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TUFTED PUFFIN POPULATION SAMPLING AT ALASKA MARITIME NATIONAL WILDLIFE REFUGE SITES 2018-2021

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INTRODUCTION

Tufted puffins (*Fratercula cirrhata*) breed from central California to the Chukchi Sea and from Japan to Siberia, but are most concentrated in Alaska, particularly in the eastern Aleutian Islands and along the Alaska Peninsula. The overall population size is estimated at almost 3 million birds (Piatt and Kitaysky 2002), although this value mostly comes from colony estimates made in the 1970's and 1980's. There is recent growing concern about the health of tufted puffin populations, as biologists have documented range contraction, colony abandonment, and population declines in the southern and eastern parts of their range (Hanson and Wiles 2015).

Monitoring numbers of tufted puffins (and other burrow-nesting seabirds) is challenging because birds nest deep underground where they cannot be easily observed. Counts of adult birds socializing on the surface of the colony or at sea around the colony are highly variable, making accurate population estimates from those data difficult (Piatt and Kitaysky 2002). Therefore, indices of breeding population size for tufted puffins are often based on counts of burrow entrances or apparently occupied burrows (usually assessed using indirect evidence of occupancy, such as feathers or guano, without being able to see into the actual nest chamber; Alaska Maritime National Wildlife Refuge 2021).

In Alaska, tufted puffin monitoring faces additional challenges due to the large number of colonies, vast expanse of the area, and remoteness of most sites. Most Alaskan puffin population estimates are decades old and often based only upon rough guesses (U.S. Fish and Wildlife Service 2021). Population trend data (usually indices of population size based on counts of burrow entrances or occupied burrows on small permanent index plots) exist at a few Alaskan puffin colonies within the Alaska Maritime National Wildlife Refuge (Dragoo et al. 2018), but even these datasets are problematic for a number of reasons. One problem is that frequent monitoring in plots may cause substantial disturbance to tufted puffins (Pierce and Simons 1986), so declines observed in annually-monitored permanent plots may be caused by researcher disturbance (A. Kettle and L. Slater, pers. comm.). Another issue is that fixed plot boundaries can be difficult to reliably sample over decades, either because the ground may slump and heave to physically move plot markers, or because different field crews interpret plot boundaries slightly different across years. In addition, the large size of some existing plots may make it difficult to accurately and consistently count burrows from one year to the next, especially with changing field crews. Finally, a number of these Refuge sites have historical particulars (such as release from predation by introduced foxes) that may make observed trends at those sites unrepresentative of puffin populations across the rest of Alaska. To effectively manage and conserve tufted puffins worldwide, we need better data on puffin population trajectories in Alaska.

Our objective was to develop a protocol to better assess changes in tufted puffin populations in Alaska. We wanted a reproducible sampling method that would generate a metric representing puffin breeding population at a colony, allowing us to quantify variability in puffin populations with reasonable power to detect biologically important levels of decline. It was also important to increase representation by sampling more than just a few plots on an island, and more than just a few islands across the Refuge. Therefore, we aimed to design a procedure low enough in disturbance to conduct annually at our monitoring sites, and simple and flexible enough to do opportunistically at other puffin colonies across the Refuge that are visited irregularly and for which detailed prior knowledge of the colony is not known. Finally, our methodology had to address biases present in previous puffin population datasets on the Refuge (i.e., disturbance, observer differences). In 2018, we started testing this new protocol at several different islands in order to assess its statistical usefulness and logistical feasibility. We followed up with additional sampling in 2019 and 2021, choosing a mix of new and repeated islands. In 2021, we also conducted reconnaissance at a number of tufted puffin colonies that had not yet been sampled, to assess to sampling possibilities.

METHODS

Protocol:

Our theoretical population metric of interest is the number of adult birds attending a breeding colony in a given year. Because counts of adult tufted puffins at the colony or at sea around the colony are highly variable (Piatt and Kitaysky 2002), we used occupied burrows to represent breeding puffins. Our sampling frame was all tufted puffin colonies on the Alaska Maritime National Wildlife Refuge, with an individual puffin colony as the sampling unit. Colony was defined as group of tufted puffins breeding in a distinct area, usually an entire island but sometimes a specific geographic area on the mainland. At some colonies, we distinguished sub-colonies as distinct patches of tufted puffin burrows separated (by distance or landscape feature) from other tufted puffin burrows.

Tufted puffins primarily nest in soil burrows but also use crevices in talus slopes, among beach boulders, and in sea cliffs. Burrows are typically located on moderate to steep slopes anywhere from above storm tide to hundreds of meters above sea level, with highest burrow densities usually along cliff edges and on steep slopes (Wehle 1980, Piatt and Kitaysky 2002). We defined puffin nesting habitat as any area with tufted puffin burrows. We defined puffin burrows as those with an entrance >14.5 cm wide and a minimum depth of 30 cm; (Alaska Maritime National Wildlife Refuge 2019). Because puffin habitat can be patchy and is rarely homogenous, we considered puffin habitat on a broad scale instead of trying to delineate small patches.

The protocol entailed sampling $1m^2$ quadrats (Figures 1 and 2) along randomly selected transects within potential puffin habitat (with the exception of crews on Buldir in 2019 and 2021, who utilized $1.5m^2$ quadrats due to extremely low density of burrows). Occupancy within each quadrat was defined on the basis of presence/absence of occupied burrows; by repeatedly measuring occupancy in this way in numerous quadrats throughout the colony, we measured a rate of quadrats containing occupied burrows (see Appendix A for the complete detailed sampling protocol used in 2018-2021).

The primary goal of this protocol is to measure and track changes in the rate of quadrats containing occupied burrows over time. Because we are still evaluating and improving this protocol for long-term implementation, we also recorded the presence of unoccupied puffin burrows in quadrats, in order to examine rates of quadrats containing *any* puffin burrows. This will help inform the details of our sampling design (e.g., quadrat size, number of quadrats needed, coverage of transects) and give us more ability to think about what constrains rates of occupied quadrats.

This new population monitoring protocol differs from that of previous tufted puffin population monitoring on the Refuge in two main ways: (a) occupancy is defined on the basis of presence/absence of occupied burrows in many small ($1m^2$ or $1.5m^2$) quadrats, rather than counting numbers of occupied burrows in fewer larger plots; and (b) sampling is conducted along randomly-located (or as close to random as logistics and terrain allow) transects throughout the extent of the colony, instead of in the same fixed plots year after year.

At some islands we had prior knowledge of where tufted puffins were concentrated. At annual monitoring camps, beginning transect locations and directions (or beginning and ending transect locations for St. Lazaria) were pre-determined, based on fairly extensive knowledge of the colony location and extent. At the intermittent sites, transects were located upon arrival to the island by assessing suitable habitat and attempting to spread sampling effort across the colony. At most colonies, we attempted to sample along randomly-placed transects spread across as much puffin habitat as logistically possible. Where puffin

habitat was limited or not contiguous (Buldir and St. Lazaria), a smaller sub-colony area was used for sampling, chosen based on considerations of accessibility, disturbance, and safety.

In 2021, we found that the current protocol was not practical when the accessible TUPU habitat was a very narrow band above steep slopes or cliffs, making it extremely difficult to traverse in a zig-zag fashion per the protocol. Traversing such a steep slope as a team was also quite destructive. A protocol variant was developed in the field in 2021 that provided a way to randomly sample occupancy along such a narrow band of habitat. The variant required the data recorder to walk along the top of the slope along the edge of the colony, holding the sampling frame. The 5m cord attached to the frame was marked in 0.5m increments, dividing it into tenths. A random number 1 to 10 was generated (e.g. using the last digit of a randomly stopped stopwatch) and the frame was lowered that number of marks down the slope into the puffin habitat, perpendicular to the direction of travel. Occupancy was then assessed and GPS coordinates taken by the second team member. To move to the next sampling location, the data recorder again selected a random number and took that many steps forward along the colony edge; then the quadrat was again randomly positioned on the downward slope for sampling. The amount of forward movement the data recorder moved between samples could also have been a fixed distance determined by amount of available habitat (~5m if there is a long stretch of habitat to cover, or shorter if the colony to be assessed is very small); a more systematic forward progression should not induce biased sampling at that colony. This narrow-band protocol variant was used on Nizki, Gibson, and areas in Kateekuk in 2021. The method is not applicable in all situations, particularly when the habitat is wider than 5m and using it would not allow sampling of all available habitat.

A second protocol variant was needed for Suklik Island in 2021. It is a mixed colony of tufted (TUPU) and horned (HOPU; *F. corniculata*) puffins, where HOPU utilize more interior parts of the island and TUPU are primarily located in a band along the edge, including down slopes and cliffs to the sea. There was no readily identifiable boundary between TUPU and HOPU habitat around the edge of the island; TUPU habitat along the edge blended in to mixed TUPU/HOPU habitat and further inshore to HOPU habitat only. The band of TUPU habitat was relatively narrow along steep but mostly accessible slopes, and would have otherwise been a good candidate for the previously described variant; however, the lack of an identifiable boundary between species created the quandary of where to have the recorder walk so as to be able to sample the full extent of the TUPU habitat. In the particular situation at Suklik, a crest could be roughly followed along the edge of the island, where the slopes gradually dropped down to the sea. Only sampling from this crest down would, however ignore the band of TUPU habitat that followed the other side of the crest, towards the interior of the island (which gradually mixed with HOPU habitat). We modified the narrow-band protocol variant described above, such that the data recorder would now walk along the crest along the edge of the island, but instead of always placing the frame down towards the sea at random lengths of 0-5m (per the narrow-band variant), it would alternatively be placed at random lengths of 0-5m toward the interior island as well. Based on observations made while on the island, it is likely this 5m distance on either side of the colony crest included mostly TUPU habitat, but it is possible that some burrows samples may have been HOPU at the edges of the mixed area. While this does introduce the possibility of bias, the numbers of HOPU burrows in that area was probably small, and it was a good compromise against other potential bias induced by ignoring the upper band of TUPU habitat and only focusing on sampling from the crest down the slopes to the sea.

While these two protocol variants may be slightly less randomized than the original protocol, they still decrease placement subjectivity. The 2022 protocol will be updated to include detailed directions to perform these new variants when situations require.

Sampling locations:

We conducted the tufted puffin population sampling protocol in colonies at a number of Refuge islands in the Aleutian Islands, along the Alaska Peninsula, and in the Gulf of Alaska. We sampled eight colonies in 2021, eight in 2019, and seven in 2018 (Figure 1; Table 1). Colonies were selected based on logistical feasibility and estimated puffin population sizes. Three colonies were on islands with annual monitoring sites staffed by summer-long field crews (Aiktak, Buldir, and St. Lazaria islands); these colonies were chosen because puffins were accessible and we had field crews already on the island to do the work. The other colonies were intermittent Refuge sites visited by survey crews on the R/V *Tiĝlax̂*; some (Gibson and Nizki) were selected for a specific focus on the Near Islands, while others (Kaligagan, Kateekuk, Midun, Poa, Puffin, Suklik, Vsevidof islands) were selected because they were known to have relatively large tufted puffin colonies (Byrd et al. 2015, U.S. Fish and Wildlife Service 2017) that were accessible for sampling, and were located in areas convenient to the schedule of the *Tiĝlax̂*.

In 2021, we also visited a number of additional islands to explore for possibilities of sampling: Alaid, Kohl, Agattu, Ogchul, Emerald, Egg, Kaligagan and Amagat islands. This reconnaissance provided valuable information on the puffin colonies at these islands, although sampling was not conducted due to lack accessible habitat or unsafe sea and/or wind conditions.

Work occurred in late July to mid-August in 2021 and August to early September in 2019 and 2018 (Table 1). This coincides with the latter half of the tufted puffin breeding season in Alaska (Table 1).

In 2021 at some islands, survey crews also opportunistically collected eggshell membranes for a tufted puffin range-wide genetics study in collaboration with the Pacific Seabird Group Tufted Puffin Technical Committee, ticks for the Alaska Tick program, and tufted puffin diet samples (either from pick-ups found while working in the colony or bill loads collected by screening burrows).

Figure 1. A sampling quadrat in puffin habitat.

Figure 2. Measuring burrows within a sampling quadrat.

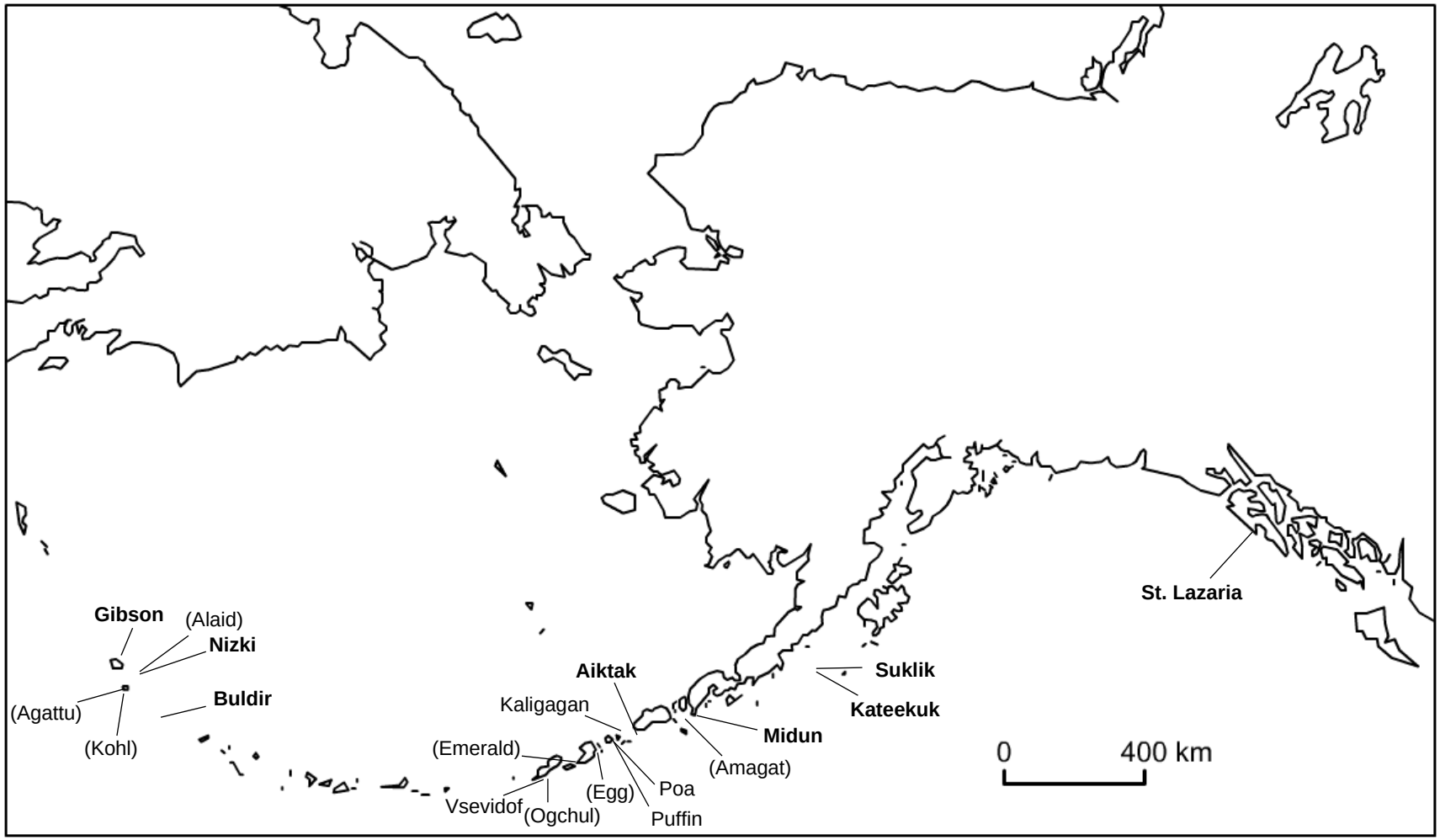

Figure 3. Puffin colonies sampled in 2018-2021 using new tufted puffin population protocol. Colonies sampled in 2021 are in bold; those visited for reconnaissance in 2021 but not sampled are in parentheses.

Table 1. Details of tufted puffin population sampling in 2018-2021.

