

Plague Management Techniques and Monitoring in Colorado's Prairie and Shrub-steppe Ecosystems

Technical Publication Number 51 August 2018





COVER PHOTOS

Top: Black-tailed prairie dog colony (Sean Streich)

Bottom, left to right: 1) Juvenile prairie dogs (Bruce Gill); 2) Insecticidal dust in prairie dog burrow (Dan Tripp)
3) Plague vaccine in drying rack (Danielle Sack) 4) Prairie dog consuming plague vaccine bait (Bruce Gill).

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Technical Publication No. 51

COLORADO PARKS AND WILDLIFE

WILDLIFE HEALTH PROGRAM

August 2018

CPW-R-T-51-18 ISSN 0084-8883

STATE OF COLORADO

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Study and Publication Funded by:

CPW and Colorado's Species Conservation Trust Fund

Suggested Citation:

Tripp, D.W., L.M. Corro, S.R. Magstadt and D.A. Sack. 2018. Plague Management Techniques and Monitoring in Colorado's Prairie and Shrub-steppe Ecosystems. Colorado Parks and Wildlife Technical Publication 51.

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Acknowledgements

Our work is supported by CPW and Colorado's Species Conservation Trust Fund. We thank R. Abbott, E. Cárdenas-Canales, A. Emslie J. Foster, K. Fox, S. Green, K. Griffin, B. Lutz, M. Markus, T. Martyn, C. Puntenny, T. Rocke, S. Streich, A. Tschirley, L.L. Wolfe and M. Zieschang for contributions to this manual. B. Dreher, A. Estep, T. Jackson, M.W. Miller, A. Poshusta and A. Seglund provided helpful comments on earlier drafts. We also thank R. Matchett and K. Kreiger for assistance in drafting protocols for 3-shooter operation and data manipulation. Photos in this bulletin were taken by L. Corro, A. Emslie, B. Gill, S. Green, S. Magstadt, C. Puntenny, D. Sack, S. Streich and D. Tripp.

Introduction

Plague, caused by the bacterium *Yersinia pestis*, affects numerous wildlife species worldwide. Its introduction has contributed to the degradation of North American grassland and shrub-steppe ecosystems (Gage and Kosoy 2005; Augustine et al. 2008; Eads and Biggins 2015). Prairie dogs (*Cynomys* spp.) in particular suffer plague-driven mass mortality that can collapse colony complexes over large geographic areas and further fragment or eliminate isolated populations (Ecke and Johnson 1952; Fitzgerald 1993). Other associated wildlife species, like the endangered black-footed ferret (*Mustela nigripes*) that rely on prairie dogs for habitat or prey, may be directly or indirectly affected by plague (Antolin et al. 2002; Biggins et al. 2010). The ability to mitigate plague at an ecologically meaningful scale has thus emerged as a critical conservation need in Colorado and range wide (Creekmore et al. 2002; Seglund and Schnurr 2010; Biggins et al. 2010; Abbott et al. 2012; Tripp et al. 2017).

Until recently, the plague management approach most widely practiced in North America was reactive use of insecticides to control fleas, the primary plague vector (Seery et al. 2003; Biggins et al. 2010). This approach can be effective in reducing mortality and spillover to domestic animals and humans, but does little to offset the broader ecological impacts of plague. Since the early 2000s, attention has shifted to developing preventive plague management approaches for prairie dog habitats via vector control (Hoogland et al. 2004; Biggins et al. 2010; Griebel 2012; Jachowski et al. 2012) and oral vaccination (Mencher et al. 2004; Abbott et al. 2012; Rocke, et al. 2010; Rocke et al. 2014; Rocke et al. 2017; Tripp et al. 2017).

