies. Fst is a relative metric of differentiation between populations and reflects both gene flow (or lack thereof) and drift, but cannot be used to parse timing of divergence. However recent research using mitochondrial genomes (see "Evolutionary History," above) demonstrated a Holocene separation between the two island spotted skunk populations and between the islands and mainland (Bolas et al. in review B). This time frame of colonization of the northern Channel Islands for island spotted skunks is similar to that of gray foxes (Goddard et al. 2015). Given that island foxes are considered a distinct species on the Channel Islands, this suggests that reevaluation of the taxonomic status of island spotted skunks is warranted. Further, evidence of biogeographic and phylogenetic endemism (see "Evolutionary History," above) and isolation over a long time period for spotted skunks on both Santa Cruz and Santa Rosa islands lends support to designating each island population a distinct subspecies.

Population Genetics

Population genetics research includes study of genetic variation, spatial organization, effective population size, genetic load, inbreeding, and recent demographic history.

The degree of genetic variation in a population is measured using metrics such as heterozygosity across several loci or nucleotide diversity. Floyd et al. (2011) assessed genetic variation on Santa Cruz Island using microsatellites at eight loci in 208 spotted skunks and found diversity in island spotted skunk populations (observed heterozygosity [Ho] = 0.45, expected heterozygosity [He] = 0.58, average number of alleles [A] = 3.17) was roughly 30% lower than in mainland populations (Ho = 0.65, He = 0.78, A = 4.93). An additional study of 115 skunks on Santa Cruz Island using microsatellites at seven loci found mean heterozygosity was 0.556 (Jones et al. 2013). Finally, analysis of the mitochondrial genome of 46 skunks from both islands found haplotype diversity of 0.733, SD 0.089 and 0.385 SD 0.099 on Santa Cruz and Santa Rosa

islands, respectively and nucleotide diversity of 0.10 (0.02) and 0.06 (0.02) on Santa Cruz and Santa Rosa islands, respectively, which was significantly lower than that of mainland populations (Bolas et al. *in review B*).

Studies of spatial genetic structure provide insights into gene flow among individuals and populations. A description of studies of population structure by island can be found in the Distribution and Taxonomy Section 3. A single investigation of spatial genetic structure on Santa Cruz found females of multiple generations did not exhibit site philopatry, as there was no evidence of a relationship between familial relationship and spatial organization (Jones et al. 2013).

Studies of effective population size, genetic load, and inbreeding are ongoing for island foxes (Robinson et al. 2016, 2018), but have not been conducted for island spotted skunks. Effective population size is partly a function of the variation in population size over generations, and describes the available genetic variation that may be inherited and the rate at which genetic diversity will be lost in the future. Genetic load assesses the accumulation of deleterious mutations within a population. Finally, inbreeding studies investigate the frequency of close-relative matings (regardless of whether by choice or small population size), which, in turn, relates to the frequency of deleterious recessive alleles coming together in homozygous form.

Low genetic diversity in island spotted skunks is expected because these are small island populations. However, it is unclear whether long-term low effective population size as compared to more recent demographic declines and bottlenecks has more of an influence in producing low genetic diversity in skunks. The functional effects (inbreeding depression) of low genetic diversity in island spotted skunks is unknown.

Current Research

As of May 2021, a team led by J. Owen, E. Bolas, and B. Sacks are analysing the nuclear genome to gain further insight into the evolutionary history of island spotted skunks. Additionally, analysis of the nuclear genome can estimate current levels of inbreeding between island spotted skunk populations on Santa Cruz and Santa Rosa islands.

Future work led by V. Bakker includes further analysis of morphometric data to clarify taxonomic distinctions.

Key Knowledge Gaps

- Timing of divergence of island spotted skunks from mainland western spotted skunk populations and timing of divergence of Santa Rosa Island and Santa Cruz Island populations of island spotted skunks. These gaps are being addressed by:
 - Further evaluation of timing of divergence using RAD sequencing
 - Further investigation of relatedness between island populations and island mainland populations using microsatellites or SNPs
 - Further study of genetic variation within island populations
- Effective population sizes, genetic load, and inbreeding levels of island spotted skunks on Santa Rosa and Santa Cruz Islands. To be addressed with:

- Whole genome studies to determine effective population size, genetic load, and inbreeding levels for island spotted skunks
- Evidence of functional effects of low genetic diversity (see Johnson et al. 2010, Robinson et al. 2018)
- Whole genome studies to examine demographic history and the relative influence of long-term small populations vs bottlenecks on low genetic diversity

e. Disease

Section Leads: Katie Prager, Julie Barnes, Kevin Crooks, Angela Guglielmino, Laura Shaskey (adapted from ESSCSG 2019)

Infectious disease can exert regulatory pressure on host populations, and in certain cases can lead to large fluctuations in host numbers (Anderson and May 1979; Anderson and May 1991; Roelke-Parker et al. 1996). In large healthy populations, these fluctuations generally pose no threat to species and population persistence; however, combined with stochastic events (environmental and demographic), the presence of pathogen reservoirs, climate change, and anthropogenic movement of pathogens and hosts, infectious disease may pose an important extinction risk, especially to threatened and endangered species (Thorne and Williams 1988; de Castro and Bolker 2005; McCallum 2012).

Small island populations present a particularly interesting case as epidemiological theory predicts that these populations – which are isolated from mainland hosts and pathogens – will be unable to maintain most pathogens in the absence of an external reservoir (e.g., Smith et al. 1993). Therefore, they would seem to be at lower risk of disease related extinction events. However, because these island populations evolve in the absence of many pathogens, they could lose innate protective immunity due to genetic drift, making them highly susceptible to – and at risk of extinction from – novel, highly transmissible and pathogenic infectious diseases that might invade (e.g., Wilkelski et al. 2004). Island spotted skunks exist at relatively low population sizes and share their ecosystem with few hosts from which pathogens may spillover or be shared (e.g., island foxes, deer mice, marine mammals). They thus have limited risk of pathogen exposure, but may be at great risk of disease-driven extinction due to stochastic spillover events.

Canine distemper virus (CDV) and rabies virus pose an important conservation risk to many threatened and endangered carnivores, having caused massive population declines, local extirpations, and mortality events in various species (e.g., black-footed ferrets, Thorne and Williams 1988; eastern spotted skunks). In fact, a CDV outbreak was recently detected in a population of eastern spotted skunks (*Spilogale putorius*) in DuPont State Recreational Forest, North Carolina in which resulted in 50% mortality of the collared animals in a study (Harris et al., 2021). Therefore these viruses are considered high risk to island spotted skunks, with a similar potential to cause major population declines. Spotted skunks are also susceptible to a number of other pathogens that may pose a threat to the health and persistence of their population (see detailed description of specific pathogens below and in Appendices 2 and 3), and as with CDV and rabies, many of these pathogens may be shared with island foxes. The potential impact of pathogens on the skunk population may be further increased if it is experiencing other,

potentially immunosuppressive stressors (e.g., malnutrition, high inter- or intra-specific competition, predation). Although island spotted skunk densities reached record highs on Santa Cruz Island in the mid-2000's (Jones et al. 2008; Figure 1) when island foxes were rare, at their current low numbers, spotted skunks are particularly vulnerable to stochastic events such as disease epidemics. In addition, rapidly increasing island fox populations over the last decade increases the potential for fox to skunk transmission of shared pathogens and parasites - both endemic and introduced (e.g., CDV and rabies virus). In order to determine which pathogens may pose the greatest risk to the island spotted skunk, it is critical to collect baseline data on pathogens circulating in the populations on Santa Cruz and Santa Rosa Islands. Equally important is to identify pathogens that are not currently circulating in skunk populations but to which skunks may be highly susceptible, for example CDV, rabies virus, amdoparvovirus, SARS-CoV-2 (Michelitsch et al. 2021; <u>CDC COVID-19 and Animals; Cases of SARS-CoV-2 in Animals in the United States</u>).