Year	Island	Replicate	Quadrats	Date(s)	Crew
2018	Aiktak	1	100	15, 16, 17 Aug	Dan Rapp, Sarah Youngren
		2	100	22, 26, 29 Aug	Dan Rapp, Sarah Youngren
	Buldir	1	100	6, 12 Aug	Briana Bode, McKenzie Mudge, Kevin Pietrzak
	Kaligagan	1	94	1 Sep	Briana Bode, Aaron Christ, Dan Rapp, Sarah Youngren
	Midun	1	94	2 Sep	Briana Bode, Aaron Christ, Dan Rapp, Sarah Youngren
	Poa	1	78	1 Sep	Briana Bode, Aaron Christ, Dan Rapp, Sarah Youngren
	St. Lazaria	1	69	12 Aug	Stacie Evans, Dan Schultz
2019	Aiktak	1	198	22, 28 Aug	Dan Rapp, Sarah Youngren
	Buldir	1	191	8, 13 Aug	Stacie Evans, Dan Schultz, Reina Galvan
		2	193	15, 19 Aug	Stacie Evans, Dan Schultz, Reina Galvan
	Kateekuk	1	215	3 Sep	McKenzie Mudge, Kevin Pietrzak, Stacie Evans, Dan Schultz, Dan Rapp, Sarah Youngren
	Midun	1	215	2 Sep	Aaron Christ, Stacie Evans, Dan Schultz, Reina Galvan, Dan Rapp, Sarah Youngren
	Puffin	1	200	1 Sep	Aaron Christ, Stacie Evans, Dan Schultz, Reina Galvan
	St. Lazaria	1	90	20 Aug	Brendan Higgins, Jillian Soller
	Vsevidof	1	47	9 Aug	Aaron Christ, Kendra Bush
2021	Aiktak	-[a]	20	18 Aug	Dan Rapp, Sarah Youngren
	Buldir	1	200	10, 11 Aug	Brendan Higgins, Jillian Soller, Zeke Smith
	Gibson	1	110	30 Jul	Brie Drummond, Nora Rojek, Erin Lefkowitz, Sarah Guitart
	Kateekuk	1	224	14 Aug	Aaron Christ, Nora Rojek, Erin Lefkowitz, Sarah Guitart, Briana Bode, Katie Stoner
	Midun	1	133	11 Aug	Aaron Christ, Nora Rojek, Erin Lefkowitz, Sarah Guitart
	Nizki	1	220	29 Jul, 2 Aug	Brie Drummond, Nora Rojek, Erin Lefkowitz, Sarah Guitart
	St. Lazaria	1	35	19 Aug	Kristina McOmber, Aspen Ellis
	Suklik	1	106	13 Aug	Brie Drummond, Nora Rojek, Erin Lefkowitz, Sarah Guitart, Briana Bode, Katie Stoner

[a]Only one transect of 20 quadrats sampled on Aiktak in 2021, so data do not represent a complete replicate.

Colony specifics:

Aiktak - Aiktak Island, in the eastern Aleutian Islands, has one of the larger populations of tufted puffins in Alaska, with an estimated 100,000 breeding individuals (Byrd et al. 2005, U.S. Fish and Wildlife Service 2021). Tufted puffin burrows ring the perimeter of the entire island and are also present on the slopes of Southwest Slope and Gull Mountain (Figure 4). Because the puffin habitat is largely contiguous around the island and the total island size is relatively small, sampling was spread across the entire island (Figure 5).

Crews sampled 2 replicates of 100 quadrats in 2018 and 1 replicate of 200 quadrats in 2019. In 2021, only one transect of 20 quadrats was sampled due to prioritization of other tasks. Results from 2021 should be interpreted with caution, as such a small sample size mean is more prone to error (as well as only able to estimate proportions in 5% increments).

Figure 4. Location of tufted puffin habitat (shown in blue) at Aiktak Island, Alaska.

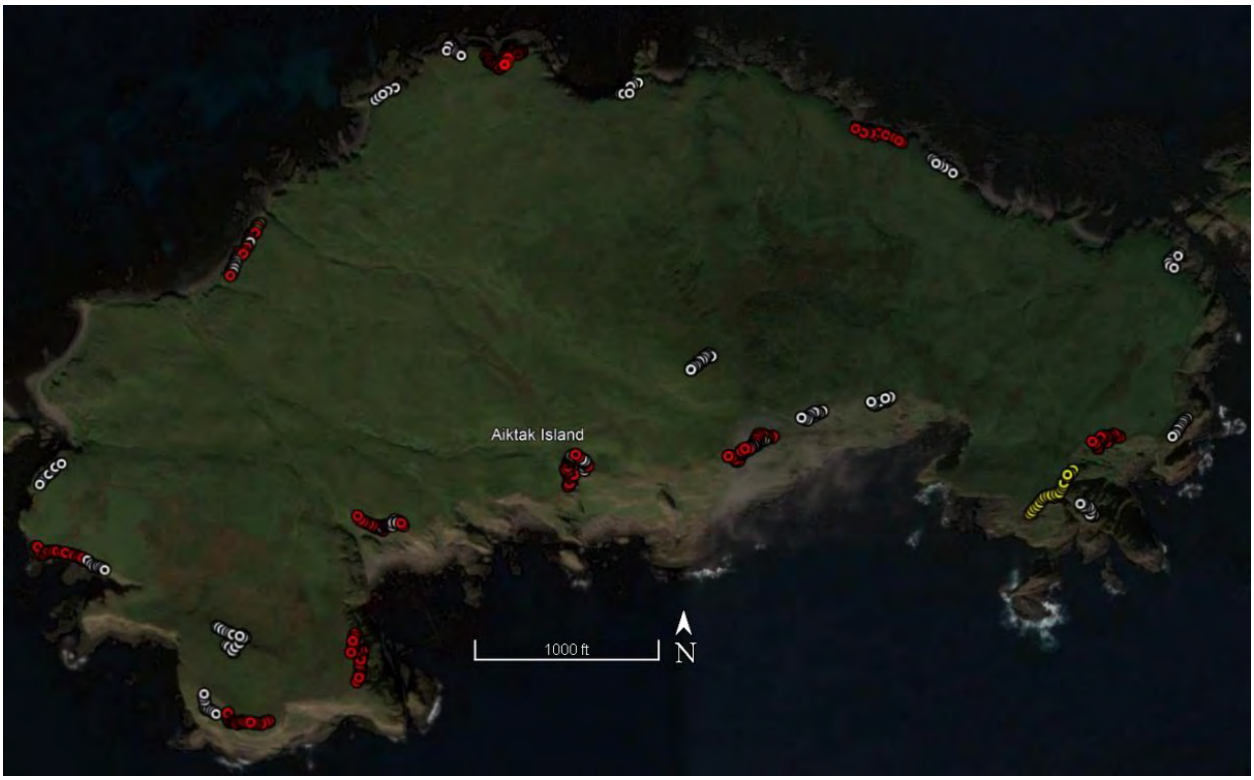

Figure 5. Quadrats sampled at Aiktak Island, Alaska in 2018-2021. Dots in white were sampled in 2018, red in 2019, and yellow in 2021.

Buldir - Buldir Island, in the western Aleutian Islands, has an estimated tufted puffin breeding population of 20,000-40,000 individuals (U.S. Fish and Wildlife Service 2021), including several nearshore islands. Puffins at Buldir are separated into a number of subcolonies, spread across the island and often with large patches of non-puffin habitat in between. Therefore, it is impractical to try to sample randomly across the entire island. We tested this protocol on Northwest Ridge in 2018 and Northwest Ridge and Crested Point in 2019-2021 (Figures 6-8), one of the more easily accessible tufted puffin habitat areas on the island.

Crews conducted single replicates of 100 quadrats in 2018, two replicates (of about 100 quadrats each, for two subcolonies) in 2019, and one replicate (of 100 quadrats each, for two subcolonies) in 2021. Sampling occurred over two days for each replicate.

Figure 6. Location of the Northwest Ridge (shown in red) and the Crested Point (shown in yellow) subcolonies sampled at Buldir Island, Alaska 2018-2021.

Figure 7. Quadrats sampled at the Northwest Ridge sub-colony, Buldir Island, Alaska in 2018-2021. Dots in white were sampled in 2018, red in 2019, and yellow in 2021.

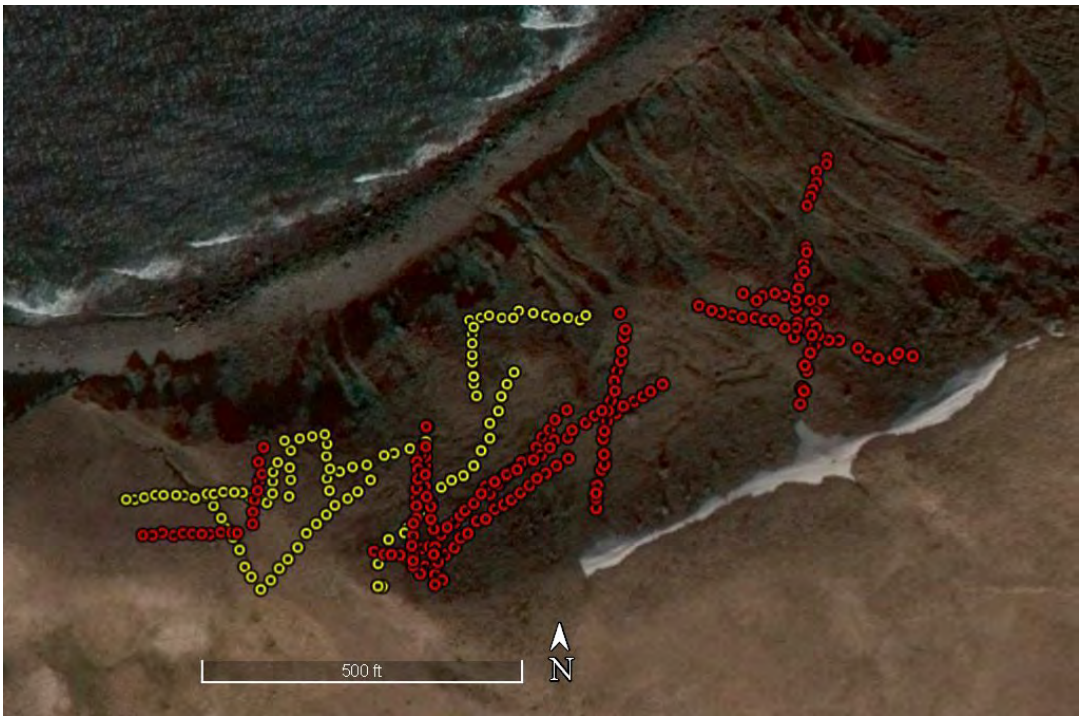

Figure 8. Quadrats sampled at the Crested Point sub-colony, Buldir Island, Alaska in 2018-2021. Dots in red were sampled in 2019, yellow in 2021.

Gibson – Gibson Island is in Chichagof Harbor off the northeastern shore of Attu Island. There are an estimated 5,000 tufted puffins breeding on the small island (U.S. Fish and Wildlife Service 2021). Gibson has historically been considered a refuge for tufted puffins and other seabirds from the rats that on Attu Island, but recent researchers noted possible rat sign on Gibson in 2013.

Gibson was sampled on 30 July 2021. Conditions started hot and sunny, and finished with a rain squall. The crew went ashore in the early afternoon and sampled the island with two teams of two, one on each side of the island. Some areas necessitated the use of the narrow-band modification. After about two hours, the survey crew had exhausted available habitat. The island contained lots of accessible tufted puffins but is just too small for much sampling with this methodology.

In addition, crews searched for and found low density of rat sign in some beach locations. In the evening, crews set out rat traps to attempt to capture rats for DNA analysis, and also deployed puffin screens for puffin diet collection. Puffin screens were retrieved just before dark (seven bill loads collected). Rat traps were collected the following morning (no rats were captured).

Figure 9. Quadrats sampled at Gibson Island, Alaska in 2021. Note that much of the island is low lying rocks, particularly the north side of the island.

Figure 10. View looking back down to access beach from saddle between two parts of Gibson Island.

Figure 11. Puffin sampling habitat on Gibson Island.

Figure 12. Puffin sampling quadrats on Gibson Island.

Kaligagan – Kaligagan Island, about 6km southwest of Aiktak in the eastern Aleutian Islands, hosts one of the larger tufted puffin colonies in Alaska with about 110,000 breeding individuals (Byrd et al. 2015, U.S. Fish and Wildlife Service 2021). A number of islets offshore (Kaligagan Islets #1-6) support an additional 20,000 puffins (Byrd et al. 2005). Tufted puffins were documented nesting around much of the perimeter of the island in 1980 (Nysewander et al. 1982). In 1996, five tufted puffin burrow plots were established and surveyed for occupancy on the west side of Kaligagan (along with two on Kaligagan Islet #2; Byrd and Williams 1996).

In 2018, crews landed on the west side of the island and sampled puffin habitat to the north and south of the access point (Figure 13), covering some of the same area as the historic plots. Two teams of two completed sampling in a few hours. In 2021, crews went ashore on Kaligagan but tide rips made access difficult and high winds were deemed too dangerous for sampling atop cliffs, so the survey was cancelled.

Figure 13. Quadrats sampled at Kaligagan Island, Alaska in 2018. Landing beach is shown in red.

Figure 14. Puffin habitat at Kaligagan Island.

Figure 15. Puffin habitat at Kaligagan Island.

Kateekuk – Kateekuk Island, in the Semidi Islands, has an estimated tufted puffin breeding population of 25,000 individuals (U.S. Fish and Wildlife Service 2021). Presence of Artic ground squirrels (*Urocitellus parryii*) likely impacts availability of habitat for burrow-nesting seabirds on Kateekuk due to habitat modification and limited predation. Kateekuk is ringed on most sides by rocky bluffs and cliffs. Access to the interior is via a cove on the eastern side, followed by a sleep climb up a vegetated slope.

Three teams of two sampled Kateekuk in 2019 and 2021. Tufted puffin burrows were restricted to the perimeter of the island. Burrow habitat was sporadic and burrow density overall was low, but patches of higher density that were worth sampling could be found along the northeastern, southeastern and southwestern coasts. In 2021, crews used a mix of standard sampling protocol and the narrow-band variant, depending on specific locations. Crews in 2021 also found five pick-up diet samples while working.

Figure 16. Quadrats sampled at Kateekuk Island, Alaska in 2019-2021. Dots in red were sampled in 2019, yellow in 2021. Landing beach is shown in red.

Figure 17. View of access beach and steep slope leading up to interior at Kateekuk Island (see arrow).

Figure 18. Hiking across island to sampling location on Kateekuk; note low vegetation.

Figure 19. Surveying puffin quadrats in *Leymus* grass along the southeast coast of Kateekuk Island.

Figure 20. Surveying puffin quadrats in putchki habitat on the northeast coast of Kateekuk Island.

Midun – Midun Island, off of the Alaska Peninsula, provides breeding habitat for 10,000-12,000 tufted puffins (Bailey and Faust 1980, U.S. Fish and Wildlife Service 2021). Puffins breed around the perimeter of the island along the clifftops.

Sampling was conducted by two teams of two 2018 and 2021, and three teams of two in 2019 (Figure 21). Puffin habitat was generally restricted to the perimeter of the island and was patchy, with good, dense puffin habitat interspersed by less dense areas. In 2021, the survey crew found it difficult to get 200 quadrats sampled with only two teams due to time needed for traversing island to connect patchy habitat. Hot sunny conditions in 2021 also made sampling in the island's thick island putchki patches difficult. The island could possibly be sampled more intensively from fewer areas to increase efficiency at the expense of sampling more of the island.

In 2021, crews also collected six diet samples by deploying burrow screens on the afternoon of 10 August, the day before conducting population sampling.

Figure 21. Quadrats sampled at Midun Island, Alaska in 2018-2021. Dots in white were sampled in 2018, red in 2019, and yellow in 2021. Landing beach is shown in red.

Figure 22. Typical putchki/*Angelica/*forb habitat on Midun Island.

Figure 23. Puffin quadrat sampling in putchki/*Angelica/*forb habitat on Midun Island.

Figure 24. Puffin quadrat sampling in grassy habitat on Midun Island.

Figure 25. Denuded vegetation in high density puffin habitat on Midun Island.