Research on plague management techniques has been ongoing within the Wildlife Health Program at Colorado Parks and Wildlife since 2008. That research has delivered in-house tools for early plague detection (Griffin et al. 2010) and demonstrated the benefits of prophylactic burrow dusting (dusting; Tripp et al. 2016). Strategies for field delivery of plague vaccine were developed (Tripp et al. 2014) and the field safety of recombinant vaccine was demonstrated (Tripp et al. 2015). Most recently, a comparison of the potential utility of dusting and vaccination in preventing prairie dog colony collapse in the face of epizootic plague was conducted (Tripp et al. 2017). Additional work focused on the development of a scaled-up vaccine bait production system (Corro et al. 2017) and assessment of antibody responses to commercial-source plague vaccine (*Yersinia Pestis* Vaccine, VLN: 188, PCN: 11Y2 R0,

Colorado Serum Company [referred to hereafter as: “plague vaccine”]; Cárdenas-Canales et al. 2017).

A fleet of prototype high pressure air (HPA) dusters were designed and built to increase reliability and precision while performing flea control. A Colorado Parks and Wildlife collaboration on the design and testing of prototype ATV mounted bait distribution equipment led to a new design increasing efficiency of vaccine bait distribution. Colorado Parks and Wildlife, Wildlife Health Program, will continue to refine and develop these and additional plague management tools as landscape level plague management programs are evaluated.

Current Plague Management Activities

Plague epizootics in prairie and shrub steppe ecosystems can negatively affect Colorado’s three prairie dog species, as well as other species of wildlife that depend on prairie dogs as prey or on landscapes modified by their activity. Understanding and controlling plague has emerged as a critical need for conserving prairie dog species and other native species of concern in Colorado.

A series of interconnected field studies in Colorado fostered development of practical approaches for limiting plague outbreaks in prairie dog colonies. As a result of that work, we now have tools for early plague detection as well as optimized approaches for annual burrow dusting of select colonies. Since 2010, Colorado Parks and Wildlife has treated 500–2,900 acres of occupied Gunnison’s prairie dog (*Cynomys gunnisoni*) habitat annually with beneficial effects on colony persistence in treated areas. To date, the majority of treated colonies have been stable or shown modest expansion since dusting began and only one has been lost to a plague outbreak.

In 2017, over 1,800 acres of occupied Gunnison’s prairie dog habitat within four Individual Population Areas (IPA – defined in Seglund and Schnurr 2010) were dusted. Also, >1,990 acres were treated with plague vaccine of which 250 acres were treated in conjunction with an ongoing field experiment to investigate the serological response of prairie dogs to vaccination. An additional >3,500 acres of black-tailed prairie dog (*Cynomys ludovicianus*) habitat at either black-footed ferret reintroduction sites or experimental research plots were treated with plague vaccine in 2017. Based on results from field studies (Rocke et al. 2017; Tripp et al. 2017) and an evaluation of cost efficiencies, plague vaccine will be used as the main tool for plague control

in the future in Colorado by Colorado Parks and Wildlife.

Annual plague management with plague vaccine will continue on identified prairie dog colonies on public and private lands where access permission for annual dusting has been granted. Plague vaccine distribution will follow established protocols. Periodic monitoring of the impacts of vaccination efforts will be conducted by mapping colony area, collecting opportunistic samples for plague surveillance and subjectively assessing prairie dog abundance using burrow activity as a proxy for counts.

In addition to ongoing work, additional colonies will be added to the program in important conservation areas by identifying suitable occupied habitat on public or private lands and working with responsible parties to establish access agreements for prairie dog conservation purposes. Continued work to develop new vector control and plague vaccine delivery tools to improve the efficacy and efficiency of plague management efforts and reduce costs will be conducted.

Our management objective for 2018 is to treat with plague vaccine and monitor at least 4,500 acres of occupied prairie dog habitat, with a long-range goal of at least 12,000 acres treated annually within the next 5

years. This long-term goal is in response to the potential for vaccinated colonies to expand in size and the need to manage plague on black-footed ferret recovery sites and Gunnison prairie dog habitat of conservation concern. For example, the prairie dog population in the Wolf Creek Wildlife Management Area in NW Colorado was severely reduced in size due to a plague epizootic in 2008. If vaccinating colonies suppresses plague mortality, then it is likely that the number of acres needing vaccine treatment will increase. These management goals are dependent on, and constrained by, available funding and staff time.