A serosurvey conducted in 2000-2001of Santa Cruz island skunks detected low prevalence of heartworm antigen and canine parvovirus (CPV) antibodies, but failed to detect antibodies against canine adenovirus (CAV), canine distemper virus (CDV), canine herpesvirus (CHV), pseudorabies virus or *Leptospira* serovars Pomona, Canicola, and Icterohaemorrhagiae (Bakker et al, 2006). More recent pathogen surveys of skunks, conducted between 2010 and 2015 on Santa Rosa Island, indicate that *Leptospira interrogans* serovar Pomona is circulating in the population and that skunks show no evidence of exposure to CDV, CAV and CPV, although sample sizes were small (Guglielmino et al., unpublished data).

A number of ectoparasites, which could act as important pathogen vectors, have also been identified on spotted skunks from both Santa Cruz and Santa Rosa Islands, including ticks (Ixodes pacificus, I. kingi), fleas (Pulex irritans, Nosopsyllus fasciatus), and lice (Neotrichodectes mephitidis) (Crooks et al. 2004; Appendix 2). Ixodes pacificus is the most commonly encountered Ixodes species in California and also occurs on island foxes (Crooks et al. 2001b). Ixodes kingi is widespread in the United States but is seldom reported in California and was not detected on island foxes. Pulex irritans, which also occurs on island foxes, is a widespread human flea that attacks a wide range of hosts, including domestic animals such as cattle, swine, and dogs, all of which have occurred on Santa Cruz and Santa Rosa Islands and may have transmitted parasites to the native carnivores. Nosopsyllus fasciatus, the rat flea, was detected on island spotted skunks on both islands. Although no rats are currently known to live on Santa Cruz and Santa Rosa Islands, spotted skunks may have acquired N. fasciatus directly from rats that might have escaped from visiting ships historically, or from the bodies of prey animals (e.g., deer mice). Neotrichodectes mephitidis, a chewing louse normally associated with the striped skunk (Mephitis mephitis), also occurs on island foxes, suggesting skunk to fox transmission. In all, these results suggest potential host-switching by ectoparasites among the native and introduced fauna on Santa Cruz and Santa Rosa Islands, demonstrating the possibility for disease transmission among species via ectoparasite vectors.

Pathogen surveys of mainland spotted skunks show evidence of exposure to a range of pathogens (Appendix 2), and spotted skunks have been identified as reservoirs for the bacteria *Francisella tularensis* (McKeever et al. 1958) and *L. interrogans* serovar Ballum (Gorman et al. 1962), and the fungus *Histoplasma capsulatum* (Emmons et al. 1949). In addition, all mainland North

American members of the genus *Spilogale* play an integral role in the enzootic and epizoonotic cycles of the rabies virus (Krebs et al. 2000, Krebs et al. 2001). Most recently, during a mortality event in DuPont State Recreational Forest, North Carolina, eastern spotted skunks have been shown to be highly susceptible to CDV (Harris et al., 2021).

We will use data on pathogens and ectoparasites identified in island spotted skunks on Santa Cruz and Santa Rosa Islands, as well as those which have been detected in mainland spotted skunks, to develop our infectious disease management plan. The aims of this plan are to: (1) establish a baseline of what is currently circulating and to understand to what diseases the population is naive – especially with regards to pathogens of major concern, e.g., rabies virus, CDV, Aleutian mink disease, skunk amdoparvovirus; (2) obtain data necessary to integrate disease risk into mathematical models to assess the impact of pathogens on spotted skunk persistence; and (3) determine whether interventions are necessary to ensure population processing, and storage guidelines to ensure consistency in data streams; and (2) bank samples for retrospective testing of other pathogens of pathogens of major concern. With these goals in mind, we identify a number of pathogens of particular concern or interest below.

Pathogens of Interest

- <u>Parvovirus:</u> When canine parvovirus mutated from the feline panleukopenia virus in the 1980s, it quickly expanded into a global pandemic, causing up to 90% mortality in some wildlife populations (Parrish 1994, Steinel et al. 2001). While the extent to which parvoviruses contributed to the decline of *S. putorius interrupta* is unclear (Gompper and Hackett 2005), mink viral enteritis, feline panleukopenia, canine parvovirus, and Aleutian mink disease parvovirus undoubtedly cause morbidity and mortality in current populations of eastern spotted skunks, western spotted skunks, and striped skunks (*Mephitis mephitis*) (Barker et al. 1983, Oie et al. 1996, Gehrt 2005, Suzán and Ceballos 2005, Bakker et al. 2006, Pennick et al. 2007, Allender et al. 2008). Furthermore, in 2010-2013, an outbreak of fatal amdoparvovirus-associated disease was documented in striped skunks in the San Francisco Bay Area of California, and additional testing detected amdoparvovirus DNA in 64.8% of sampled striped skunks across the state, with the first test-positive skunks detected from 2004 samples (Glueckert et al. 2019). It is unknown to what degree this newly discovered skunk amdoparvovirus may also be affecting spotted skunk populations.
- <u>Canine distemper virus (CDV) and rabies virus:</u> These two viruses pose an important conservation risk to many threatened and endangered carnivores, having caused massive population declines, local extirpations, and mortality events in various species (e.g., Rabies: Alexander et al. 1996; Gascoyne et al. 1993; Johnson et al. 2010; Randall et al. 2004; CDV: Gilbert et al. 2014; Goller et al. 2010; Laurenson et al. 1998; Williams et al. 1988; Thorne and Williams 1988; Harris et al., 2021). Although neither CDV nor rabies virus was a likely cause of the initial rapid decline of *S. putorius* (Gompper and Hackett 2005), these viruses are most likely contributing factors in continued morbidity and mortality in eastern spotted skunk populations. In fact, Harris et al. (2021) describe high CDV-related mortality in their study population in DuPont State Recreational Forest, North Carolina. Members of the genus *Spilogale* are among the principal hosts of rabies

(Krebs et al. 2000, Krebs et al. 2001, Suzán and Ceballos 2005) and distemper is widespread in North American mephitids overall (Goss 1948, Helmboldt and Jungherr 1955, Verts 1967, Diters and Nielsen 1978, Gehrt 2005).

- SARS-CoV-2: COVID-19 is an emerging infectious viral disease caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) causing a global pandemic in 2020. Infection and clinical disease has been reported in domestic dogs and cats, farmed mink, ferrets, asiatic small clawed otters and several other species (Michelitsch et al. 2021; https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/sa one health/sars-cov-2animals-us.). The disease has caused mild to moderate respiratory disease in most species with animals making a full recovery except for mink where it has caused acute severe interstitial pneumonia resulting in increased mortality on mink farms (Molenaar et al. 2020) There is still more to learn about this newly emerging disease and the risk to other species, but given that the family Mephitidae which includes skunks is closely related to the Mustelidae family, it is reasonable to suspect that skunks could be susceptible to SARS-CoV-2 and development of severe clinical disease. Clinical signs suspicious of SARS-CoV-2 infection in animals include fever, coughing, difficulty breathing or shortness of breath, lethargy, inappetance, sneezing, wheezing, nasal discharge, ocular discharge, vomiting and diarrhea. Currently there are no vaccines available for use in animals for this disease but they are under development. Based on the available information to date, the risk of animals spreading COVID-19 to people is considered to be low, but we know that it can spread from people to animals in some situations, especially during close contact (e.g., mink, lions, tigers). Therefore people with suspected or confirmed COVID-19 should avoid contact with animals and appropriate biosecurity measures should be in place when working in close proximity to these animals which includes wearing an appropriate mask and gloves, disinfection of equipment that comes in contact with the animal, and vaccination of personnel working in close proximity to these animals.
- <u>Other viral diseases</u>: Viruses such as infectious canine hepatitis, canine herpesvirus, and rotaviruses have been detected in striped skunks (Alexander et al. 1972, Karstad et al. 1975, Charlton et al. 1977, Diters and Nielsen 1978, Evans 1984). However, their prevalence and impact on population health in spotted skunks is unknown.
- <u>Leptospira spp</u>.: Leptospira interrogans serovar Pomona is known to circulate in the spotted skunks on Santa Rosa Island, but has not been detected in skunks on Santa Cruz Island (Guglielmino et al., unpublished data). However, the health impact of this spirochete on the skunk population is unknown and may be minor or absent. Leptospirosis has been described in *S. putorius* (Gorman et al. 1962).
- <u>Filarial diseases</u>: Despite the importance placed on heartworm (*Dirofilaria immitis*) disease in domestic animal veterinary medicine, the impact of *D. immitis* and other filarial diseases on wildlife populations remains largely unstudied (Venco et al. 2015). Microfilarial diseases have been recorded in striped skunks (Chandler 1947, Webster and Beuregard 1964, Saito and Little 1997), and heartworm antigen was detected in an island spotted skunk (*S. g. amphiala*) (Bakker et al. 2006). The geographic areas of highest *D. immitis* prevalence in dogs overlaps heavily with the current range of *S. p. interrupta* (Bowman et al. 2016).
- <u>Other mosquito- and fly-borne diseases</u>: In addition to filaria, North American mephitids host several other mosquito-borne pathogens, most notably West Nile virus in striped and