Nizki – Nizki Island, in the Near Islands, is located about 40 km east of Attu in the far western Aleutian Islands. The estimated population of tufted puffins on Nizki is about 600 individuals (Byrd et al. 2005). This relatively low number likely reflects years of predation pressure from introduced arctic foxes (*Vulpes lagopus*), which were removed in 1976 (Bailey 1993).

Survey crews went ashore on 29 July 2021 on the northwest end of Nizki (Figure 26) to attempt sampling, but there was not enough accessible puffin habitat--most was too steep. Crews walked along the north coast for a while in thick vegetation but found only small groups of tufted puffins in steep areas, nothing worth sampling. After skiffing along the north coast looking for tufted puffin breeding areas large enough for quadrat sampling, crews found low bluffs in Eider Cove with tufted puffin burrows on lower slopes on the northeast side. Two teams of two sampled a narrow band of habitat for about three hours. Progress was slow, and habitat only allowed only about 60 quadrats (Figure 27). This was the first area we developed and tried the modified narrow-band protocol. In the evening, crews skiffed the rest of the shoreline of Nizki looking for suitable sampling locations. Based on the skiff surveys, we found only a few areas on the southeastern coast that seemed possibly worth sampling; otherwise, tufted puffins on Nizki were scattered or found nesting in relatively inaccessible steep bluff/cliff habitat

On 2 August 2021, survey crews returned to a section of puffin habitat in Camp Cove on the southeast coast identified during skiff reconnaissance. Two teams of two surveyed using the narrow-band method; one team started at the landing beach, and the other hiked down coast and worked backwards. Crews sampled ~4 hours recording just under 100 quadrats each (Figure 28). Three bill load pick-ups were found and collected while conducting the population sampling.

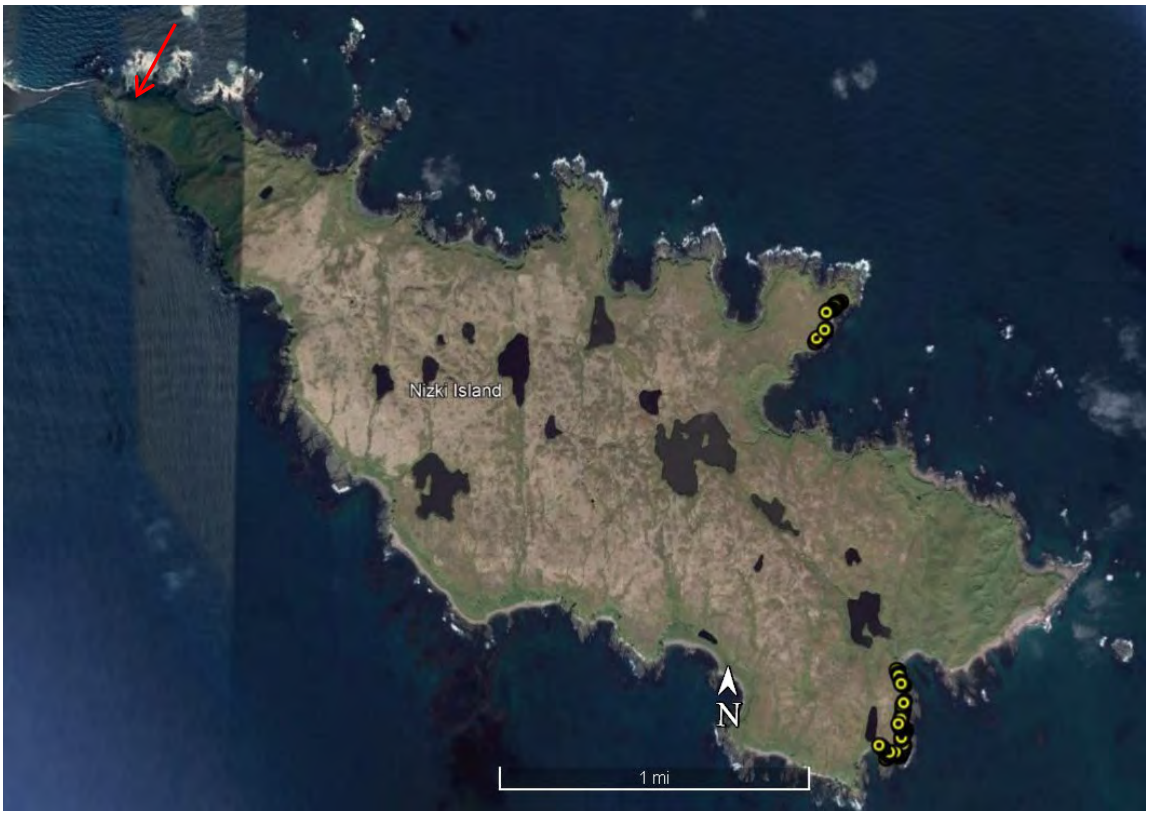

Figure 26. Quadrats sampled at Nizki Island, Alaska in 2021. Red arrow indicates where survey crews first went ashore but found poor sampling.

Figure 27. Quadrats sampled on eastern coast at Nizki Island, Alaska in 2021.

Figure 28. Quadrats sampled on southeastern coast at Nizki Island, Alaska in 2021.

Figure 29. View looking east from northwest end of Nizki Island.

Figure 30. Puffin habitat on eastern sampling location on Nizki Island.

Figure 31. Puffin habitat on southeastern sampling location on Nizki Island.

Poa – Poa Island, in the eastern Aleutian Islands, has about 33,000 breeding tufted puffins (Byrd et al. 2015, U.S. Fish and Wildlife Service 2021). European rabbits (*Oryctolagus cuniculus*) were introduced around 1940 and probably competed with tufted puffins for burrows (U.S. Fish and Wildlife Service); some rabbits were trapped in eradication efforts in 2010 and 2011 (Stevens et al. 2010, 2011). Puffins breed around the perimeter of the island on steep slopes and above clifftops.

In 2018, crews landed on the northwest side of the island and accessed the puffin colony by climbing to the top of the island along a drainage. Two teams of two sampled along the southern side of the island (Figure 32) for several hours. Traversing the island greatly reduced time for sampling.

Figure 32. Quadrats sampled at Poa Island, Alaska in 2018. Landing beach is shown in red.

Figure 33. Sampling at Poa Island, Alaska.

Figure 34. Sampling at Poa Island, Alaska.

Puffin – Puffin Island, in the eastern Aleutian Islands near the southeastern side of Akun, has an estimated tufted puffin population of about 35,000 individuals (U.S. Fish and Wildlife Service 2021).

Two teams of two surveyed Puffin Island 1 September 2019. Crews were able to sample most of the island in about 3.5 hours. While much of the colony was in steep terrain, elevations were rather low, so exposure was greatly reduced.

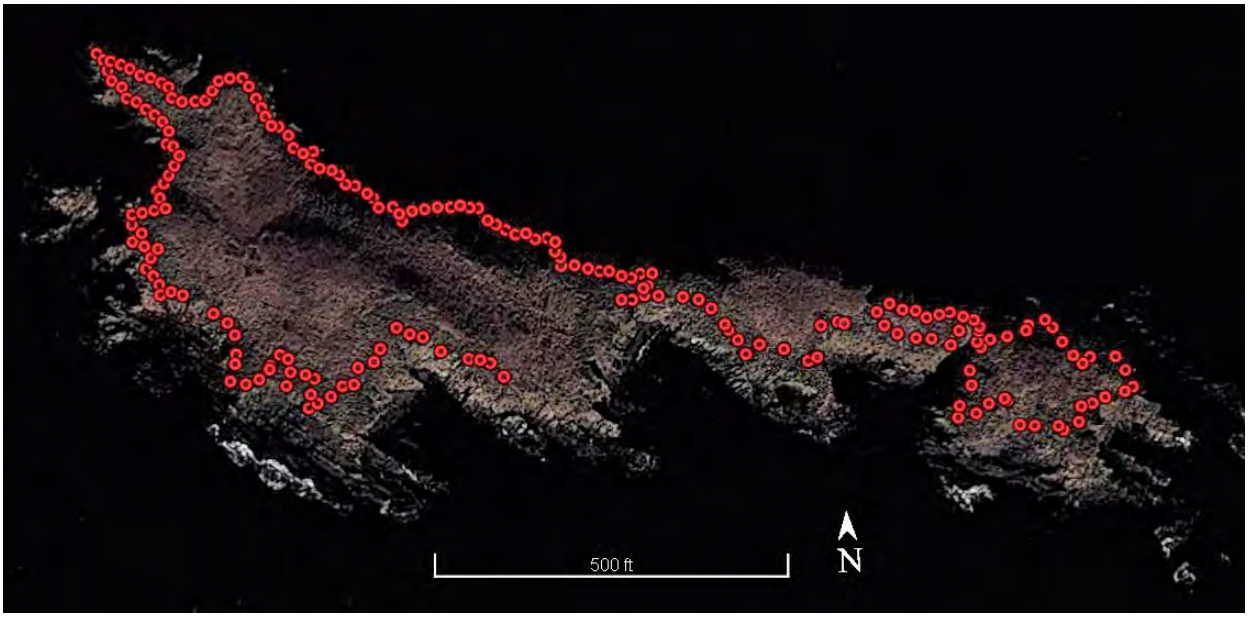

Figure 35. Quadrats sampled at Puffin Island, Alaska in 2019.

Figure 36. Sampling at Puffin Island, Alaska in 2019.

Figure 37. Puffin habitat at Puffin Island, Alaska in 2019.

St. Lazaria - St. Lazaria Island has the largest tufted puffin breeding colony in Southeast Alaska, with an estimated 7,000-10,000 individuals (U.S. Fish and Wildlife Service 2021). Most puffins on the island nest in burrows on steep slopes at the top of cliffs on the western half of the island overlooking the gull colony. The puffin habitat at St. Lazaria is challenging to sample because some areas are too steep to safely access or too fragile to work in, whereas others are covered by salmonberry thickets that are difficult to traverse. As a result, the size of workable habitat on the island is small.

A small area was sampled in 2018 using a modified transect methodology. This area was expanded in 2019 and 2021 to include more colony edge habitat rather than just the center of the colony. Even in this expanded area, however, fewer than 100 quadrats were sampled in each year.

Figure 38. Map showing tufted puffin sampling area (grey polygon) at St. Lazaria Island, Alaska.

Figure 39. Quadrats sampled at St. Lazaria Island, Alaska in 2018-2021. Dots in white were sampled in 2018, red in 2019, and yellow in 2021.

Suklik – Suklik Island, in the Semidi Islands, has an estimated tufted puffin population of about 40,000 individuals. In addition, about 250,000 horned puffins breed on Suklik (U.S. Fish and Wildlife Service 2021). Tufted puffins are mostly concentrated around the perimeter of the island, while horned puffins are more in the island interior, but some mixing does occur, making it difficult to ascertain which burrows belong to which species in some cases. As one of the few Semidi Islands without Arctic ground squirrels, the vegetation on Suklik is much taller and thicker than on neighboring Chowiet and Kateekuk (both of which have ground squirrels present). The habitat soil in some parts of the island is very fragile and it is easy to crush burrows underfoot. Suklik is ringed by steep rocky cliffs and access to the island interior is provided via a steep grassy chute through nesting habitat of northern fulmars on the west side.

After setting out burrow screens for diet sampling, in the morning on the west side just above the access chute, surveys crews hiked up to the peak of the island on the east side. From there, following a detailed discussion on modifying the protocol due to mixed-species colony, three teams of two conducted population sampling in different parts of the island. Time constraints limited sampling time so only 106 quadrats were sampled, but crews were able to test the new modified methodology.

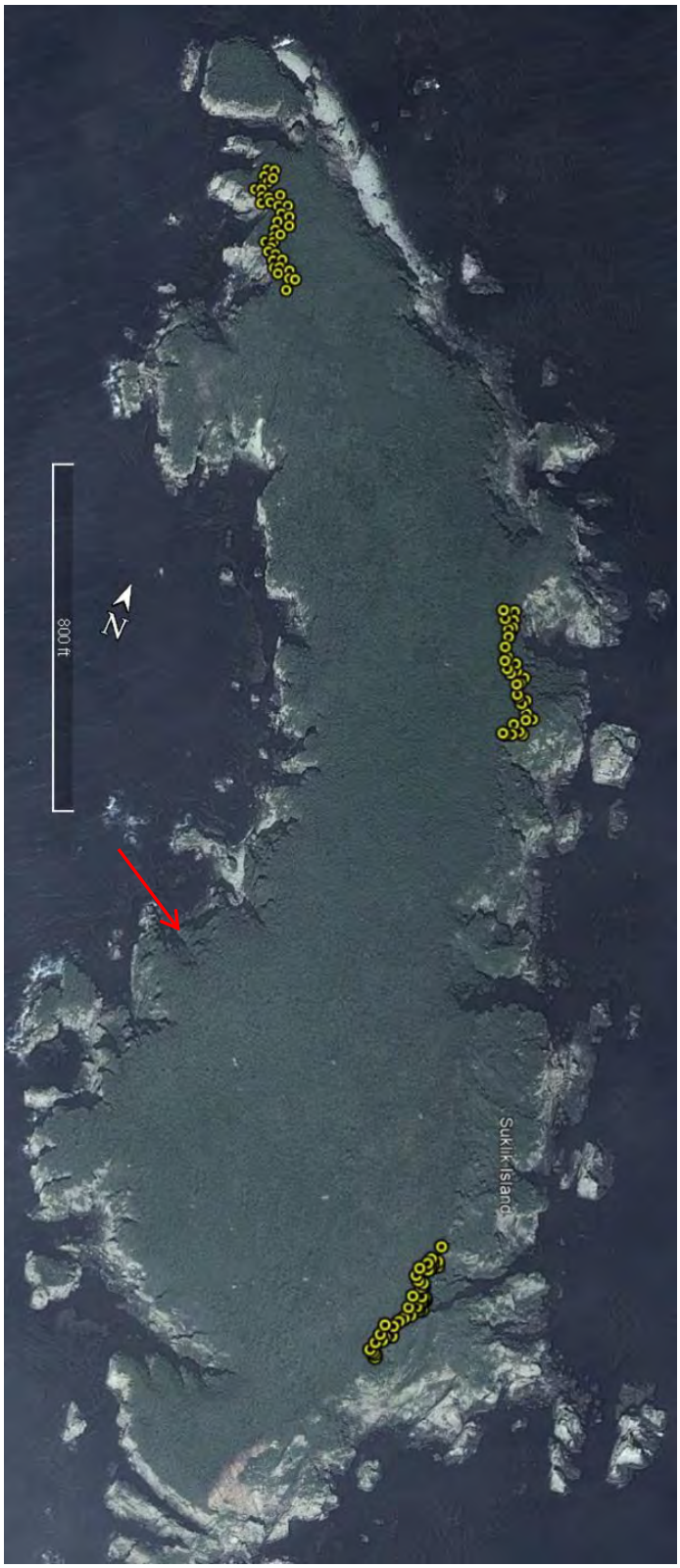

Figure 40. Quadrats sampled at Suklik Island, Alaska in 2021. Red arrow indicates landing access.

Figure 41. Fragile tufted puffin habitat along northwest corner of Suklik Island.

Figure 42. Looking south along ridge showing tufted puffin habitat on Suklik Island.

Figure 43. Descending access chute to skiff pickup location at Suklik Island.

Vsevidof – Vsevdof Island is located in the eastern Aleutian Islands just south of Umak Island. The most recent tufted puffin population estimate is about 65,000 birds (U.S. Fish and Wildlife Service 2021). Tufted puffins were documented as breeding around much of the perimeter of the island in 1980 (Nysewander et al. 1982).

Vsevidof was surveyed in 2019 by a single team of two. Due to time constraints and limited survey crew members, only 47 quadrats were sampled. Waist high vegetation made movement on the island difficult. Much of the habitat observed was on grassy slopes too steep for sampling. The puffin habitat surveyed was on the eastern tip of the island (Figure 44).