western spotted skunks and an undetermined *Flavivirus* in striped and hooded skunks (*Mephitis macroura*) (Anderson et al. 2001, Bentler et al. 2007, Gabriel et al. 2008). The increased periods of drought punctuating future climatic patterns may exacerbate the severity of West Nile virus outbreaks throughout the United States (Paull et al. 2017). Increased periods of drought, high temperatures, and dry weather patterns likewise amplify the spread of sand-fly-borne diseases, such as leishmaniasis (Cardenas et al. 2006, Kaffenberger et al. 2017). *Leishmania* strains have been recovered from hog-nosed skunks, *Conepatus chinga* (Buitrago et al. 2011), and pygmy spotted skunks (*Spilogale pygmaea*) and eastern spotted skunks have been identified as probable reservoirs for the protozoan parasites in Mexico (Stephens et al. 2009). The distribution of canine and human leishmaniasis in North America largely overlaps with the range of *S. p. interrupta*, increasing the likelihood of this subspecies' exposure to the disease (Baneth and Solano-Gallego 2012). However, many of these pathogens are likely low risk to the island spotted skunk due to geographic isolation.

- Ectoparasite-borne diseases: Q fever, Powassan virus, plague (Yersinia pestis), babesiosis (Babesia spp.), Rocky Mountain spotted fever (Rickettsia rickettsii), tick-borne relapsing fever (Borrelia turicatae), Tularaemia (Francisella tularensis), and Lyme disease (Borrelia burgdorferi) have been isolated in western spotted skunks, eastern spotted skunks, and/or striped skunks (McKeever et al. 1958, Holbrook and Frerichs 1970, Alexander et al. 1972, Riemann et al. 1978, Magnarelli et al. 1983, Smith et al. 1984, Johnson 1987, LoGiudice et al. 2003, Salkeld and Stapp 2006, Brinkerhoff et al. 2009, Clark et al. 2012, Wormser and Pritt 2015, Gulas-Wroblewski et al. 2017). Additionally, the ectoparasite taxa found on island spotted skunks (see Appendix 2) are known vectors of a diverse array of infectious pathogens (Norman et al. 1999, Parola and Raoult 2001, Bitam et al. 2010, Eisen and Gage 2012). For example, Pulex irritans fleas are a vector of *Yersinia pestis*, the causative agent of plague, *Ixodes pacificus* ticks are a vector of Lyme disease (Borrelia burgdorferi), and chewing lice are active ectoparasites that can produce intense irritation with secondary bacterial infection (Crooks et al. 2001b; Burgdorfer et al. 1985). Climate change models predict shifting distribution of ectoparasites and their associated diseases, which will impact the degree and diversity to which mainland spotted skunks, and perhaps island spotted skunks, will be exposed to ectoparasite-borne pathogens in the future (Esteve-Gassent et al. 2016).
- Other endoparasites (protozoans and helminths): Spirocerca and Acanthocephala have been detected in island foxes and have been associated with significant pathology. Although Acanthocephala are limited to San Miguel island foxes, Spirocerca infections have been severe in foxes across the Northern Channel Islands, including foxes from Santa Rosa and Santa Cruz Islands. Spirocerca and other endoparasites may pose a risk to island spotted skunks although this has not been reported in the limited number of island skunk necropsies performed so far (Dr Leslie Woods, CAHFS, personal communication). *Baylisascaris columnaris* and *Skrjabingylus chitwoodorum* (sinus nematode) have both been well documented in mainland skunks (Lesmeister et al. 2008; Gehrt et al. 2010; Hughes et al. 2018; Higdon and Gompper 2020), but it is unclear how these parasites may be impacting island spotted skunks. The sinus nematode can cause a swelling of the frontal sinuses in mustelid hosts, potentially reducing braincase volume (Bowman and Tamlin 2007). Within mainland spotted skunks, western clades have a higher prevalence of the sinus nematode, with gastropods (snails) as the intermediate host (Higdon and

Gompper 2020). Evidence of this nematode is present in the majority of island spotted skunk skulls in the Santa Barbara Museum of Natural History collection, from both Santa Cruz and Santa Rosa Islands.

- <u>Fungal disease</u>: Fungal infections are emerging as driving forces in population crashes of a variety of wildlife species (Hayman et al. 2016, Lorch et al. 2016, Scheele et al. 2017). *Histoplasma capsulatum* was recovered from an eastern spotted skunk (Emmons et al. 1949), while cases of histoplasmosis and aspergillosis have been reported in striped skunks (Durant and Doll 1939, Emmons et al. 1955, Menges et al. 1955, Verts 1967). To date, the type, clinical manifestations, and influence on overall health of pathogenic fungi in island spotted skunks are unknown. A comprehensive assessment of mycosis in this species is needed, especially as new and more heat-tolerant fungal pathogens evolve in response to rising global temperatures (Robert et al. 2015).
- <u>Toxoplasmosis</u>: As its primary definitive hosts, domestic cats (*Felis catus*), spread across the North American continent, so too does the coccidian parasite *Toxoplasma gondii*. There is serologic evidence of exposure to *T. gondii* in western spotted and striped skunks (Franti et al. 1976, Tizard et al. 1976, Diters and Nielsen 1978, Riemann et al. 1978, Suzán and Ceballos 2005, Gabriel et al. 2008), and the parasite has caused clinical illness and significant population declines in closely-related wild mustelids (Burns et al. 2003, Conrad et al. 2005). However, there are no resident cats on Santa Cruz and Santa Rosa Islands, therefore this protozoal pathogen is likely of very low risk to these populations.
- <u>Chagas disease</u>: Infections with the protozoan parasite *Trypanosoma cruzi* (Chagas disease) typically lead to acute or chronic cardiac disease in mammalian hosts and resulted in myocarditis in a striped skunk in California (Ryan et al. 1985). A plains spotted skunk (*S. p. interrupta*) tested positive for Chagas disease in Texas (Gulas-Wroblewski et al. 2017), and the extended range of eastern spotted skunks includes regions with confirmed *T. cruzi*-infected triatomid insect vectors, domestic dogs (*Canis familiaris*), humans, and other wild mammals (Bern et al. 2011, Snowden and Kjos 2011, Garcia et al. 2015). Currently the triatomid insect vectors for this pathogen are absent from Santa Cruz and Santa Rosa Islands, hence Chagas disease is of low concern.
- <u>Other bacterial diseases:</u> *Staphylococcus* and *Listeria* infect striped skunks (Bolin et al. 1955, Osebold et al. 1957, Verts 1967, Aarestrup 2001), and *Brucella abortus* infects western spotted and striped skunks (Moore and Schnurrenberger 1981). Whether these pathogens are seen in island spotted skunks needs to be determined.