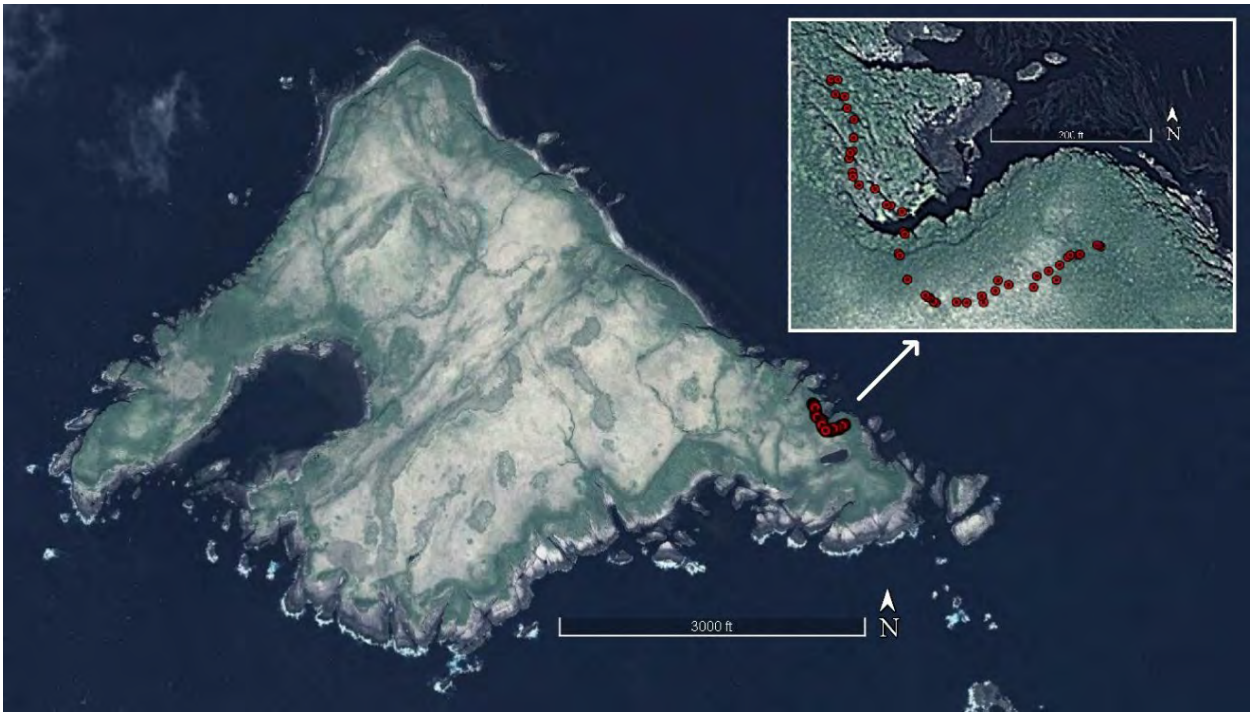

Figure 44. Quadrats sampled at Vsevidof Island, Alaska in 2019.

Figure 45. Puffin burrows at Vsevidof Island, Alaska in 2019.

Figure 46. Puffin habitat at Vsevidof Island, Alaska in 2019.

Additional island reconnaissance:

Agattu – Agattu Island is located in the Near Islands at the western end of the Aleutian Island chain. Introduced arctic foxes were removed in 1977 (Bailey 1993), at which time tufted puffins remained only in inaccessible steep cliff habitat and offshore rocks (Early et al. 1980). By the 1980's, tufted puffins had begun to increase their distribution on the main island, and in 1990, Refuge staff monitored tufted puffins on offshore Kohl and Tower islands and mainland colonies near Island Cove and Cape Sabak (Byrd et al. 1992). The most recent estimated population of tufted puffins on Agattu (including offshore islands) is about 37,000 individuals (Byrd et al. 2005).

On 1 August 2021, we did a skiff- and ship-based reconnaissance of the east and south shores of Agattu, where past information noted more puffin colonies occurred. We attempted to access Kohl Island in the morning but there was too much swell to safely access the island by bumping off on the rocks. From a skiff, we then surveyed along the southeast shore but found puffins nesting too scattered or too steep for surveying. We cruised the south side of the island all the way to the southwest tip at Gillon Point, and then viewed the east side, but although tufted puffins were present in scattered numbers, little seemed accessible. The only place we sampled on Agattu was on a small offshore unnamed islet at Gillon Point, where we found a somewhat accessible colony but decided that it was too small an area for quadrat sampling. Despite some indications of expansion in the 1990s (Byrd et al. 1992), the overall impression at Agattu was that puffins have not recolonized much of the island after fox removal and instead are still mostly nesting in inaccessible locations, with only a few low-density burrow areas in places that may be safely sampled.

The survey crew went ashore in the late afternoon on an offshore islet off Gillon Point to set burrow screens, which were retrieved right before dark for 28 bill load samples.

Alaid – Alaid Island, in the Near Islands, is next to Nizki to the west and about 40 km east of Attu in the far western Aleutian Islands. The estimated population of tufted puffins on Alaid is about 600 individuals (Byrd et al. 2005). This relatively low number likely reflects years of predation pressure from introduced arctic foxes (*Vulpes lagopus*), which were removed in 1976 (Bailey 1993).

On the evening of 29 July 2021, we circumnavigated Alaid in a skiff to reconnoiter for tufted puffin areas we could sample. No obvious suitable areas for sampling puffins were found; tufted puffins on Alaid were scattered in small numbers or found nesting in relatively inaccessible steep cliff habitat. It does not appear that birds have recolonized much of the island after fox removal, although there were some indications of expansion in the 1990s (Byrd et al. 1992).

Amagat – Amagat Island is off the Alaska Peninsula south of Cold Bay. An estimated 100,000 tufted puffins and 140,000 horned puffins breed on the island (U.S. Fish and Wildlife Service 2021).

Survey crews conducted a skiff-based reconnaissance of Amagat first thing in the morning on 9 August to look for landing spots and tufted puffin habitat. We found many potential landing beaches and a few places to scramble up into the interior, the best of which is a low saddle on the east side of the island. On 10 August, crews went ashore on the east beach, which looked deceivingly good from afar, but ended up being shallow and reefy (doable in calm conditions but would be difficult in any amount of surf). We hiked up to the saddle between two main peaks of the island, fighting through heavy vegetation at lower elevations that gave way to easier walking closer to the saddle. From the saddle there were good views of the western side of island. On the highest peak to the south, a large, dense colony of mixed tufted and horned puffins was visible, with hundreds of birds and numerous burrows visible through binoculars amidst an apparent glaucous-winged gull colony. The puffin colony extended all the way around the peak to where the cliffs start on south side. Accessing the colony would require a hike up a steep chute on left side of the peak, likely a 45-60-minute hike from saddle, and would need a dry, calm day to be safe. From afar, there was no obvious delineation between tufted and horned puffin habitat, and so it would be impossible to sample only tufted puffins at that colony. We refrained from attempting population sampling until further thought could be put into whether it was worth sampling in such a mixed colony, since the results would not allow tracking population changes of either species.

After assessing the large mixed colony from afar, we hiked further north around the island to search for additional tufted puffin areas to survey. Hiking up over the peak on the north side, we followed the perimeter of the steep slopes long the east side, finding several small pockets of good exclusively tufted puffin habitat along the tops of the slopes of the northeast side of the island and along the top of the northwest ridge. Such areas could be good locations for diet screening, perhaps would comprise a large enough area for population sampling if all habitat patches were combined.

There was also a large horned puffin colony in an overgrown rocky talus area on the north side of the island, visible from the water. A few storm-petrel-sized burrows were found in the chute leading up to the saddle, and a dead intact fork-tailed storm-petrel was found in the grass near the beach. Several deposits of fresh river otter scat were observed on the beach and a few trails leading into the vegetation could be seen; otters likely were coming over from the mainland.

Overall, Amagat is a large puffin colony with potentially accessible habitat and may be worth returning to sample if a) we decide how to deal with a mixed colony, or b) we attempt sampling the numerous patches of tufted puffin patches along the north part of the island.

Figure 47. Saddle on east side of Amagat to access island interior.

Figure 48. Southern peak on Amagat, location of large mixed horned and tufted puffin colony (see arrow).

Figure 49. Ridge running north towards additional tufted puffin habitat on Amagat Island.

Figure 50. Tufted puffin habitat on northeast coast of Amagat Island.

Egg – Egg Island is in the eastern Aleutian Islands, just off the eastern tip of Unalaska Island. The tufted puffin breeding population is estimated at over 160,000 individuals (U.S. Fish and Wildlife Service 2021). Tufted puffins were documented as breeding all around the perimeter of the island in 1980 (Nysewander et al. 1982), and a small number of puffin density plots were surveyed in 1987 and 1991 (Byrd et al. 1992).

We accessed Egg Island from a beach on the north side of the island on 7 August 2021 and hiked up into the island interior by climbing up a steep streambed. Under clear skies, survey crews hiked along almost the entire perimeter of the island, much of it thick waist-high vegetation. Tufted puffins were numerous but almost all located in inaccessible cliff habitat that could not be safely sampled. No areas of accessible puffin habitat big enough for sampling were found.

Figure 51. Northeast portion of Egg Island, viewed from peak on west side. Arrow shows access route to island interior.

Figure 52. Tall thick grass covering much of Egg Island.

Figure 53. Traversing southern coast of Egg Island.

Emerald – Emerald Island is in the eastern Aleutian Islands, just off the western tip of Unalaska Island. The number of tufted puffins breeding on Emerald is estimated to be about 32,000 birds (U.S. Fish and Wildlife Service 2021). Tufted puffins were documented as breeding around much of the perimeter of the island in 1981 (Nysewander et al. 1982)

The survey crew landed on a beach on the north end of Emerald in the early afternoon on 6 August 2021. Weather conditions were poor, with heavy rain and strong winds out of the southeast. We climbed up the vegetated bluff to the interior of the island above the beach, and then hiked across the island to what was assumed to be the best puffin area on south end of island. To get across the island, we had to traverse extremely fragile storm-petrel habitat, and many burrows were crushed. There were several patches of tufted puffin habitat on the south side that may have been surveyable, but extreme wind gusts on that side of the island were too strong to safely work atop the cliffs. Crews split into two teams and walked back to the landing spot in opposite directions along the perimeter, scoping out for additional tufted puffin locations. Puffin habitat was patchy along coast; some areas were potentially surveyable, but the ground was extremely fragile and prone to burrow collapse underfoot. It is difficult to see how one could conduct puffin sampling on Emerald without destroying lots of burrow-nester habitat along the way.

Figure 54. Southern coast of Emerald Island.

Figure 55. Tufted puffin habitat at south end of Emerald Island.

Figure 56. Northwestern coast of Emerald Island.

Ogchul – Ogchul Island is in the eastern Aleutian Islands just south of Umak, next to Vsevidof. The estimated tufted puffin population is about 60,000 birds (U.S. Fish and Wildlife Service 2021). Tufted puffins were documented as breeding around much of the perimeter of the island in 1980 (Nysewander et al. 1982)

We arrived at Ogchul on 6 August on a dark, ominous morning with strong horizontal driving rain. Access to the island was via a bump onto the rocks and then a climb up a columnar basalt series of steps/ledges up to the top of the island. Due to the poor conditions, although a skeleton survey crew of three permanent Refuge biologists went ashore and scurried up the basalt to the interior of the island, they decided not to risk sampling in such slippery conditions. In better weather conditions, this island has potential for sampling.

Figure 57. Access steps/ledges on Ogchul Island. Photo by Nora Rojek.

RESULTS

Sampling:

Guano was the primary evidence of occupancy, with feathers and eggshells also commonly found (Table 2). Prey appeared the least, with only a few instances documented. The search for evidence was never intended to be exhaustive, as once occupancy was established no further evidence was needed, although multiple forms of evidence were noted in many cases. The rates listed should be viewed accordingly and indicate which form of evidence was more readily identifiable rather than an overall rate of occurrence.

Rates of quadrats containing occupied burrows ranged from a high of 84% in St. Lazaria to a low of 9% at Buldir (Table 3). There was also a wide range of rates of any puffin burrows (occupied or not) among the different islands, St. Lazaria again having the highest at 88% and Buldir the lowest with only 31%. While the rate of quadrats containing any burrows doesn't measure burrow density specifically, the two should increase or decrease roughly in parallel.

Buldir experienced extremely low productivity in 2018, which could somewhat account for low quadrat occupancy, but low density of any puffin burrows was undoubtedly a major factor. The rate of quadrats containing occupied burrows is necessarily limited to be at most the rate at which any burrows were present in quadrats. Low rates of occupied burrows pose issues with the power of detecting trends (see power analysis section below).

Rates of quadrats containing any burrows (Table 4) can provide some information on the realistic maximum occupancy rates one might expect at a site, which is helpful in considering sampling efforts (see also Drummond and Christ 2019 for power analysis results). However, it is difficult to make many inferences from the relationship between the two measures. Because the rate of occupied quadrats and the rate of quadrats containing any burrows are based on presence/absence, the two measures do not relate to the actual ratio of occupied to unoccupied burrows.

For example, a quadrat could have (1) a single occupied burrow, or (2) a single occupied burrow plus multiple unoccupied burrows. Both observations would yield the same recorded data (occupied burrows: "present"; all burrows: "present"). Therefore, while it is tempting to interpret high rates of both occupancy and burrow presence at a colony (such as was found at St. Lazaria) as representing a thriving colony saturating its nesting habitat, and conversely interpreting lower occupancy to burrow presence rate ratios elsewhere as colonies in decline or with poor reproduction, these types of inferences are not appropriate with these data.

Instead of looking at all surveyed quadrats that are occupied (i.e., the overall occupancy rate, Table 3) we can also examine the occupied rate using only quadrats that have burrows, occupied or not as the basis (Table 5). This could give a rough idea of the usage of available burrows, but it still suffers from not having any information about numbers of burrows within a given quadrat as well as any ratio of occupation within that quadrat.

Table 2. Percent of evidence types found in quadrats with evidence across all years. More than one type of evidence can be recorded at a burrow so values may not add up to 100%.

Island	Guano	Feathers	Eggshells	Egg	Chick	Adult	Prey
Aiktak	53%	22%	24%	5%	5%	6%	3%
Buldir	69%	11%	20%	-	6%	11%	-
Gibson	65%	38%	30%	-	3%	-	3%
Kaligagan	40%	8%	40%	18%	-	13%	-
Kateekuk	78%	38%	12%	4%	4%	1%	1%
Midun	55%	31%	12%	1%	3%	3%	-
Nizki	90%	3%	7%	1%	2%	7%	1%
Poa	27%	27%	23%	18%	9%	18%	-
Puffin	63%	21%	44%	-	5%	5%	-
St. Lazaria	42%	65%	3%	3%	1%	1%	-
Suklik	73%	27%	18%	3%	3%	3%	3%
Vsevidof	86%	14%	-	-	-	-	-
All combined	61%	27%	18%	4%	4%	5%	1%

Table 3. Percentage quadrat occupancy by island and year. Values represent percent of quadrats with an occupied burrow (quadrats occupied/quadrats sampled).

Island	2018	2019	2021
Aiktak	30% (59/200)	38% (76/198)	20% (4/20)[a]
Buldir NW Ridge	9% (9/100)	17% (33/191)	2% (2/100)
Crested Point	-	5% (9/193)	1% (1/100)
combined	-	11%	2%
Gibson	-	-	34% (37/110)
Kaligagan	43% (40/94)	-	-
Kateekuk	-	14% (31/215)	19% (42/224)
Midun	30% (28/94)	28% (61/215)	27% (36/133)
Nizki N side	-	-	38% (22/58)
SE side	-	-	40% (65/162)
combined	-	-	40%
Poa	28% (22/78)	-	-
Puffin	-	22% (43/200)	-
St. Lazaria	58% (40/69)[b]	27% (24/90)	29% 10/35
Suklik	-	-	31% (33/106)
Vsevidof	-	15% (7/47)	-

[a]Only one transect of 20 quadrats sampled on Aiktak in 2021, so data do not represent a complete survey.

[b]Only a small area of high-density habitat was surveyed in 2018 data may not be comparable with 2019-2021 when a broader area of habitat was used.

Table 4. Percentage quadrat with burrows by island and year. Values represent percent of quadrats with a burrow, regardless of occupancy (quadrats with burrows/quadrats sampled).

Island	2018	2019	2021
Aiktak	77% (153/200)	77% (152/198)	75% 15(/20)[a]
Buldir NW Ridge	31% (31/100)	27% (52/191)	9% (9/100)
Crested Point	-	8% (16/193)	4% (4/100)
combined	-	18%	7%
Gibson	-	-	68% (75/110)
Kaligagan	70% (66/94)	-	-
Kateekuk	-	20% (44/215)	25% (55/224)
Midun	53% (50/94)	42% (90/215)	54% (72/133)
Nizki N side	-	-	57% (33/58)
SE side	-	-	60% (98/162)
combined	-	-	60%
Poa	60% (47/78)	-	-
Puffin	-	39% (77/200)	-
St. Lazaria	88% (61/69)[b]	32% (29/90)	60% (21/35)
Suklik	-	-	48% (51/106)
Vsevidof	-	45% (21/47)	-

[a]Only one transect of 20 quadrats sampled on Aiktak in 2021, so data do not represent a complete survey.
[b]Only a small area of high-density habitat was surveyed in 2018 data are not comparable with 2019-2021 when a broader area of habitat was used.

Table 5. Percentage of quadrat-containing burrows also showing evidence of occupancy by island and year. Values represent the percent of quadrats containing a burrow that had an occupied burrow (quadrats occupied/quadrats with burrows).

Island	2018	2019	2021
Aiktak	39% (59/153)	50% (76/152)	27% (4/15)[a]
Buldir NW Ridge	29% (9/31)	63% (33/52)	22% (2/9)
Crested Point	-	56% (9/16)	25% (1/4)
combined	-	62%	23%
Gibson	-	-	49% (37/75)
Kaligagan	61% (40/66)	-	-
Kateekuk	-	70% (31/44)	76% (42/55)
Midun	56% (28/50)	68% (61/90)	50% (36/72)
Nizki N side	-	-	67% (22/33)
SE side	-	-	66% (65/98)
combined	-	-	66%
Poa	47% (22/47)	-	-
Puffin	-	56% (43/77)	-
St Lazaria	66% (40/61)[b]	83% (24/29)	48% (10/21)
Suklik	-	-	65% (33/51)
Vsevidof	-	33% (7/21)	-

[a]Only one transect of 20 quadrats sampled on Aiktak in 2021, so data do not represent a complete survey.
[b]Only a small area of high-density habitat was surveyed in 2018 data may not be comparable with 2019-2021 when a broader area of habitat was used.

Variability:

Between-replicate variability

Limited multiple replicate information is from annual monitoring sites. Due to changes in task prioritization, plans to sample replicates were not fully realized. Only Aiktak in 2018 and Buldir (two sub-colonies) in 2019 each sampled two replicates of about 100 quadrats per replicate (Table 6). Buldir NW Ridge showed a situation similar to Aiktak's in 2018, namely, that the burrow rate was similar between replicates, but the rate of occupancy was noticeably different. Buldir Crested Point showed little difference but is also much lower density than the other two locations so even a single percentage point difference could actually represent a large relative change. This potential for marked variability reinforces the importance of maintaining larger sample sizes. Another potential source of this variability is a change in amount of apparent evidence. Guano, the predominant sign of occupancy, could easily become less apparent or disappear after marked rainfall. Sampling after an extended period of dry weather could increase the probability of observing some signs of evidence.

Inter-annual variability in occupancy rate

Some islands exhibited very little inter-annual variability while others showed marked variability in occupancy rate (Table 3). Occupancy rate is measured in the latter half of the TUPU breeding season, so a poor year for productivity, especially early egg failure, could affect occupancy. Aiktak showed a 25% increase in occupancy between 2018 and 2019, and that reflects a modest increase in max potential reproductive success (.81 to .91, Youngren et al 2019). Similarly, Kateekuk showed a 36% increase between 2019 and 2021. Presuming productivity of the nearby Chowiet Island reflects that of Kateekuk, we can see another modest increase in max potential reproductive success (.77 to .81, Bode et al 2021). Midun (all three years) and St Lazaria (2019, 2021) maintained similar occupancy rates over the multiple years sampled, but unfortunately no productivity data for those sites have been collected.

Buldir was the least consistent through the years sampled by nearly doubling occupancy between 2018 and 2019 before dropping precipitously in 2021. This initially aligns with reproductive success at Buldir, where 2018 was a complete failure (0% max potential reproductive success) and 2019 was average (48% max potential reproductive success), but 2021 occupancy data do not reflect the above-average max potential reproductive success (78%, second highest of all years recorded, AMNWR unpublished data). This could be due to difficult sampling conditions and very low burrow density found at Buldir, which is markedly lower than other sampled colonies (see Table 4). Low burrow density necessitates much larger sample sizes to get any appreciable power, so it is possible this discrepancy is due to insufficient sample collection. Alternative sampling regimes and/or increased sample sizes should be examined to ameliorate this.

Table 6. Comparisons of multiple replicates sampled in a single year.

	Replicate	
	1	2
Aiktak 2018		
Proportion quadrats with:		
Occupied puffin burrows	0.24	0.35
Any puffin burrows	0.74	0.79
# quadrats sampled	100	100
Buldir 2019 –NW Ridge		
Proportion quadrats with:		
Occupied puffin burrows	0.21	0.14
Any puffin burrows	0.28	0.26
# quadrats sampled	95	96
Buldir 2019 –Crested Point		
Proportion quadrats with:		
Occupied puffin burrows	0.04	0.05
Any puffin burrows	0.08	0.08
# quadrats sampled	96	97

DISCUSSION

Sampling in 2021 experienced wide differences in conditions of islands and colonies, and the need for a more flexible protocol to handle these different conditions. There are, however, some colonies that clearly support a larger TUPU population than we can sample because the accessible amount of habitat is too limited. We can break down islands into several basic categories.

1. Islands with colonies large enough for sampling with standard protocol:
   Using the standard protocol as written.

2. Islands with accessible but narrow bands of habitat:
   Use modifications in order to accommodate.

3. Islands with insufficient accessible habitat for sampling with standard protocol:
   More intensive sampling might be possible, but one should first ensure the accessible habit is representative enough of the colony to be monitored.

4. Islands without much accessible habitat:
   Nothing can be really done on such islands using this protocol.

5. Islands with accessible habitat but mixed puffin species:
   Might be possible to mostly separate (e.g., Suklik) and use current protocol. Other islands where there is marked mixing and separation is not possible (e.g., Amagat), would need to monitor a metric that reflects both species together. Whether this is useful enough for management purposes is an open question.

Additionally, large islands like Buldir with multiple sub-colonies that show markedly different densities should avoid aggregating whole-island results. This is particularly problematic if sampling between years is not consistent between the sub-colonies.

While there are instances where sampling must be curtailed for time or logistical reasons, effort should be given to sample at levels necessary to detect trends, following the IUCN definition of Critically Endangered (CE, 80% decline over 10 years or 3 generations, whichever is greater, when the cause is unknown or has not stopped), that could indicate such a decline (Drummond and Christ 2019). Using the rates of occupancy commonly seen, sampling only 100 quadrats/year isn't sufficient to get even 80% confidence and 60% power to detect such a decline in 20 years. 200 quadrats/year would be sufficient for most colonies encountered so far. It is preferable to monitor less frequently at a higher level than to monitor annually at an insufficient level.

For successful survey preparation, there was little substitute for on-the-ground observations prior to surveying. Simply knowing an island historically supported puffin nesting gave no information about how accessible it was for sampling. Close observation while circumnavigating could give some idea but was still no guarantee of appropriate sampling habitat. We still encountered islands that looked possible from the water but ended up not having enough available habitat to sample. Aerial imagery from small drones could be an avenue to provide faster assessment than circumambulating a colony on foot.

Future revisions of the protocol should include the two modifications developed this year. First, as long as the accessible habitat is being appropriately sampled, there is little problem with mixing protocol variants within a single survey. The precise details still need to be determined by the specific area being sampled. Second, whether to walk forward a random distance or a uniform distance would really depend on the amount of area attempting to be sampled. Fixed distances are easier to determine so it is likely preferable if for no other reason that simplicity.

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Appendix A. Tufted puffin plot occupancy rate protocol used in 2021

Wildlife Inventory Plan
Alaska Maritime National Wildlife Refuge
Protocol #32

Version 1.2

Parameter: Quadrat occupancy rate (surrogate for annual breeding population)

Species: Tufted puffin

PURPOSE

To use the rate of occupied quadrats as a surrogate for annual tufted puffin breeding population, in order to detect biologically important levels of decline in puffin populations with reasonable power. Specifically, we want the power to detect within 10 years a decline in puffin populations of 80% over 3 generations (65 years for tufted puffins; BirdLife International 2018), corresponding to the IUCN definition of Critically Endangered (IUCN 2001).

BACKGROUND

Tufted puffins breed from central California to the Chukchi Sea and from Japan to Siberia, but are most concentrated in Alaska, particularly in the eastern Aleutian Islands and along the Alaska Peninsula. The overall population size is estimated at almost 3 million birds (Piatt and Kitaysky 2002), although this value mostly comes from colony estimates made in the 1970's and 1980's. There is recent growing concern about the health of tufted puffin populations, as biologists have documented range contraction, colony abandonment, and population declines in the southern and eastern parts of their range (Hanson and Wiles 2015). As a result, tufted puffins are currently being evaluated for listing as federally endangered (U.S. Fish and Wildlife Service 2015).

Monitoring numbers of tufted puffins (and other burrow-nesting seabirds) is challenging because birds nest deep underground where they cannot be easily observed. Counts of adult birds socializing on the surface of the colony or at sea around the colony are highly variable, making accurate population estimates from those data difficult (Piatt and Kitaysky 2002). Therefore, indices of breeding population size for tufted puffins are often based on counts of burrow entrances or apparently-occupied burrows (usually assessed using indirect evidence of occupancy, such as feathers or guano, without being able to see into the actual nest chamber; see Burrow-nester Population Protocol).

In Alaska, tufted puffin monitoring faces additional challenges due to the large number of colonies, vast expanse of the area, and remoteness of most sites. Most Alaskan puffin population estimates are decades old and often based only upon rough guesses (U.S. Fish and Wildlife Service 2017). Population trend data (usually indices of population size based on counts of burrow entrances or occupied burrows on small permanent index plots) exist at a few Alaskan puffin colonies within the Alaska Maritime National Wildlife Refuge (Dragoo et al. 2018), but even these datasets are problematic for a number of reasons. One problem is that frequent monitoring in plots may cause substantial disturbance to tufted puffins (Pierce and Simons 1986), so declines observed in annually-monitored permanent plots may be caused by researcher disturbance (A. Kettle and L. Slater, pers. comm.). Another issue is that fixed plot boundaries can be difficult to reliably sample over decades, either because the ground may slump and heave to physically move plot markers, or because different field crews interpret plot boundaries slightly different across years. In addition, the large size of some existing plots may make it difficult to accurately and consistently count burrows from one year to the next, especially with changing field crews. Finally, a number of these Refuge sites have historical particulars (such as release from predation by introduced foxes) that may make observed trends at those sites unrepresentative of puffin populations across the rest

of Alaska. To effectively manage and conserve tufted puffins worldwide, we need better data on puffin population trajectories in Alaska.

Our objective is to develop a protocol to better assess changes in tufted puffin populations in Alaska. We wanted a reproducible sampling method that would generate a metric representing puffin breeding population at a colony, allowing us to quantify variability in puffin populations with reasonable power to detect biologically important levels of decline. It was also important to increase representation by sampling more than just a few plots on an island, and more than just a few islands across the Refuge. Therefore, we aimed to design a procedure low enough in disturbance to conduct annually at our monitoring sites, and simple and flexible enough to do opportunistically at other puffin colonies across the Refuge that are visited irregularly and for which detailed prior knowledge of the colony is not known. Finally, our methodology had to address biases present in previous puffin population datasets on the Refuge (i.e., disturbance, observer differences).

GENERAL OVERVIEW

Our theoretical population metric of interest is the number of adult birds attending a breeding colony in a given year. Because counts of adult birds at the colony or at sea around the colony are highly variable for tufted puffins (Piatt and Kitaysky 2002), we use occupied burrows to represent breeding puffins. Our sampling frame is all tufted puffin colonies on the Alaska Maritime National Wildlife Refuge, with an individual puffin colony as the sampling unit. Colony is defined as group of tufted puffins breeding in distinct area, usually on an island-scale but sometimes a specific geographic area on the mainland. At some colonies, sub-colonies are distinguished as distinct patches of tufted puffin burrows separated (by distance or landscape feature) from other tufted puffin burrows.

Tufted puffins primarily nest in soil burrows but will also use crevices in talus slopes, among beach boulders, and in sea cliffs. Burrows are typically located on moderate to steep slopes anywhere from above storm tide to hundreds of meters above sea level, with highest burrow densities usually along cliff edges and on steep slopes (Wehle 1980, Piatt and Kitaysky 2002). We define puffin nesting habitat as any area with tufted puffin burrows (an entrance >14.5 cm wide with a minimum depth of 30 cm; see Burrow-nester Population Protocol). Because puffin habitat can be patchy and is rarely homogenous, we consider puffin habitat on a broad scale instead of trying to delineate small patches.

The protocol entails sampling small (generally $1m^2$) quadrats along randomly selected transects within potential puffin habitat. Occupancy within each quadrat is defined on the basis of presence/absence of occupied burrows; by repeatedly measuring occupancy in this way in numerous quadrats throughout the colony, we can measure a rate of quadrats containing occupied burrows (i.e., 100 out of 200 quadrats are occupied). Comparing average rates of occupied quadrats through time should provide a rough indication of population trend. Performing the survey multiple times during a season is important to estimate annual variability in the average occupancy, and is necessary to estimate the power this has to evaluate trend.

Although the primary goal of this protocol is to measure and track changes in the rate of quadrats containing occupied burrows over time, because we are still evaluating and improving this protocol for long-term implementation, we are also interested in recording the presence of unoccupied puffin burrows. This will allow us to examine rates of quadrats containing *any* puffin burrows, in order to inform the details of our sampling design (e.g., quadrat size, number of quadrats needed, coverage of transects) and give us more ability to think about what constrains rates of occupied quadrats.

This new population monitoring protocol differs from previous tufted puffin population monitoring on the Refuge in two main ways: (a) occupancy is defined on the basis of presence/absence of occupied burrows in many small (i.e., $1m^2$) quadrats, rather than counting numbers of occupied burrows in fewer larger plots; and (b) sampling is conducted along randomly-located (or as close to random as logistics and terrain allow) transects throughout the extent of the colony, instead of in the same fixed plots year after year.

PROCEDURE

The goal is to sample 200 quadrats per replicate at each colony (based on a power analysis using 2018 sampling; Drummond and Christ 2019) along a number of transects (usually 10 transects of 20 quadrats each). Preferably the number of quadrats per transect should be consistent at a given site. The number of transects is a trade-off between more potential diverse areas across the colony and a higher degree of randomness (with more transects) vs less potential disturbance and sampling time (with fewer transects). Colonies covering a large area may be more appropriate for fewer long transects, while for narrower colonies it might be more appropriate to do a larger number of shorter transects. Should the colony shape be quite linear (e.g., a narrow band along a hillside), it would be appropriate to make transects zig-zag from edge to edge rather than make transects straight down the centerline. The important part is to not sample in a way to generate obvious bias. The time finding and getting to transect start locations will likely be greater than that spent sampling quadrats in a straight line once on the transect.

Sampling along transects is a compromise: ideally, quadrats would be randomly chosen throughout the extent of the colony, but this would require knowing the extent of the colony, unacceptable levels of disturbance, and difficult logistics. When possible, transects are randomized throughout the colony (which necessitates knowing the colony boundaries ahead of time); when that is not possible, transects should be chosen so as not to over-emphasize any particular quality of habitat. For example, always starting transects at the outer edge of a colony could over-sample from marginal habitat with lower density. Likewise, only running transects through the highest density sections of the colony will bias the average occupation higher than the true average. The same transects and quadrats will not be repeatedly sampled from year to year, thus minimizing the collective impact of walking through the same areas of the colony. This method relies upon randomization to get an unbiased estimate of the average occupancy rate, and larger sample sizes help to reduce the variability of estimates.

Quadrats should be $1m^2$ in most cases, except when otherwise noted. It is important than use the same quadrat size for an entire replicate, and at the same colony across years. Quadrats are kept small to increase efficiency and reduce disturbance when sampling, but in colonies with low densities, a larger quadrat may be needed to reliably detect puffin occupancy.