Disease Management Plan

Diseases of concern in island skunk populations are poorly understood due to limited disease studies and limited availability of carcasses for pathological studies. Hematology and biochemistry results are published for thirty-six island skunks (with and without sedation) between 1999-2001 (Crooks et al. 2003). Baseline data will be helpful to understand if there are current diseases of concern that could be affecting the current population size and potential disease threats in the future. Sharing of habitat and interactions with island foxes also leads to potential interspecies transmission and shared disease threats.

There is a concern that the newly discovered skunk amdoparvovirus (related to Aleutian disease) may be an emerging disease in free-living striped skunk in California (LaDouceur et al. 2014, Glueckert et al. 2019) and it is unknown if amdoparvovirus is present in island skunk populations. Testing to determine the presence or absence of this pathogen will be helpful in disease management. Based on limited studies, heartworm infection appears to be of low incidence in skunks on Santa Cruz island (1/31 skunks tested positive for heartworm antigen in 2006) (Bakker et al. 2006). Further testing will help determine if this is a pathogen of concern or not. Storage of serum samples will aid retrospective studies in the event of disease outbreaks and to understand threats in the future.

Based on the pathogens known to impact mainland skunks as well as those found in island spotted skunks, we recommend the following plan:

- 1) Regular sampling of island spotted skunks using an established protocol for consistency across sites, research groups and managers (see also Appendix 4).
 - a) At a minimum collect the following from all animals in hand
 - i) Blood for serum and clot (plain tube)
 - ii) Vibrissae (+/- fur)
 - iii) Feces if present in trap
 - b) When time, skill, and animal stress permit collect the following additional samples:
 *Up to 5 ml can be collected from an animal greater than 600 gm, however most handlers typically collect 1-3 ml. CBC/chemistry panel can be run on a small sample (1 ml or less) in a heparin tube.
 - i) Blood for CBC (EDTA or heparin)/ hemoparasites (EDTA)/ heartworm screen (EDTA).
 - ii) Urine
 - iii) Rectal swab
 - iv) Ectoparasites
- 2) Pathogens of concern for screening
 - a) High priority
 - i) CDV
 - (1) Serum antibody (viral neutralization)
 - (2) +/- viral antigen (PCR) in tissues or urine
 - ii) CPV
 - (1) Serum antibody
 - iii) Aleutian mink disease virus/ new skunk amdoparvovirus strain
 - (1) Viral antigen to determine viral carriers for Aleutian mink disease PCR by Zoologix Lab (whole blood, feces, rectal swab, urine or tissues) or for other skunk amdoparvovirus strains possibly at Dr Janet Foley's Lab, UC Davis.
 - (2) Serum antibody (ELISA) for Aleutian mink disease to determine exposure (not infection) - University of Georgia. Need to contact the lab to find out if they can test for antibodies to any other skunk amdoparvovirus strains.
 - (3) Coordinate with Deanna Clifford on amdoparvovirus testing efforts in California
- b) Lower priority
 - i) Endoparasites fecal flotation/ova and parasite screen
 - ii) Dirofilaria immitus
 - (1) Antigen ELISA (canine/feline) not validated for skunks and unknown if canid or felid test is more suitable for skunks. (Serum)
 - (2) Microfilaria blood smear or Modified knotts test (EDTA)
- 3) Necropsy all available carcasses sent to Leslie Woods at CAHFS
- 4) Consider vaccination
 - a) Vaccination of exotic carnivores with the recombinant canary pox vector vaccine (Purevax Ferret Distemper Vaccine; Merial) and the killed rabies virus vaccine (IMRAB; Merial) is standard protocol in zoos and is considered safe. This protocol should be considered, but safety and efficacy should be further assessed by performing a thorough literature review prior to adopting this protocol (e.g., assess safety and efficacy in: blackfooted ferret, red panda, island fox studies).
 - b) Then perform mathematical modeling to assess the most effective vaccination strategy, i.e., total number of animals to vaccinate, frequency, distribution across locations and age and sex classes.
 - c) Once safety is confirmed, consider the cost/benefit of simply vaccinating all skunks that are handled during captures, as is done with the island foxes.

Key Knowledge Gaps

- Systematic literature review of pathogens/parasites in the mainland western spotted skunk (this has been completed for the eastern spotted skunk; ESSCSG 2019)
- Baseline data on pathogens currently circulating in island spotted skunk populations
- Identification of pathogens, to which skunks may be highly susceptible, which are not currently circulating in their population
- Integration of disease risk into mathematical models to assess impacts of pathogens on skunk population persistence
- Determination of which interventions are necessary to ensure population persistence, including the potential for rabies and/or CDV vaccinations
- Possible transmission of pathogens from island foxes to skunks (i.e. assess serosurvey and pathogen detection PCR results from sampled foxes).

f. Diet

Section Leads: Juliann Schamel and Paul Collins

On the mainland, the eastern spotted skunk is an omnivorous species that can have a varied diet (ESSCSG 2019). Insects are a particularly important food source for the species, with beetles (Coleoptera) and grasshoppers (Orthoptera) major components of their diet (Howell 1906, Crabb 1941, Kinlaw 1995). Other recorded food items include small mammals, birds, lizards,

salamanders, fungi, carrion and plant material (Howell 1906, Pellett 1913, Selko 1937, Crabb 1941, McCullough and Fritzell 1984).

To date, there have been only two published studies on the diet of island spotted skunks, both on Santa Cruz Island (see Natural History Section 5). Island spotted skunk diet was investigated on Santa Cruz Island via scat analysis in 1991-1992, during a period of low population density (Crooks and Van Vuren, 1995). Analysis of 41 scat contents showed skunks were consuming primarily deer mice and insects along with occasional lizards and birds. Fruits and berries were absent (Crooks and Van Vuren 1995). Diet was calculated as percent frequency of occurrence in scat at each study site and season (wet / dry). Some seasonal variation in diet was found. Deer mice (88% / 88%) and insects (60% / 75%) were the primary food items in both the wet and dry season. Other food items found in scat were lizards (12% / 31%) and birds (4% / 0%). The insects consisted of: Jerusalem cricket (52% / 81%), other Orthoptera (36% / 19%), Coleoptera (8% / 44%), Lepidoptera (20% / 25%), Dermaptera (4% / 0%), Hymenoptera (11% / 33%). No fruits or other plants were found in island spotted skunk scat on Santa Cruz Island in 1991-2.

A second inquiry into island spotted skunk diet via scat analysis was carried out on Santa Cruz Island in 2003-2004, when fox density was very low and skunk density was very high (Jones et al. 2008). This study found a different and broader range of foods in use, including a high occurrence of invertebrates, a moderate occurrence of vertebrates, and a low occurrence of plants (fruits and berries), particularly in the dry season (Jones et al. 2008). Insects (100% / 93%) made up the majority of the diet in 2003-2004, in both the wet and dry seasons. Lizards and salamanders (42% / 45%) dominated the vertebrate portion of the diet. Deer mice (19% / 14%) were also found, but no birds. Insects consisted of: Jerusalem cricket (100% / 83%), other Orthoptera (58% / 45%), Coleoptera (69% / 38%), Lepidoptera caterpillars (50% / 7%), Dermaptera (54% / 55%), and other insects (58% / 24%). In 2003-2004, some plant matter was also found in skunk scat (26% / 29%), suggesting that skunks may have expanded their dietary niche breadth in response to intra-specific competition (Jones et al. 2008). It is interesting to note that in 2003-2004, insects, lizards, and salamanders were the most frequently used food items, rather than deer mice, which were found to be the most important food source in 1991-92.

The only other known records of island spotted skunk diet are from three field expeditions in 1927, 1936, and 1948. H. H. Sheldon recorded the stomach contents of eight island spotted skunks collected on Santa Rosa Island in 1927, which he found to contain "75% crickets, grasshoppers, and potato bugs, and 25% seeds of the cactus pear" (Sheldon, 1927). This is the only known island spotted skunk dietary study from Santa Rosa Island. The stomach contents of one skunk trapped by Rett in 1936 on Santa Cruz Island contained "nothing but insects, beetles, and bugs" (Rett, 1936). Pearson's 1948 unpublished field notes include the stomach contents of six skunks collected on Santa Cruz Island, which were found to contain "insects (Jerusalem crickets), deer mice, carrion (unspecified), grapes (*Vitis* spp.), and summer holly (*Comarostaphylis diversifolia*) stems and berries" (Collins, 1998). It is interesting to note that *Opuntia* was recorded as a food source on Santa Rosa Island in 1927, but has not been detected in any studies from Santa Cruz Island. It is possible that island spotted skunk diet varies between islands, as has been found in island fox populations (Cypher et al. 2014). Cypher et al. (2014) found that deer mice and insects were the most important food items to island fox in all seasons on Santa Rosa Island, whereas fruits and insects were the most important food items to island fox

in all seasons on Santa Cruz Island, with deer mice showing up at >10% frequency only in the spring season.

A current investigation of island spotted skunk diet on Santa Rosa Island is using stable isotope analysis of whisker samples collected from 2011 to present to assess seasonal diet, dietary niche breadth, and individual specialization across all major habitat types (Schamel, Newsome, and Guglielmino, ongoing). This study will assess dietary niche breadth and individual specialization across a gradient of population densities in island spotted skunk, to evaluate the effect of intra-specific competition on dietary resource (see Interaction with Foxes Section 8c for more details). This is the first study of skunk diet on Santa Rosa Island, aside from the record of eight stomach contents from 1927. Historical whisker samples from museum specimens (1927, 1936, 1948) are also being analyzed (Schamel, Newsome, and Collins, ongoing) to assess historical diet and the use of *Opuntia* cactus as a dietary item from a time when the cactus was more prevalent on the landscape. This study hopes to provide some context for *Opuntia* restoration, which was suggested as a potential management action at the January 2020 workshop.

Key Knowledge Gaps

- Current and historical seasonal skunk diet on Santa Rosa Island (prior published studies conducted only on Santa Cruz Island)
 - Research underway via stable isotope analysis of whiskers
- Current seasonal skunk diet on Santa Cruz Island
- Comparison of skunk diet across habitat types
 - Some data from Santa Cruz Island from 1991-2 and 2003-4
 - Research underway on Santa Rosa Island via stable isotope analysis of whiskers
- Temporal trends in skunk diet with changes in skunk population density, fox population density, and environmental characteristics
 - Research underway on Santa Rosa Island via stable isotope analysis of whiskers
- Potential switch in skunk diet with population fluctuations of deer mice (see Prey Population Dynamics Section 8g)
 - Will be explored to a some extent with current stable isotope research on Santa Rosa Island, but a more robust study would incorporate habitat-specific density index for deer mice on Santa Rosa and Santa Cruz Islands (see Prey Population Dynamics Section 8g) and reliable skunk population density estimate (see Population Monitoring Methods Section 8a)

Potential Research Methods

- Scat Collection: scat analysis and/or DNA metabarcoding
 - Scat collection at den sites located via VHF telemetry
 - Scent detection dogs to locate and collect island spotted skunk scat. Below are notes from Marie Tosa's project on western spotted skunks in the Pacific Northwest:
 - Scat detection dogs (Conservation Canines, handlers and dogs are now with Rogue Detection Dogs) worked best when handlers were able to

direct detection dogs to past den sites and dogs searched the den site structure and surrounding areas.

- Scats found by the detection dogs were typically close to the den site structure, sometimes in it, or sometimes in a nearby structure. Defecation areas were typically areas with vertical cover (e.g., under bark, under log, in a hole). Rarely were they in open areas.
- Stored scat in ziplock bags and froze samples as soon as possible. Can also dry samples and store at room temperature, but best to freeze.
- Whisker Analysis
 - Continue stable isotope analysis of whiskers to assess individual diet in different habitats and across a variety of environmental factors
- Hair Collection
 - Stable isotope analysis of hair samples collected at hair snare stations
 - Benefits: non-invasive, cheaper lab analysis
 - Drawbacks: the sample cannot be linked to a known individual, and would only provide one C and N value rather than the longitudinal data provided by a whisker sample; resulting dietary inferences would be much broader in scope

g. Prey Population Dynamics

Section Leads: Laura Shaskey and John Orrock

As is characteristic of oceanic islands, Santa Cruz and Santa Rosa Islands possess an unbalanced and depauperate fauna, thereby limiting prey diversity for island spotted skunks (Crooks and Van Vuren 1995; Jones et al. 2008; Schwemm et al. 2018). For example, the only common small mammal on Santa Cruz and Santa Rosa Islands is the deer mouse. On both islands, knowledge is limited on the population dynamics of skunk prey, as well as skunk foraging preferences relative to prey availability (see Diet Section 8f). Prior to the decline of the island fox population and when skunk density was low, skunk scat collected from Santa Cruz Island in 1992 documented deer mice and invertebrates as primary food items, and lizards as secondary food items (Crooks and Van Vuren 1995), suggesting that some prey types may be particularly important for skunk persistence. When fox density on Santa Cruz Island was very low and skunk density was high in 2003-2004, scat analysis showed that invertebrates made up the majority of skunk diet, lizards dominated the vertebrate portion of the diet, and deer mice were found to a lesser extent (Jones et al. 2008). This shift suggests the skunks may be flexible in their diet and can shift predation towards more abundant prey. Fluctuations in both fox and skunk densities over time could influence prey population dynamics, and thus fox and skunk foraging choices. Climate variation (e.g., annual precipitation) also influences prey availability.

Given a relatively species-poor prey base, and the proportion of mice in skunk diet, it is possible that skunk populations may respond to population dynamics of island deer mice. Long-term monitoring grids for island deer mice and herpetofauna have been maintained on San Miguel, Anacapa, and Santa Barbara Island for nearly three decades by the National Park Service. However, long-term monitoring programs for these species have not yet been established on Santa Rosa and Santa Cruz Islands, and predator-prey dynamics and available habitats do vary by island. On the other islands, deer mouse populations are known to go through significant

seasonal, annual, and multi-annual population cycles (Coonan 2016; Schwemm et al. 2018; Shaskey, *in prep*), which are influenced by precipitation patterns, terrestrial mammalian predators, and reproductive cycles (e.g., Drost and Fellers 1991; Orrock et al. 2011). High winter rainfall encourages plant growth and provides food resources, while drought reduces plant growth and limits mouse productivity. However, abundant winter rain combined with cold temperatures may actually increase winter mortality and reduce the number of mice that survive from fall to spring. Thus, it is possible that island spotted skunk populations may not only respond to population fluctuations of deer mice, but also to the climatic conditions that drive those fluctuations.

San Miguel Island is the only island with a long-term deer mouse monitoring program and a terrestrial mammal predator (i.e., the island fox), so patterns in deer mouse populations on Santa Rosa and Santa Cruz Islands could be inferred from what is observed on San Miguel. A significant negative relationship has been documented between fox abundance and deer mouse abundance (Orrock and Fletcher 2014). With island fox recovery and significant increases in fox densities on San Miguel Island, deer mouse populations decreased compared to periods when foxes were less abundant (Coonan 2016). Thus, it is conceivable that if fox predation also limits mice populations on Santa Cruz and Santa Rosa Islands, this might heighten competition and resource limitation for island spotted skunks (Jones et al. 2008).