Some colonies are occupied by other species such as storm-petrels. In these cases, care should be taken to ignore burrows associated with these other species, although it is worthwhile noting that they exist in that quadrat. A burrow board or tape measure may be required to assist in making the determination that a burrow is likely from a tufted puffin.

Sampling should be conducted in August towards the latter half of the breeding season. To maximize likelihood of detecting occupancy, ideally sampling would occur at the end of the chick-rearing period but before chicks begin fledging. Timing may be optimized at different sites based on difference in puffin timing of breeding and field crew schedules.

Before doing any work, please **double check that your GPS is set to WGS84 with decimal degrees**; if it is anything different, change it.

Delineating the colony:

Before beginning, scan the colony to get a rough idea of colony boundaries. This will help determine where the first transect should begin, how not to generate obvious bias, etc.

If time allows (either before or after sampling), it would be particularly valuable to walk the perimeter of the puffin colony with a GPS track on. This will provide us detailed information about the colony extent, which will allow us to fine-tune our transect locations in the future and maximize our sampling effort. At sites where puffin burrows are patchy and not contiguous, it may be difficult to determine where the colony boundary actually is. We are looking for a broad picture rather than an extremely detailed picture of small patches: think of trying to walk a rough polygon encompassing the existing puffin breeding area, even with patches of unused habitat between. If puffin breeding areas are distinctly separated by large spaces, then dividing the island into distinct sub-colonies would be appropriate. It may be helpful to take occasional GPS waypoints as you walk the colony perimeter, to help delineate the boundary and to make any necessary notes. If you cannot walk the entire boundary of the colony due to terrain, unsafe areas, etc., please make notes of this so that we can build this information into our island maps.

Where to do transects:

You will be provided a list of transect start locations and bearings for your site. These will be latitude/longitude locations randomly selected within what is believed to be puffin habitat, based on our best knowledge of that colony (information from some sites will be better than others). It may not be possible to access all locations on your list for a number of reasons: you can't get to the location due to some physical barrier, the location isn't safe to work at (e.g., perched on the edge of a cliff), our information about the location or extent of the puffin colony is inaccurate, etc. If you can't get to a starting point, just do your best to get as close as possible, *record the new starting location* on your datasheet, and proceed from there. If you can't even get reasonably close to a point or have no idea where the location is, move on to the next potential starting point on your list.

Once you have a start location for your transect, walk in a straight line (or as straight as possible given the terrain) along the assigned direction, using your compass or GPS as a guide (use a compass if possible because it is more accurate). Stop at approximately 5m intervals to assess occupancy within a quadrat (the precise spacing is not critical, but it should be relatively consistent within a survey). It can be helpful to use a 5m length of cord tied to the sampling square – have one person hold the end of the cord and another walk the transect line until the cord is taut.

How to assess occupancy within a quadrat:

At each quadrat along the transect:

(1) Place the sampling frame on the ground to define your quadrat boundaries (Figure 1). We use a **1m^2 sampling frame** (square of 1m on each side**) at MOST sites** (see Buldir for an exception).

- It is important that you are not influenced by the burrows you may see (e.g., do not tweak your quadrat placement to include particular burrows). To do this in a consistent way without bias, position the center of the closest edge of the frame even with the tip of your right toe (wherever you stopped on your last foot step) and aim the rest of the frame away from you.
- To have a consistent quadrat area (i.e., 1m^2), the frame must be parallel to the ground. If vegetation or terrain make the frame lay unevenly on the ground (which is likely in August), adjust the frame so that it is parallel (in an extreme situation, you may need one person to hold the sampling frame even while another searches the quadrat underneath). On a slope, the sampling frame should be parallel to the slope (ground), not the horizon.

(2) Take a waypoint and record the latitude/longitude in your notebook.

(3) Search the quadrat for puffin burrows.

- By Refuge definition, puffin burrows are >14.5 cm wide with a minimum depth of 30 cm. In colonies with mixed species, it may be necessary to measure questionable burrows with a wooden "burrow board" (Figures 2 and 3) to determine size (a measuring tape can be used in a pinch).
- Burrow entrances completely within the sampling frame are considered within the quadrat.
- Burrow entrances on the border of the sampling frame (only partially within the sampling frame) are considered within the quadrat if they are on the *marked/painted half of the sampling frame* (for a square quadrat, two adjacent legs and two corners of the frame will be marked to differentiate them from the opposite legs; see Figure 4). (The reason for this is that if we include all partially contained entrances, then our sampled area is actually greater than the area of the frame because included burrows extend beyond the border. Partially covered entrances, presuming an equally random probability of location on the border will thus be excluded on average half of the time, maintaining sampling area consistency.)
- If there is at least one puffin-sized burrow within the quadrat, proceed to #4. If there are no puffin-sized burrows within the quadrat, record "N" under "Burrow (Y/N)" in your notebook and proceed to #5.

(4) Using a flashlight, assess puffin-sized burrows within the quadrat for occupancy (see below). You *only need to see evidence of occupancy in a single burrow* to call the quadrat occupied; there

is no need to look in every burrow once you have found evidence of occupancy, nor to record how many burrows are present. However, please record in your notebook which types of evidence you find (this will help us improve the protocol and ensure quality control).

- Evidence of an occupied burrow is defined as the presence of one or more of the following *inside a burrow*:
    - o feathers [F]
    - o guano (often a "pocket" at the end of a tunnel) [G]
    - o prey (fish/squid) deep inside the burrow [P]
    - o egg shell fragments or membrane(s) [S]
    - o egg(s) [E]
    - o chick(s) [C]
    - o adult(s) (inside burrow) [A]

  Note that fresh digging and prey at the *entrance* of a burrow are *not* indicators of occupancy (fish are tricky because they may have been dropped by a bird flying over, so be sure fish are deep enough in a burrow that they couldn't have simply fallen from above before using them as evidence of occupancy). Also note that signs of occupancy do not necessarily need to be contained in the quadrat as long as the associated burrow is considered within the quadrat.
- If you find no evidence in any puffin-sized burrows in the quadrat, enter "N" under "Evidence Y/N".

(5) If a quadrat on a transect looks like it contains a portion of non-habitat, record the amount of habitat to the nearest quartile, that is, estimate percent habitat to for bins: 0-24%, 25-49%, 50-74%, 75-99% (or simply 100% if it is all habitat). This information will be used to help estimate the efficiency of the methodology in light of sampling assumptions.

Continue sampling quadrats at 5m intervals until you have done the required number of quadrats. When the first transect is complete, evaluate where the next transect should start by continuing down the sequential list of transect start locations. Repeat as above until all transects for the replicate (i.e., 10 transects to give you 200 quadrats) are completed.

Hopefully there will be sufficient time to repeat the survey at least two times. Replications should be separated by several hours, preferably on different days, but not weeks apart. If possible, use different transects for each replication. Replications are crucial to evaluating variability of the occupancy metric and will help determine efficacy and applicability of the survey.

How to record data:

Record all data in a Rite-in-the-Rain® notebook as outlined in Figure 5. Specifically:

Transect: Sequential number of transect you are on (1, 2, 3, etc.) When you complete a replicate (200 quadrats), continue sequential transect numbering for the next transect (i.e., if you did 10 transects in the first replicate, start the second replicate with #11).

Quadrat: Sequential number of quadrat you are sampling (1, 2, 3, etc.). Continue sequential numbering until you have completed all quadrats in a replicate (generally 200), then restart at 1 for any additional replicates.

Lat.: Latitude of quadrat (WGS84).

Long.: Longitude of quadrat (WGS84).

Waypoint: Name of waypoint in GPS for reference.

Burrow (Y/N): Record if *any* large (>14.5cm) burrows is found in quadrat, regardless of occupancy, in Y/N format.

Evidence (Y/N): Record if *evidence for occupancy* (see below) is found in quadrat, in Y/N format.

Evidence type: Record which evidence for occupancy was observed (record all you happen to see, but this does *not* mean you need to search the entire quadrat exhaustively).

% Habitat: Estimate % habitat in quartiles (i.e., 0-24%, 25-49%, 50-74%, 75-99%, 100%)

Comments: Record any comments or notes.

Difficulties you may encounter:

(1) Difficulty placing sampling frame on ground - There may be instances when it is difficult or impossible to lay the sampling square down on terrain, due to uneven ground, large tussocks, vegetation, rocks, etc. Just do the best you can. You may have to approximate the quadrat area by having one person hold the sampling square parallel to the ground surface at some higher-than-ideal height. In some situations (e.g., high *Leymus* grass or thick salmonberry bramble), it may be possible to take the sampling square apart and reassemble it at ground level around the difficult vegetation.

(2) Transect leads you out of puffin habitat - Puffin habitat can be patchy and is rarely homogenous, and following a straight-line transect through a colony may take you in and out of puffin breeding areas. While it is important to document the absence of puffins in suitable habitat, we do not have time to sample infinite quadrats in non-habitat along a transect. If you sample three consecutive quadrats with no puffin-sized burrows at all (not simply no occupied burrows), look around you to assess the surrounding habitat. If it seems that you are just in a small unused patch and are about to enter puffin habitat again in the next 5-10m, then you can continue along the same transect line. If not, then go back to the last quadrat (approximately, as we are not permanently marking quadrats) where you found a puffin-sized burrow (whether occupied or not). From there, turn at a right angle (90 degrees) back towards the occupied part of the colony and continue sampling along that new transect direction. Repeat this process anytime you have three consecutive quadrats with no burrows.

There may be situations when you need to change the direction of your transect more quickly. For instance, your transect may lead you off the island or into an area that is not safe to survey (e.g., too steep or cliff's edge). In this case, simply go back to the last quadrat you sampled, turn at a right angle (90 degrees) back towards the occupied part of the colony and continue sampling along that new transect direction.

(3) Need more transect start locations/bearings - It may be possible that our best guess of the location and extent of the puffin colony is incorrect at a site and therefore the provided list of transect start locations and bearings are not useful. Alternatively, we may opportunistically sample an island for which we have not had a chance to generate random locations. If you find yourself lacking randomly-generated transect start locations and bearings, simply find a reasonable place within the puffin colony to start, choose an arbitrary direction, and go from there. It won't be completely random, but you will still be able to follow the protocol enough to give us valuable feedback on the sampling scheme and teach us about the puffin colony at that site.

Note that in cases in which our prior knowledge of the puffin colony is woefully inaccurate, it would be particularly valuable to walk the perimeter of the colony with a GPS.

(4) Crushing many burrows - This protocol was designed to minimize disturbance, by (a) using transects rather than random points to prevent crews from having to traipse across the entire colony, (b) by using small sampling squares to prevent disturbing a large area, and (c) not repeatedly sampling in the same plots over time. However, it is inevitable that your presence in the colony will cause some level of disturbance, and you may crush burrows no matter how carefully you walk. Carefully dig out any burrow you crush to make sure there isn't a bird trapped inside and do your best to affect any repairs you can. In general, we consider this an unfortunate consequence of working in burrow-nesting colonies. However, we don't want our sampling to destroy a colony, so if you think you are sampling in an ultra-sensitive area that is suffering devastating effects from our protocol, please stop and contact the biologists to discuss the issue.

(5) Colony is very small - This protocol is optimized for sampling across large areas and we generally prioritize sampling on islands with large tufted puffin colonies. However, it is possible to come to a site with a much smaller colony than expected, or to have a site that may be large but be very restricted in areas that we can actually sample. For a number of reasons, this sampling scheme does not work well for very small areas. In these cases, we would either decide not to sample at that island, or use randomized points instead of quadrats along transects.

(6) This isn't working at all!! - Hopefully not, but in rare cases there may be times when this protocol makes you want to scream in frustration and you can't believe your supervisor is asking you to do this. Please bear with us. This protocol is still in the developmental phases and we need to see what does and doesn't work. All of your input, both positive and negative, are greatly appreciated and will go towards creating a sampling scheme that is both useful and feasible. Undoubtedly there will be kinks to iron out, but our end goal is an important one: to find a better way to track tufted puffin populations across the Refuge. This is a big and potentially crucial data gap that we have wanted to tackle for many years. So please hang in there when things are hard, give us as much feedback as you can, and know that you are helping create something that could make a difference for management and conservation of tufted puffins.

Specific Requirements:

Dates: Occupancy sampling: August.
Walk perimeter of colony: Any time.

Optimal sample size: 200, generally 10 transects of 20 quadrats each (except at some colonies).

Time of day: Any time.

Weather: Any weather.

Equipment needed: Rite-in-the-Rain® notebook, at least two pencils, GPS (WGS84), compass, burrow board measurer, flashlight, spare flashlight batteries, square frame made from PVC tubing and 90 degree elbows (two adjacent sides and two corners will be marked to differentiate them from the opposite two).

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Figure 1. Sampling quadrat in puffin habitat.

Figure 2. Measuring burrows within sampling quadrat.

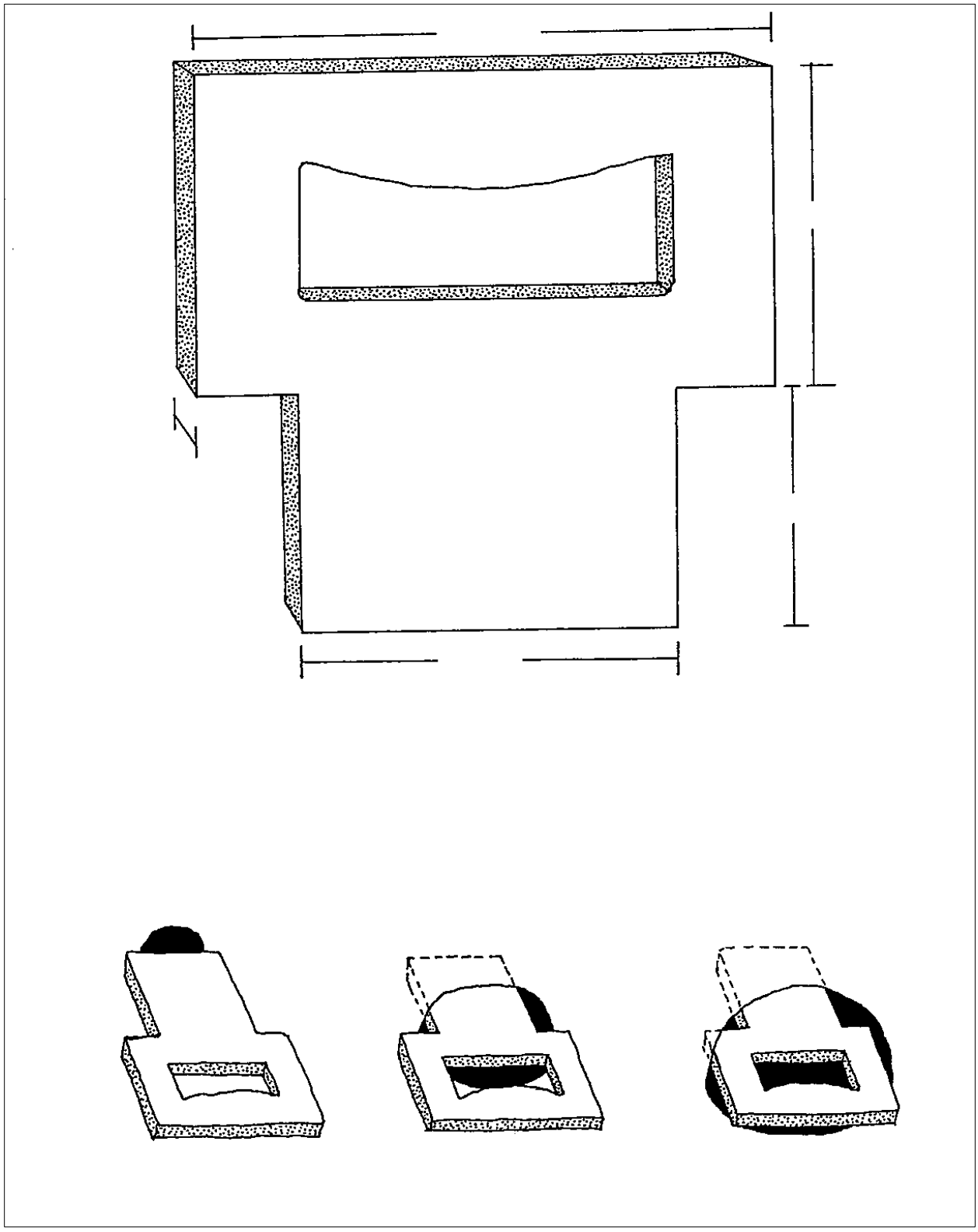
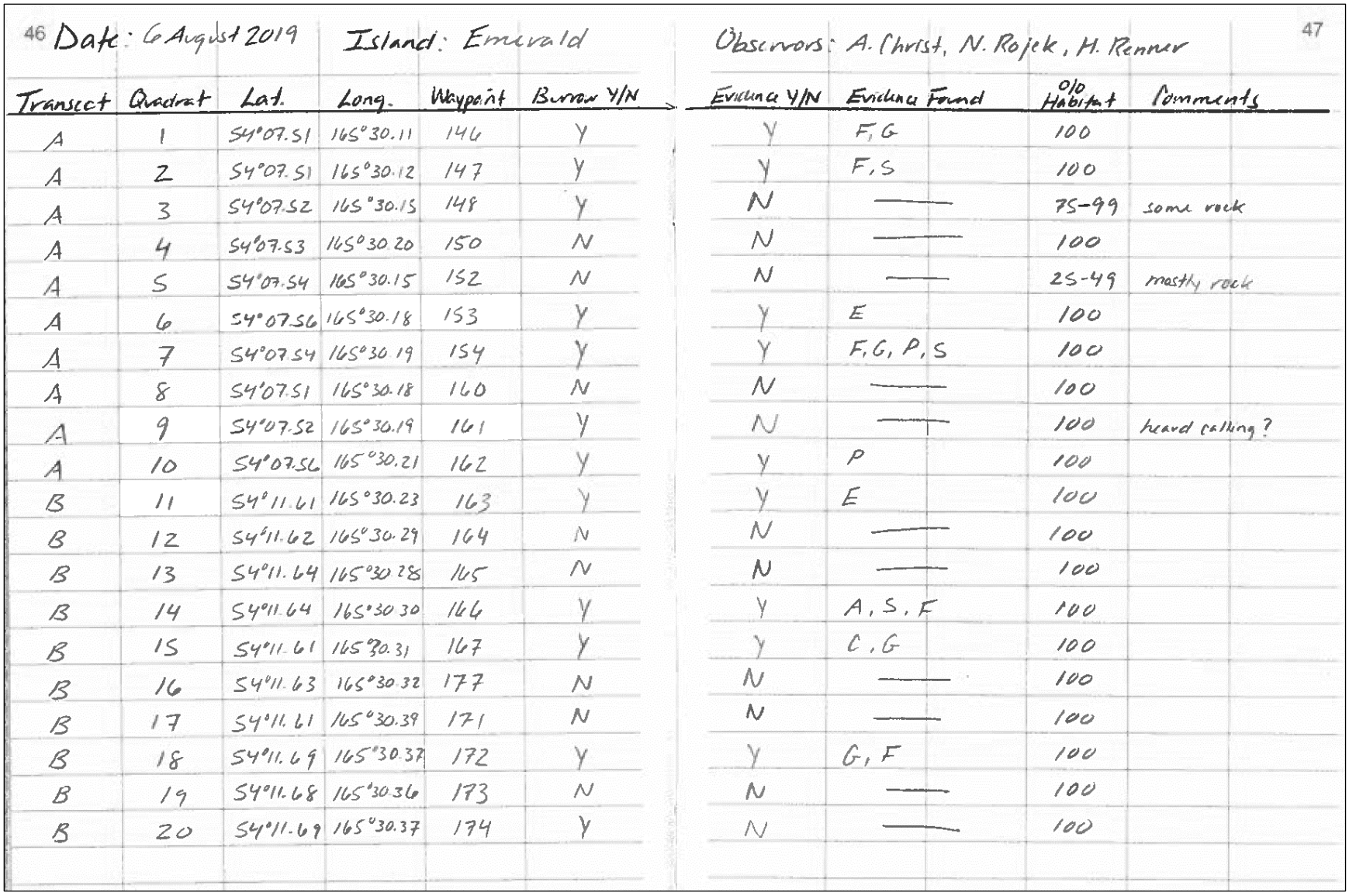


Figure 3. Diagram of burrow-measuring device and examples of burrows with entrances of different sizes.

a)

b)

Figure 4. a) Square quadrat, burrows bisected by black frame included, burrows bisected by red frame excluded. Note the added area (within black dotted lines) is approximately equal to the removed area (within the red dotted lines). b) Because of how annular area is calculated, the difference between included and excluded regions is unequal and increases by the square of the average burrow diameter. Therefore, using a rod and string to define plots makes determining inclusion and exclusion of burrows quite complicated and is not advised.



46 Date: 6 August 2019 Island: Emerald Observers: A. Christ, N. Rojek, H. Renner 47

Transect	Quadrat	Lat.	Long.	Waypoint	Burrow Y/N	Evidence Y/N	Evidence Found	% Habitat	Comments
A	1	54°07.51	165°30.11	146	Y	Y	F, G	100	
A	2	54°07.51	165°30.12	147	Y	Y	F, S	100	
A	3	54°07.52	165°30.15	148	Y	N	—	75-99	some rock
A	4	54°07.53	165°30.20	150	N	N	—	100	
A	5	54°07.54	165°30.15	152	N	N	—	25-49	mostly rock
A	6	54°07.56	165°30.18	153	Y	Y	E	100	
A	7	54°07.54	165°30.19	154	Y	Y	F, G, P, S	100	
A	8	54°07.51	165°30.18	160	N	N	—	100	
A	9	54°07.52	165°30.19	161	Y	N	—	100	heard calling?
A	10	54°07.56	165°30.21	162	Y	Y	P	100	
B	11	54°11.61	165°30.23	163	Y	Y	E	100	
B	12	54°11.62	165°30.29	164	N	N	—	100	
B	13	54°11.64	165°30.28	165	N	N	—	100	
B	14	54°11.64	165°30.30	166	Y	Y	A, S, F	100	
B	15	54°11.61	165°30.31	167	Y	Y	C, G	100	
B	16	54°11.63	165°30.32	177	N	N	—	100	
B	17	54°11.61	165°30.39	171	N	N	—	100	
B	18	54°11.69	165°30.37	172	Y	Y	G, F	100	
B	19	54°11.68	165°30.36	173	N	N	—	100	
B	20	54°11.69	165°30.37	174	Y	N	—	100	

Figure 5. Set-up of data notebook for tufted puffin quadrat occupancy rate sampling.

Attachment A. Aiktak Island specifics (includes Figure A1 and Table A1)

PROCEDURE DETAILS SPECIFIC TO AIKTAK

Aiktak Island has one of the largest populations of tufted puffins in Alaska, with an estimated 100,000 breeding individuals (Byrd et al. 2005, Goyert et al. 2017, U.S. Fish and Wildlife Service 2017). Tufted puffin burrows ring the perimeter of the entire island and are also present on the slopes of Southwest Slope and Gull Mountain (see Figure A1). On Aiktak, peak egg laying occurs in mid-June, hatch in late July or early August, and fledging in late August through September. **Surveys should be conducted from mid- to late August**, when most chicks will have hatched but before many fledge.

The colony at Aiktak covers a lot of area and is largely contiguous, so aim for replicates of 200 quadrats. Because puffins are mostly restricted to a narrow band around the perimeter of the island (with some exceptions), transects will probably need to turn several times to remain within puffin habitat. Due to the large colony size and the restricted perimeter width, **aim for 10 transects of 20 quadrats each** to ensure most spatial coverage across the island. Use a $1m^2$ sampling frame (1m on each side) for your quadrats.

Normally, we would provide a list of random transect start locations and ask you to start at the beginning and work your way down the list. However, because the colony at Aiktak encompasses the coastline of the entire colony, using completely random transects at Aiktak would require a huge effort in trekking back and forth across the island. Therefore, we are providing sets of 10 randomly-derived transect start locations and directions (see Figure A1), but you may choose to do them in whatever order you wish (i.e., moving around the island in one direction, or in blocks depending on other work in areas of the island); if you wish to sample them consecutively with minimal back-tracking, start at the first location and work your way sequentially in a counter-clockwise direction around the island. To further minimize sampling effort, rather than providing extra transect start locations to make up for ones you may not be able to access, **simply get as close to each of the start locations as you can and start from there** (make sure you record where the start point actually is). Refer to Table A1 for randomly-derived transect start locations. Use the first set of 10 start locations for the first replicate of 200 quadrats, and the second set of start locations for a second replicate of 200 if you are able. If you can fit it in with your other work, try for two replicates will allow us to look at variability in the data. Aiktak may be one of our more "ideal" colonies for this sampling protocol, so it would be especially helpful to get replicates here if possible.

The boundary of the colony was walked with a GPS track on in 2018, so it is not necessary to repeat this exercise in 2021.

Specific Requirements at Aiktak Island:

Dates: Occupancy sampling: Mid- to late August.

Optimal sample size: At least one replicate of 200 (each with 10 transects of 20 quadrats each). A second replicate of 200 is a bonus.

Time of day: Any time.

Weather: Any weather.

Equipment needed: Rite-in-the-Rain® notebook, at least two pencils, GPS (WGS84), compass, burrow board measurer, flashlight, spare flashlight batteries, square frame made from PVC tubing and 90 degree elbows resulting in 1 m^2 area (two adjacent sides and two corners will be marked to differentiate them from the opposite two).

Literature Cited

Byrd, G. V., Renner, H. M., and M. Renner. 2005. Distribution patterns and population trends of breeding seabirds in the Aleutian Islands. Fisheries Oceanography 14, 139-159.

Goyert, H. F., E. O. Garton, B. A. Drummond, and H. M. Renner. 2017. Density-dependence and changes in the carrying capacity in Alaskan seabird populations. Biological Conservation 209:178-187.

U.S. Fish and Wildlife Service. 2017. North Pacific Seabird Colony Database – computer database and colony status record archives. U. S. Fish and Wildlife Service, Migratory Bird Management, Anchorage, Alaska. Accessed 15 February 2017. [axiom.seabirds.net/maps/north-pacific-seabirds/]

Figure A1. Randomly-derived transect start locations for the first (shown in red) and second (shown in blue) replicates of tufted puffin quadrat occupancy sampling at Aiktak Island in 2021. The light blue polygon shows the best estimate of the extent of the tufted puffin colony on the island.

Table A1. Randomly-derived transect start locations (WGS84) and directions for tufted puffin quadrat occupancy sampling at Aiktak Island in 2021. Note that the name of each transect also contains the start direction for convenience (i.e., A-280 means start transect A by walking in a direction of 280°).

Label	Lat.	Long.	Start direction (degrees)[a]
REPLICATE 1			
A-091	54.18545	-164.84494	91
B-247	54.18112	-164.84431	247
C-073	54.17931	-164.84234	73
D-184	54.17930	-164.83795	184
I-009	54.18254	-164.82933	9
J-027	54.18366	-164.82614	27
L-041	54.18204	-164.82198	41
M-128	54.18265	-164.81999	128
R-140	54.18772	-164.82363	140
T-056	54.18930	-164.83668	56
REPLICATE 2			
E-343	54.18066	-164.83887	343
F-030	54.18178	-164.83950	30
G-058	54.18190	-164.83825	58
H-090	54.18277	-164.83240	90
K-102	54.18372	-164.82509	102
N-162	54.18204	-164.81884	162
O-084	54.18347	-164.81780	84
P-359	54.18463	-164.81695	359
Q-134	54.18670	-164.82044	134
S-158	54.18914	-164.82800	158

[a]Direction is based on 0-359 degrees, with 0° = N.

Attachment B. Buldir Island specifics (includes Figure B1-B3 and Table B1)

PROCEDURE DETAILS SPECIFIC TO BULDIR

Buldir Island has an estimated tufted puffin breeding population of 20,000-40,000 individuals (Goyert et al. 2017, U.S. Fish and Wildlife Service 2017), including several nearshore islands. Highest concentrations of puffins are found at Northwest Point, on the slopes above Crested Point and Main Talus, along Sharp's Ridge, and on either side of Peregrine Point (Figure B1). On Buldir, most chicks hatch from early to late July and fledge beginning in mid- to late August. **Surveys should be conducted from early to mid-August**, when chicks will have hatched but before they start fledging.

Because puffins at Buldir are separated into a number of "sub-colonies", spread across the island and often with large patches of non-puffin habitat in between, it is impractical to try to sample randomly across the entire island. *In 2021,* we are asking you to sample two of the more easily accessible areas: Northwest Ridge and the area about Crested Point and Main Talus. Both are relatively small areas with steep slopes, so **aim for 5 transects of 20 quadrats in each sub-colony area**. You may have to reflect transects off the edge of the colony numerous times. Transect start locations and bearings have been randomly derived (see Table B1 and Figures B2-B3) based on our best estimate of colony location (*we have hopefully provided more start locations than you will need because you may not be able to access all locations or some may not be in puffin habitat; just go down the list sequentially until you complete 5 transects*). If start locations are grossly in error, do the best you can by getting as close as you can, or in the worst case just find someplace to start (see #3 in "Difficulties you may encounter" above).

Because the density of puffin burrows is lower at Buldir than at other colonies we sample, it is necessary to use a larger sampling frame to capture enough puffin burrows within your quadrats to make sampling useful. **Use a 1.5m^2 sampling frame** (1.22m on each side) for your quadrats.

Buldir is such a large island and puffin habitat is so scattered that it is difficult to optimize sampling without a good idea of colony and sub-colony boundaries. **It will be extremely helpful for you to walk the boundary of tufted puffin sub-colonies with a GPS track on**. Try to do this both for the sub-colonies you are sampling this year (Northwest Ridge and Crested Point/Main Talus), and also a potential sampling area at Spike Camp around Peregrine Point.) *This can be done at any time during the season* (not necessarily concurrent with sampling for quadrat occupancy, although it can be). While determining the perimeter of other sub-colony areas, consider any logistical considerations to sampling those areas that might provide us valuable feedback in determining the structure of our puffin sampling at Buldir.

Although multiple replicates of 200 quadrats would be great, it is likely unrealistic at Buldir due to other work load requirements. If you can complete one replicate of 200 quadrats and walk the boundaries of the sub-colonies, we will be thrilled.

Specific Requirements at Buldir Island:

Dates: Occupancy sampling: Early to mid-August.
Walk perimeter of sub-colonies: Any time.

Optimal sample size: One replicate of 200 (5 transects of 20 quadrats each, in each of two sub-colonies).

Time of day: Any time.

Weather: Any weather.

Equipment needed: Rite-in-the-Rain® notebook, at least two pencils, GPS (WGS84), compass, burrow board measurer, flashlight, spare flashlight batteries, square frame made from PVC tubing and 90 degree elbows resulting in 1.5 m^2 area (two adjacent sides and two corners will be marked to differentiate them from the opposite two).

Literature Cited

Goyert, H. F., E. O. Garton, B. A. Drummond, and H. M. Renner. 2017. Density-dependence and changes in the carrying capacity in Alaskan seabird populations. Biological Conservation 209:178-187.

U.S. Fish and Wildlife Service. 2017. North Pacific Seabird Colony Database – computer database and colony status record archives. U. S. Fish and Wildlife Service, Migratory Bird Management, Anchorage, Alaska. Accessed 15 February 2017. [axiom.seabirds.net/maps/north-pacific-seabirds/]

Figure B1. Map showing locations of major tufted puffin sub-colonies at Buldir Island.

Figure B2. Randomly-derived transect start locations for the first replicate of tufted puffin quadrat occupancy sampling at Northwest Ridge, Buldir Island in 2021. Only 5 transects need to be sampled but extra start locations are provided in case some are out of the colony or inaccessible.

Figure B3. Randomly-derived transect start locations for the first replicate of tufted puffin quadrat occupancy sampling at Crested Point and Main Talus, Buldir Island in 2021. Only 5 transects need to be sampled but extra start locations are provided in case some are out of the colony or inaccessible.

Table B1. Randomly-derived transect start locations (WGS84) and directions for tufted puffin quadrat occupancy sampling at Buldir Island in 2021. Note that the name of each transect also contains the start direction for convenience (i.e., A-280 means start transect A by walking in a direction of 280°). Only 5 transects need to be sampled from each sub-colony but extra start locations are provided in case some are out of the colony or inaccessible.