Comparative data across all of the Channel Islands suggests that precipitation and terrestrial mammalian predators are important for affecting not only the abundance of deer mice, but also stress physiology and prevalence of diseases (Orrock et al. 2011). Sin Nombre virus prevalence in deer mice is a significant function of island area and rainfall, most likely because large islands that receive a lot of precipitation have the largest rodent populations and hantavirus spreads more readily in dense rodent communities (Orrock et al. 2011).

Although mice are important prey for skunks and foxes (Crooks and Van Vuren 1995), we know the least about their ecology on the two islands where foxes and skunks are found. In 2004-2006, capture rates of both western harvest mice and island deer mice on Santa Cruz Island were higher than previously recorded on the island in 1991-1995 (Drost et al. 2009), which could be a function of both habitat recovery from the removal of feral animals and decreased predation pressure while foxes were at very low densities. On Santa Cruz Island, deer mice population density also varied among habitats (Mayfield et al. 2000). Deer mice were abundant in habitat dominated by woody vegetation (e.g., chaparral, oak woodland, and coastal sage scrub) but rare or absent from habitat dominated by herbaceous vegetation (grassland, fennel-grassland). Thus, ongoing vegetative recovery following removal of non-native herbivores likely has benefited mice. In turn, skunks also frequent areas with woody vegetation, so vegetative recovery, and increased deer mice populations, may benefit skunks by improving habitat quality and prey availability (Jones et al. 2008). Preliminary evidence suggests that remotely sensed vegetation data may be highly useful for predicting deer-mouse population on Santa Cruz Island but not Santa Rosa Island (J. Orrock, unpublished data). These data suggest that island-specific factors may drive variation in mouse populations. Filling this knowledge gap may be important to determine skunk population viability and the ultimate outcome of fox-skunk interactions.

Skunk detections decreased dramatically on both Santa Cruz and Santa Rosa Islands from 2012 to 2019 (see Population Trends Section 6), which also co-occurred with a severe multi-year

drought. During this same period, island foxes increased to record densities on both islands. One scenario is that rapidly increasing densities of foxes during drought years impacted prey availability, resulting in increased competition for resources and possibly increased fox predation of skunks. A standardized monitoring protocol for deer mice on both Santa Cruz and Santa Rosa Islands could lend valuable insight into population dynamics of island spotted skunks. Analysis of temporal trends of deer mice abundance as a predictor of island spotted skunk population dynamics on both islands would be interesting.

A recommendation generated from the January 2020 workshop was to consider designing a monitoring program for mice on Santa Cruz and Santa Rosa Islands (see Monitoring Recommendations Section 9). Schwemm et al. (2018) suggest that a simple density index (the unique number of animals individuals detected during the sampling period) can provide a simple and cost-effective approach for determining seasonal and annual changes in island deer mouse abundance. For example, trapping grids of 25 traps in each of 3-4 habitats, trapped 1-2 times a year, could provide valuable information on deer mice populations; small mammal traps to use in this effort might be available from UC Davis (Dirk Van Vuren, personal communication). NPS is interested in collaborating with researchers, students, and/or island field stations to develop a long-term monitoring program for mice on Santa Cruz and Santa Rosa Islands.

Key knowledge gaps

- Seasonal and annual deer mouse population dynamics on Santa Rosa and Santa Cruz islands are currently unknown. Standardized mouse monitoring grids are needed on both islands. In addition to important population data, monitoring grids provide the opportunity to sample rodents for diseases and parasites that can infect skunks, foxes, and humans. Santa Cruz and Santa Rosa Island are also the two islands where mice have the greatest prevalence of Sin Nombre virus (Orrock et al. 2011; Orrock and Allan 2008).
- Design and feasibility of deer mouse trapping grids on Santa Cruz and Santa Rosa Islands
- Relationship between population dynamics of deer mice and island spotted skunks
- How climate might mediate the relationship between deer mice and island spotted skunk populations
- How deer mice might mediate potential competition between island foxes and island spotted skunks
- Seasonal and annual population dynamics of invertebrate prey species are currently unknown. Invertebrates make up a significant portion of island spotted skunk diet (Crooks and Van Vuren 1995, Jones et al. 2008), therefore research on the dynamics of invertebrate prey availability in different habitat types and climate conditions is needed.
- Lizard monitoring on Santa Rosa and Santa Cruz islands is limited, and the population status of amphibian species are currently unknown. Further monitoring of herpetofauna on both islands is necessary.
- Due to diverse habitats and the large area of both Santa Rosa and Santa Cruz Island, prey availability studies should be focused in regions with long-term skunk population monitoring (remote cameras and/or traps).

h. Habitat Use

Section Leads: Kevin Crooks, Ellie Bolas, Adam Dillon, Calypso Gagorik, Tad Theimer

To date, there have been only two published studies on habitat use of island spotted skunks, both on Santa Cruz Island (see Natural History Section 5). Based on a VHF radio-telemetry study in 1992, skunks on Santa Cruz Island showed a preference for ravines dominated by coastal sage scrub (Crooks and Van Vuren 1994, 1995). Radio-collared skunks in 2003-2004 used a wide variety of habitat types, although they tended to avoid open habitats compared to more heavily vegetated sites (Jones et al. 2008). High skunk densities during this period may have forced them to use a broader array of habitats than they did in 1992.

Currently, there are several ongoing analyses of habitat use of island spotted skunks. In 2015-2017, microhabitat associations for skunks were investigated at camera and trap sites across both islands. Skunks had positive associations with measures of rugged topography (terrain roughness and proximity to stream courses) and woody vegetation including low shrubs and trees and stumps (Bolas et al. *in review a*). The use of these microhabitats may reflect skunks using ground-level cover provided by terrain or vegetation in order to avoid competitive interactions with foxes.

Additionally, a team led by C. Gagorik is using GPS telemetry collected 2018-2019 to assess habitat use of skunks and foxes on Santa Cruz Island (see Population Monitoring Methods Section 8a). Incomplete datasets from 6 GPS collars were recovered. Number of GPS points varied across individuals and species due to collar damage and malfunctions. Preliminary data suggest that skunks tended to occupy areas of high slope, high cover, or both. Skunks also displayed restricted movements by only using a small percentage of their overall home range during the course of a week. As of May 2020, data analysis is in progress.

Finally, a team led by A. Dillon is analyzing macrohabitat variables associated with skunks captured on fox trapping grids on Santa Cruz Island (2008-2019) and Santa Rosa Island (2009-2019). Macrohabitat variables will be included as covariates in spatially explicit capture-recapture (secr) models predicting skunk density. Habitat-specific densities can then be extrapolated across the island to generate habitat maps for island spotted skunks and island-wide population estimates. As of June 2021, data analysis is in progress.

Key knowledge gaps

- Microhabitat use by island spotted skunks
- Habitat partitioning between skunks and foxes
- Habitat-specific densities
- Extrapolation of habitat-specific densities to generate island-wide habitat maps and population estimates on both Santa Cruz and Santa Rosa Island

9. Monitoring Recommendations

Section Leads: Kevin Crooks, Christina Boser, Laura Shaskey, Lara Brenner

With the development of this plan and associated background information, a key next step is the prioritization and implementation of specific actions to advance the conservation of the island spotted skunk, including both monitoring and management. In this section, we list monitoring

recommendations generated during the January 2020 workshop, with subsequent group input; this list will be updated annually. We emphasize this list represents a brainstorm of monitoring recommendations and that, ultimately, decisions on monitoring plans will be determined by the land management agencies, NPS and TNC. For each monitoring recommendation, we identify the potential timeline of pursuing the activity:

- 1. VHF mortality monitoring
 - a. Monitoring of skunks fitted with VHF collars with mortality sensors will allow for:
 - i. Estimation of survival rates
 - ii. Identification of cause of mortality (although retrieving body can be challenging)
 - iii. Identification of den sites (to potentially find skunk scats for diet or genetic analyses, and to place remote cameras at the den to possibly evaluate juvenile recruitment and fox predation on den sites)
 - b. Note that the use of VHF and GPS collars for location tracking (e.g., to estimate movement patterns and home ranges) is challenging and likely not an immediate priority for skunk monitoring.
 - c. <u>Timeline</u>: In 2020, NPS piloted VHF mortality surveys on Santa Cruz Island, at the Scorpion campground. A total of 5 skunks were collared (3 males, 2 females) between February 2020 and February 2021. No skunk mortalities were detected during the study. As of March 2021 there are no active collars in this area. There are plans to expand mortality monitoring to Santa Rosa Island in the future.
- 2. Island-wide camera monitoring
 - a. Camera grids, stratified by habitat type and operated continuously, can yield valuable information on:
 - i. Occupancy/distribution
 - ii. Habitat use (via occupancy models and relative activity indices);
 - iii. Extinction/colonization dynamics (via dynamic multi-season occupancy models);
 - iv. Spatio-temporal interactions with foxes (via multi-species occupancy models);
 - v. Daily activity patterns (via time stamps on photographs);
 - vi. Relative abundance (via detection rates)
 - vii. Population size (if skunks are individually identifiable on camera)
 - b. <u>Timeline</u>: There are several recent and ongoing local-scale camera projects on Santa Cruz and Santa Rosa Islands. Design and implement systematic island-wide camera surveys in future years. A workshop to discuss the design of camera surveys will be conducted in summer/fall 2021.
 - c. <u>Rationale</u>:
 - i. Bolas et al. (2020) suggested that cameras may be more efficient than traps for monitoring skunks, as the rugged terrain of both islands poses logistical challenges for trapping, especially during the wet season.

- ii. Further, remote cameras may be stationed over larger areas or longer durations with much less effort than traps.
- iii. In addition, Bolas et al. (2020) found that skunk detections with cameras increased from summer to early winter in 2017, which suggests that winter monitoring may be preferable, and since winter rains and wet conditions can make trapping difficult or impossible, wildlife cameras may be superior for gathering data on skunks at this time of year
- 3. Trapping grids
 - a. Ongoing fox trapping grids have provided consistent information on skunk population trends, but their design is not ideal for skunks.
 - <u>Timeline</u>: Fox grids, conducted annually each summer/fall, will be conducted at least through 2021 when the post-delisting monitoring plan for foxes is reevaluated. NPS has plans to continue trapping grids on Santa Rosa Island in perpetuity as part of their long-term monitoring program, but is considering reducing the number of grids from 18 to 12. On Santa Cruz Island, TNC reduced the number of grids from 10 to 8 on TNC property in 2020 (omitting two grids added in 2017). These 8 grids will be operated in 2021.
 - b. Another option is to design trapping grids targeted for skunks specifically. This could include conducting trapping grids in winter when skunk capture success is higher. Also, consider closing traps each morning to reduce fox captures during the day.
 - i. <u>Timeline</u>: If established, skunk trapping grids might not need to be conducted annually, but regularly every 2-5 years?
- 4. Disease monitoring (see Disease Section 8e)
 - a. Develop standardized protocol to collect and bank samples for disease monitoring
 - i. <u>Timeline</u>: Protocol not completed in 2020 due to personnel turnover at TNC & NPS; will begin in 2021 after NPS has onboarded a new CHIS Wildlife Biologist
 - b. Build long-term disease data set for skunks
 - i. <u>Timeline</u>: Dataset not completed in 2020 due to personnel turnover at TNC & NPS; will begin to build in 2021 after NPS has onboarded a new CHIS Wildlife Biologist and continue to build long-term
- 5. Deer mice monitoring
 - a. Analyze historical data on temporal trends of deer mouse population abundance on San Miguel Island as predictor of population dynamics of island spotted skunks on Santa Cruz Island and Santa Rosa Island since the early 1990's.
 - i. <u>Timeline</u>: Complete analyses in future years.
 - b. Design and implement monitoring program for deer mice on Santa Cruz and Santa Rosa Islands
 - i. <u>Timeline:</u> Consider as monitoring effort in future years.
- 6. Non-invasive genetic monitoring

- a. Use hair snares (e.g., modified PVC design) to collect genetic samples?
- b. Use scat detection dogs to find skunk scats for genetic samples?
- c. <u>Timeline</u>: In consideration to be a potential monitoring effort in future years.

10. Management Options

Section Leads: Kevin Crooks, Christina Boser, Laura Shaskey, Lara Brenner

Based on discussion during the January 2020 workshop and subsequent group input, the following management options have been identified. We emphasize that this list represents a brainstorm of possible options and not necessarily recommendations to pursue such actions in the short- or long-term; the list will be updated annually. For each option, we identify the potential timeline of pursuing the management action:

- 1. Develop standardized protocol for skunk handling and data collection
 - a. Ensure consistency in data collection between land management agencies and islands
 - b. Protocols to collect data on weight, sex, blood, hair, fecal swabs, photos (for individual id), morphometrics. See Disease Section 8e for disease sampling protocol.
 - c. <u>Timeline</u>:Protocol not completed in 2020 due to personnel turnover at TNC & NPS; will begin in 2021 after NPS has onboarded a new CHIS Wildlife Biologist.
- 2. Develop standardized protocol for data management
 - a. Develop shared curated database
 - b. <u>Timeline</u>: Shared database not developed in 2020 due to personnel turnover at TNC & NPS; will begin in 2021 after NPS has onboarded a new CHIS Wildlife Biologist
- 3. Form island spotted skunk working group
 - a. <u>Timeline</u>: Working group was formed in Spring, 2020 and will continue to meet annually
- 4. Develop island spotted skunk working group website
 - a. <u>Timeline</u>: Website not developed in 2020 due to personnel turnover at TNC; will be developed in 2021.
- 5. Develop island spotted skunk conservation plan to inform upcoming monitoring and management plans
 - a. <u>Timeline</u>: Island spotted skunk conservation plan was completed in Summer, 2020.
- 6. Submit petitions for listing the island spotted skunk at the State and/or Federal level
 - a. State: petition to uplist from Species of Special Concern to Threatened/Endangered status
 - i. <u>Timeline</u>: Consider submitting petition in 2021

- b. Federal: petition to list as Threatened/Endangered subspecies under ESA
 i. Timeline: Consider submitting petition in 2021
- 7. Habitat management options
 - a. Opuntia restoration to provide skunk habitat and protective cover from foxes?
 - i. <u>Timeline</u>: Consider in future years if continued concerns about skunk viability
 - b. Fox-proof artificial den sites?
 - i. <u>Timeline</u>: Consider in future years if continued concerns about fox impacts to skunk viability
 - c. Continued recovery of shrub communities (quality skunk habitat) on islands
 - i. <u>Timeline</u>: TNC is currently working on removal of non-native vegetation on Santa Cruz, and will begin to restore rare, native plants with the construction of a new nursery in 2021. NPS has several active projects to remove non-native vegetation and restore native plants and communities on both Santa Cruz and Santa Rosa Islands.
- 8. Disease management (see Disease Section 8e)
 - a. Vaccinations for rabies and/or distemper, similar to fox protocol
 - i. <u>Timeline</u>: Reach out to CDFW in 2021 to begin permitting process for CDV vaccination in island spotted skunks
- 9. Captive breeding (on-island or in mainland zoos)
 - a. Establish captive breeding program either on-island or in mainland facilities/zoos
 - b. <u>Timeline</u>: Threat not sufficiently urgent to warrant at this time. Consider in future years if risk of extinction is unacceptably high
- 10. Establish skunk "reserve" on island
 - a. Create skunk reserve by building fox-proof fence and excluding foxes on part of island
 - b. <u>Timeline</u>: Threat not sufficiently urgent to warrant at this time. Consider in future years if risk of extinction is unacceptably high
- 11. Reintroduce skunks to San Miguel Island
 - a. To spread risk of extinction, reintroduce skunks to their historic range on San Miguel Island
 - b. <u>Timeline</u>: Threat not sufficiently urgent to warrant at this time. Consider in future years if risk of extinction is unacceptably high?