Label	Lat.	Long.	Start direction (degrees)[a]
REPLICATE 1 – NORTHWEST POINT			
A-316	52.37322	175.87555	316
B-100	52.37287	175.87355	100
C-232	52.37314	175.87432	232
D-127	52.37345	175.87261	127
E-204	52.37284	175.87299	204
F-200	52.37427	175.87113	200
G-184	52.37305	175.87433	184
H-119	52.37317	175.87462	119
REPLICATE 2 – CRESTED POINT AND MAIN TALUS			
A-066	52.37245	175.89640	66
B-337	52.37224	175.89676	337
C-182	52.37188	175.89395	182
D-358	52.37211	175.89533	358
E-310	52.37253	175.89729	310
F-262	52.37173	175.89404	262
G-232	52.37183	175.89456	232
H-019	52.37138	175.89466	19
A-066	52.37245	175.89640	66

[a]Direction is based on 0-359 degrees, with 0° = N.

Attachment C. St. Lazaria Island specifics (includes Figures C1-3 and Table C1)

PROCEDURE DETAILS SPECIFIC TO ST. LAZARIA

St. Lazaria Island has the largest tufted puffin breeding colony in Southeast Alaska, with an estimated of 7,000-10,000 individuals (Goyert et al. 2017, U.S. Fish and Wildlife Service 2017). Most puffins on the island nest in burrows on steep slopes at the top of cliffs on the western half of the island overlooking the gull colony.

The puffin habitat at St. Lazaria is challenging to sample because some areas are too steep to safely access or too fragile to work in, whereas others are covered by salmonberry thickets that are difficult to traverse. In 2018, we sampled puffin burrows in 69 quadrats in a small subset of potential tufted puffin habitat. A larger boundary of possible workable puffin habitat was delineated with a GPS at the beginning of the season in 2019 (Figure C1) to try to expand the sampling area to fit in more quadrats. Even with expanding the potential sampling area, the size of puffin habitat that is workable on the island was too small for our transect design, so we attempted a modified transect technique in 2019, in which the crew sampled every 5m along pre-selected transect lines (Figure C2). This ended up being a lot of work (vegetation made it difficult to stick to a specific straight line) and yielded fewer quadrats than hoped. In 2021, we are going to attempt to simplify sampling at St. Lazaria. In short, you will start at one or the other end of the tufted puffin habitat, choose a direction to start sampling, and simply continue until you have traversed the entire area.

The 2021 modifications in detail:

a) Flip a coin (or some two sided object) to determine whether you start nearest the trail at point A, or farthest from the trail at point U (see Figure C2 and Table C1).

b) Choose a direction that will lead you further into tufted puffin habitat.

c) Sample every 5m in a straight direction. If you reach a cliff edge or leave tufted puffin habitat (determined by three consecutive quadrats with no puffin-sized burrows per the protocol), reflect back into the colony at a 90 degree angle and continue sampling.

d) Stop when you have traversed the entire area of tufted puffin habitat.

Please ***BE SAFE*** and do not sample too close to the cliff edge. No data is worth potential accidents. Be especially cautious of wet vegetation that may be slippery and tussocks that may look secure but are actually overhanging the cliff.

Our goal would be to sample 100 quadrat, but really you will just be able to do what you can do. You will be limited by available habitat. Just do whatever fits, spacing sampling about every 5m, and call it good. We don't want to sample quadrats closer together to up the sample size, as the area is so small and we don't want to be tramping down every inch of puffin habitat.

This protocol will be time consuming and frustrating - making your way through the vegetation at St. Lazaria is not easy. Sampling in the salmonberry is going to be difficult but try the best you can: it will probably be necessary to take the sampling square apart and reassemble it at ground level at the base of the salmonberry bushes to get around some of the higher vegetation. Try not to mentally curse your Unit Biologist too much…

On St. Lazaria, most chicks probably hatch from late July to mid-August. Surveys should be conducted around mid-August, when chicks will have hatched but before they start fledging.

Specific Requirements at St. Lazaria Island:

Dates: Occupancy sampling: Mid-August.
Optimal sample size: One replicate of about 100? (as many as fits).
Time of day: Any time.
Weather: Any weather.
Equipment needed: Rite-in-the-Rain® notebook, at least two pencils, GPS (WGS84), compass, burrow board measurer, flashlight, spare flashlight batteries, square frame made from PVC tubing and 90 degree elbows resulting in 1 m^2 area (two adjacent sides and two corners will be marked to differentiate them from the opposite two).

Literature Cited

Goyert, H. F., E. O. Garton, B. A. Drummond, and H. M. Renner. 2017. Density-dependence and changes in the carrying capacity in Alaskan seabird populations. Biological Conservation 209:178-187.

U.S. Fish and Wildlife Service. 2017. North Pacific Seabird Colony Database – computer database and colony status record archives. U. S. Fish and Wildlife Service, Migratory Bird Management, Anchorage, Alaska. Accessed 15 February 2017. [axiom.seabirds.net/maps/north-pacific-seabirds/]

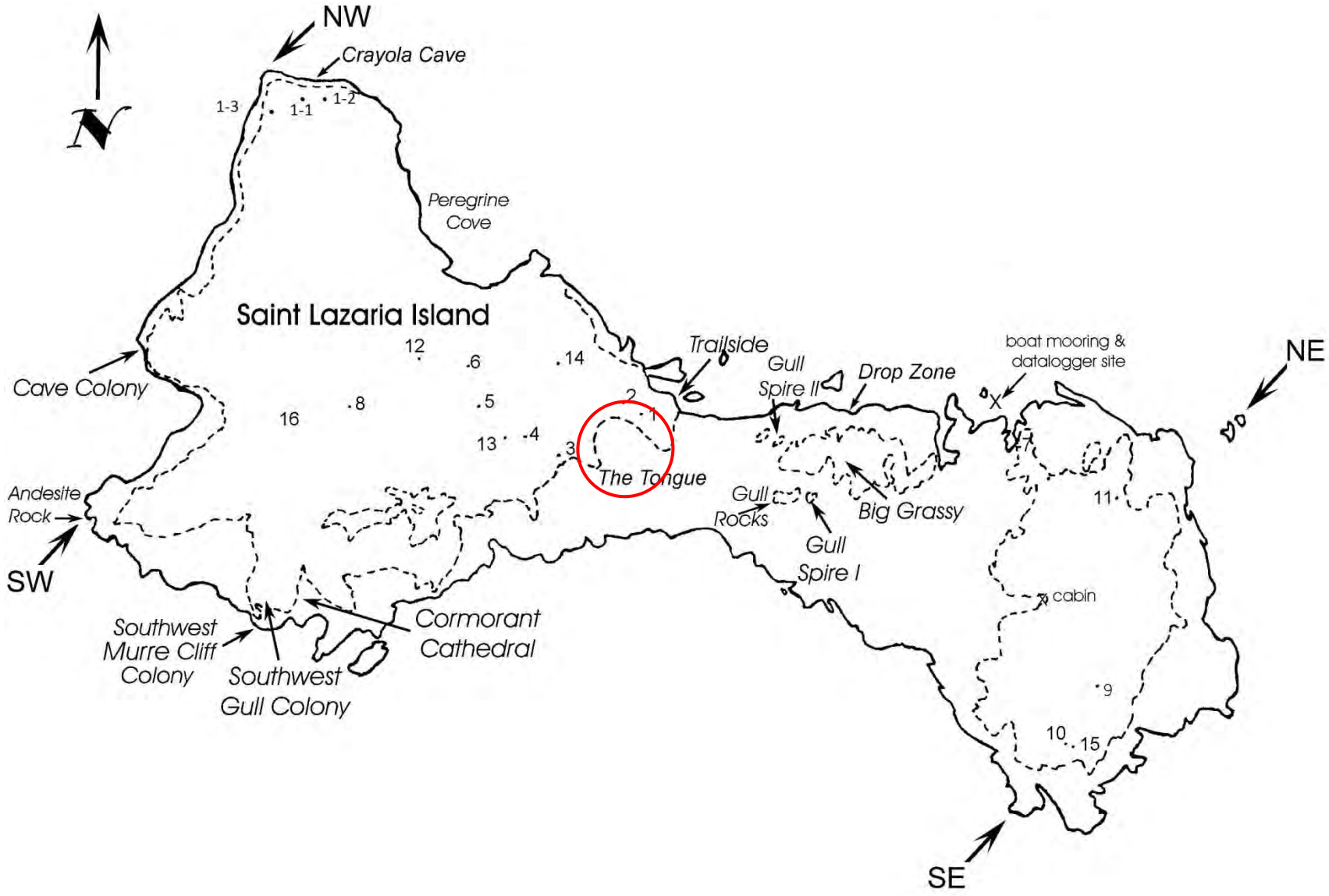
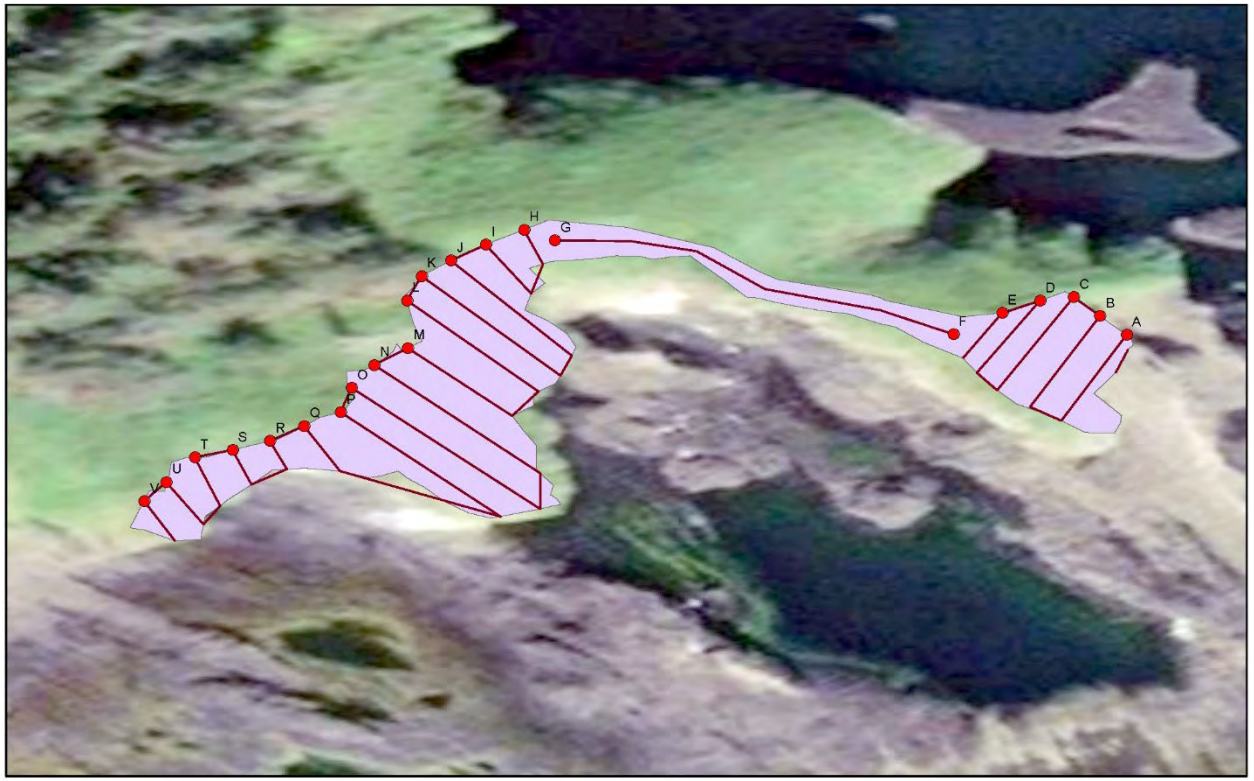


Figure C1. Map showing tufted puffin sampling area (red) at St. Lazaria Island.



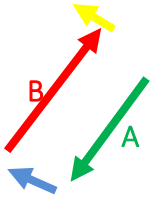


Figure C2. Transect locations for tufted puffin quadrat occupancy sampling at St. Lazaria Island in 2019. Arrows show the path of sampling; colored letters indicate transect names associated with start or end points.

Table C1. Transect start or end locations (WGS84) for tufted puffin quadrat occupancy sampling at St. Lazaria Island in 2019.

Point name	Lat.	Long.
A	-135.70806	56.98700
B	-135.70811	56.98703
C	-135.70816	56.98707
D	-135.70822	56.98706
E	-135.70829	56.98704
F	-135.70838	56.98700
G	-135.70913	56.98717
H	-135.70919	56.98719
I	-135.70926	56.98716
J	-135.70932	56.98713
K	-135.70938	56.98711
L	-135.70941	56.98706
M	-135.70940	56.98697
N	-135.70947	56.98694
O	-135.70951	56.98690
P	-135.70953	56.98685
Q	-135.70960	56.98682
R	-135.70966	56.98680
S	-135.70973	56.98678
T	-135.70980	56.98677
U	-135.70986	56.98672
V	-135.70990	56.98668

Attachment D. Intermittent Island specifics

PROCEDURE DETAILS SPECIFIC TO INTERMITTENT ISLANDS

This protocol was designed to sample puffin colonies not just at annual monitoring sites where puffin habitat is fairly well delineated, but also at other islands where Refuge staff may visit only rarely and extent of puffin habitat may be unknown. At these islands, puffin colony boundaries will probably not be well established, and no starting locations for transects will exist.

Access to islands is usually restricted to one or a few places where crews can get ashore on an island. From any previous knowledge of the island, proceed to areas of known puffin habitat (or lacking that, proceed to likely puffin habitat and traipse around until you find some). Create a waypoint for all access points used.

Once at puffin habitat, you will need to determine starting location and direction for transects, using some sort of random number generator to avoid human bias where possible. For instance, use a stopwatch function on a watch, hitting the stop button at any point and looking at the hundredths of a second digit. This will be how many meters you travel into the colony to start sampling. Do this again to determine which compass direction you will follow to start sampling (broken down into ten sections, such that 0 = 0°, 1 = 36°, 2 = 72°, etc). For example, if the stopwatch first stops at 00:05.96, you would travel 6m into the colony to start your transect. Stopping it again at 00:15:39 tells you to start at a heading of 324° (9x36° = 324°).

Once you have your transect start location and direction, proceed to sample following the protocol. If the accessible colony extent seems large, sample 10 quadrats to a transect; if accessible colony extent seems small, sample 20 quadrats to a transect. Remember to reflect back into the colony if your sampling leads you out of puffin habitat or to an inaccessible area (see “Transect leads you out of puffin habitat” on page 6). Once you complete one transect, move 50-100m farther down the coast in the general direction you were already moving and repeat the procedure to start sampling the second transect. On larger islands, it may be preferable to move even farther, but such considerations involve questions of staying within or moving between large sub-colonies, and will need to be directed on a case-by-case basis by the project leader.

Aim for at least 200 quadrats sampled (more is always better). Sampling is most efficient in teams of two; if multiple teams are present, crews should sample in different areas, in order to cover more of the geographic extent of the colony. Depending on the colony, this may mean heading off in different directions from one starting point, or having one team walk to a completely different place in the colony from which to start. It is always more efficient and less disruptive to use multiple beach access points when possible rather than crossing an island on foot, but heading in opposite directions is usually preferable to traversing the island prior to starting due to time constraints.

If time or personnel allows, it is helpful to walk the boundary of the colony with a GPS to delineate puffin habitat, but this is a lower priority than sampling quadrats.

Specific Requirements at Intermittent Islands:

Dates: Occupancy sampling: August/early September
Optimal sample size: 200 quadrats
Time of day: Any time.
Weather: Any weather.
Equipment needed: Rite-in-the-Rain® notebook, at least two pencils, GPS (WGS84), compass, burrow board measurer, flashlight, spare flashlight batteries, square frame made from PVC tubing and 90 degree elbows resulting in 1 m^2 area (two adjacent sides and two corners will be marked to differentiate them from the opposite two).

Protocol Revision History Log

Revision Date	Changes made	New version #
April 2021	Updated transect start locations for Aiktak and Buldir, modified St. Lazaria, added intermittent sampling details	1.2
July 2019	Updated sample sizes, rewrote some intro/methods for clarity, changed terminology of plot to quadrat	1.1
May-July 2018	Protocol developed for pilot year, includes Aiktak, Buldir, and St. Lazaria attachments	1.0