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12. Appendices

Appendix 1. Members of the Island Spotted Skunk Working Group (2021)

Name	Affiliation
Lara Brenner	The Nature Conservancy
David Jachowski	Clemson University
Kevin Crooks	Colorado State University
Adam Dillon	Colorado State University
Ben Murley	Kings River Fisher Project
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Ken Convery	National Park Service, CHIS
Annie Little	National Park Service, CHIS
Ethan McKinley	National Park Service, CHIS
Juliann Schamel	National Park Service, CHIS
Melissa Marshall	National Park Service, CHIS
Laura Shaskey	US Fish and Wildlife Service
Paul Collins	Natural History Museum of Santa Barbara
Calypso Gagorik	Northern Arizona University

Tad Theimer	Northern Arizona University
Marie Tosa	Oregon State University
Julie Barnes	Santa Barbara Zoo
Heather Alford	Student Conservation Association, CHIS
Bridget Parrino	Student Conservation Association, CHIS
Christie Boser	The Nature Conservancy
Michael Hague	The Nature Conservancy
Scott Morrison	The Nature Conservancy
John Randall	The Nature Conservancy
Ellie Bolas	UC Davis
Ben Sacks	UC Davis
Dirk Van Vuren	UC Davis
Angela Guiglielmino	UCLA
Katie Prager	UCLA
Mario Castellano	UCSB
Angel Rameriz	UCSB
Cristina Soto-Balderas	UCSB
Dan Doak	University of Colorado Boulder

Delegence	LIC Fish and Wildlife Complex
Robert McMorran	US Fish and wildlife Service
Bill Kendall	USGS
Bill Hoyer	US Navy
Scott Meyler	The Nature Conservancy
Estelle Sandhaus	Santa Barbara Zoo
Katrina Olthof	Wildlands Conservation Science
Julia Owens	UC Davis
Stephen Harris	Clemson University
Shea Miller	National Parks Service, CHIS
Jory Brinkerhoff	University of Richmond
Elizabeth Forrey	University of Richmond

Appendix 2 Ecto- and endoparasites of the eastern spotted skunk (*Spilogale putorius*). Those also identified in island spotted skunks (*Spilogale gracilis amphialus*) are indicated by an asterisk. Adapted from ESSCSG 2019.

Ectopa	oparasites Citation Endoparasites		Citation		
	Species Species		Species		
Fleas	Ctenocephalides felis	1,2	Protozoans	Eimeria mephitidis	20
	Ctenophtalmus pseudagyrtes	3		Isospora sengeri	20, 21
	Echidnophaga gallinacea	1,4, 5		Isospora spilogales	20, 21
	Orchopeas howardi	1		Sarcocystis sp. indet.	20
	Orchopeas leucopus	3		Trypanosoma cruzi	29
	Polygenis gwyni	1, 2, 5	Helminths	<i>Acanthocephala</i> sp. indet.	22
	Pulex irritans*	1, 30		Baylisascaris columnaris	20
	Nosopsyllus fasciatus*	30			
	Xenopsylla cheopis	1		Capillaria aerophila	20
Lice	Neotrichodectes (Trichodectes) osborni	1, 6-10		Capillaria putorii	20
	Neotrichodectes (Trichodectes) mephitidis*	11, 30		Capillaria procyonis	20
Mites	Androlaelaps casalis	12		Centrorhynchus conspectus	23
	Androlaelaps fahrenholzi	12, 13		Crenosoma sp. indet.	20
	Androlaelaps [Haemolaelaps] geomys	1, 12		Mesocestoides sp. indet.	22
	Androlaelaps [Haemolaelaps] glasgowi	1		Molineus sp. indet.	20, 22
	Androlaelaps [Haemolaelaps] megaventralis	1		Physaloptera maxillaris	22
	Echinonyssus staffordi	12, 13		Physaloptera sp. indet.	20
	Eucheyletia bishoppi	13		Placoconus lotoris	20
	Eulaelaps stabularis	1, 12, 13		Skrjabingylus chitwoodorum	22
	Haemogamasus reidi	13		Skrjabingylus sp. indet.	20, 24, 25

	Hirstionyssus staffordi	1
	Ornithonyssus bacoti	1, 12
	Pygmephorus designatus	13
	Xenoryctes latiporus	13
Ticks	Amblyomma americanum	1
	Amblyomma auricularium	14
	Dermacentor variabilis	1, 15, 16
	Ixodes bishoppi	1
	Ixodes cookei	1, 15-17
	Ixodes minor	18
	Ixodes kingii*	30
	Ixodes pacificus*	30
	Ixodes scapularis	19

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Appendix 3: Pathogens of island spotted skunks

Viral Pathogens

Amdoparvovirus (Aleutian disease virus – ADV – like virus)1,2CDV3CPV3Influenza A virus1Rabies virus (REF – can steal from eastern spotted skunk plan)West Nile Virus (REF – can steal from eastern spotted skunk plan)

Protozoal Pathogens

Coccidia³ Eimeria mephitidis⁴ Isospora sengeri⁴ Isospora spilogales⁴ Sarcocystis sp. ⁴ Sarcocystis neuroa (seen in 1 striped skunk) ^{3,5} Toxoplasma gondii^{3,6–8} *Trypanosoma cruzii* (REF – can steal from eastern spotted skunk plan)

Nematodes

Baylisascaris columnaris^{3,4} Capillaria sp. ^{3,4} Crenosoma sp. ^{3,4} Dirofilaria *Dipetalonema mephitis*³ Molineus sp. ⁴ Physaloptera maxillaris^{3,4} Placoconus lotoris⁴ Skrjabingylus chitwoodorum^{3,4} Strongyloides papillosus³

Cestodes

Mesocestoides sp.³

<u>Bacteria</u>

Bartonella⁹ Francisella tularensis (REF – can steal from eastern spotted skunk plan) Helicobacter³ Leptospira^{1,3} Salmonella¹

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Appendix 4: Sample collection to consider depending on diseases of concern

- 1. Blood
 - a. EDTA
 - i. Genetics (DNA paper)
 - ii. CBC/haemoparasites
 - iii. HW microfilaria
 - iv. Aleutian disease/amdoparvovirus-PCR
 - b. LiHep
 - i. CBC/chem (for small samples)
 - c. Serum
 - i. Chemistry panel
 - ii. HW Antigen ELISA
 - iii. Lepto serology
 - iv. Viral serology (distemper, parvovirus, amdoparvovirus, coronavirus, adenovirus)
 - v. Store serum in 0.3-0.5 ml aliquots
 - d. Blood clot from serum tube (fresh or frozen) genetics
- 2. Feces
 - a. O&P (eg: Skrjabingylus, Bayliascaris, Spirocerca, Acanthocephala, Capillaria)
 - b. Direct exam only if fresh
 - c. Viral PCR (Aleutian disease, Distemper, Parvovirus)- depending on viral shedding
- 3. Urine
 - a. Urinalysis
 - b. Leptospira PCR
 - c. Aleutian disease PCR
- 4. Rectal swab (can freeze swabs)
 - a. Fecal culture
 - b. Aleutian disease/amdoparvovirus PCR- depending on viral shedding
 - c. Distemper/Parvo PCR depending on viral shedding
- 5. Cheek/saliva swab
 - a. DNA
- 6. Nasal swab/oropharyngeal swab
 - a. SARS-CoV-2 PCR
- 7. Hair pluck
 - a. DNA
- 8. Vibrissae
 - a. Diet study
- 9. Tissues (fresh/frozen/fixed)
 - a. Histopathology (fixed tissues)
- b. Toxicology (fresh or frozen tissues)
- c. Viral PCR (fixed tissues less reliable)