Plague Management Recommendations

Insecticidal Dusting

Colorado Parks and Wildlife research demonstrates that deltamethrin treatments may be more effective as a conservation tool when applied in advance of epizootic plague (Tripp et al. 2016). Reactive insecticide application (after discovery of carcasses or observations of diminished activity in prairie dog colonies) likely will be too late to be effective. Prairie dog social dynamics, burrow densities, precipitation frequency/intensity and soil type likely have direct and indirect impacts on dusting efficacy.

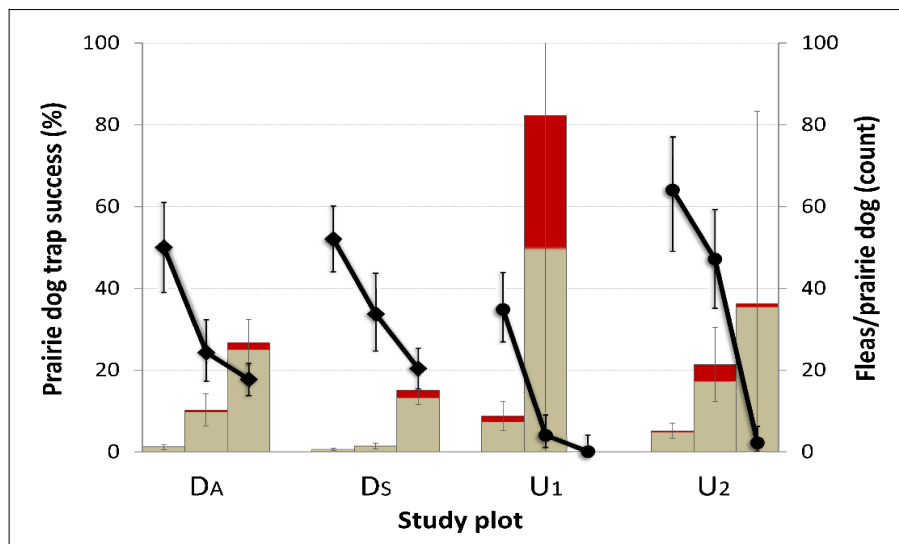


Figure 1. Mean white-tailed prairie dog (*Cynomys leucurus*) trap success (total captures/total trap days) on the individual study areas (black lines, left axis). Prairie dogs were captured in June, July and September 2009 in areas that received deltamethrin in autumn 2008 (DA) or spring 2009 (DS), and on adjacent undusted colonies (U1, U2). Mean flea abundance on captured prairie dogs is under-laid (bars, right axis); the portion of each bar shaded in red represents the overall proportion of flea pools (from prairie dogs or their burrows) PCR-positive for *Yersinia pestis* DNA at each plot-sampling point, shown as an index of relative plague activity. All error bars are 95% confidence intervals. Figure from: Tripp et al. 2016, Season of deltamethrin application affects flea and plague control in white tailed prairie dog colonies. *Journal of Wildlife Diseases* 52: 553–561.

Autumn dusting may offer advantages over spring applications in suppressing overwinter or early spring flea activity that may facilitate epizootic plague (Figure 1). Timing of dusting should be adjusted in hibernating species such that application precedes the late autumn seasonal decline in aboveground activity in order to assure sufficient dispersion of deltamethrin deeper into burrow chambers by prairie dogs. Large colony complexes or collections of occupied but fragmented habitat may benefit from a dusting strategy that includes

both spring and autumn dusting to provide maximum flea suppression for at least part of the complex year-round.

Dusting combined with oral vaccination (Tripp et al. 2017) may present the most effective, risk adverse and likely financially unsustainable option for comprehensive plague management to conserve prairie dog species (Tripp et al. 2017).

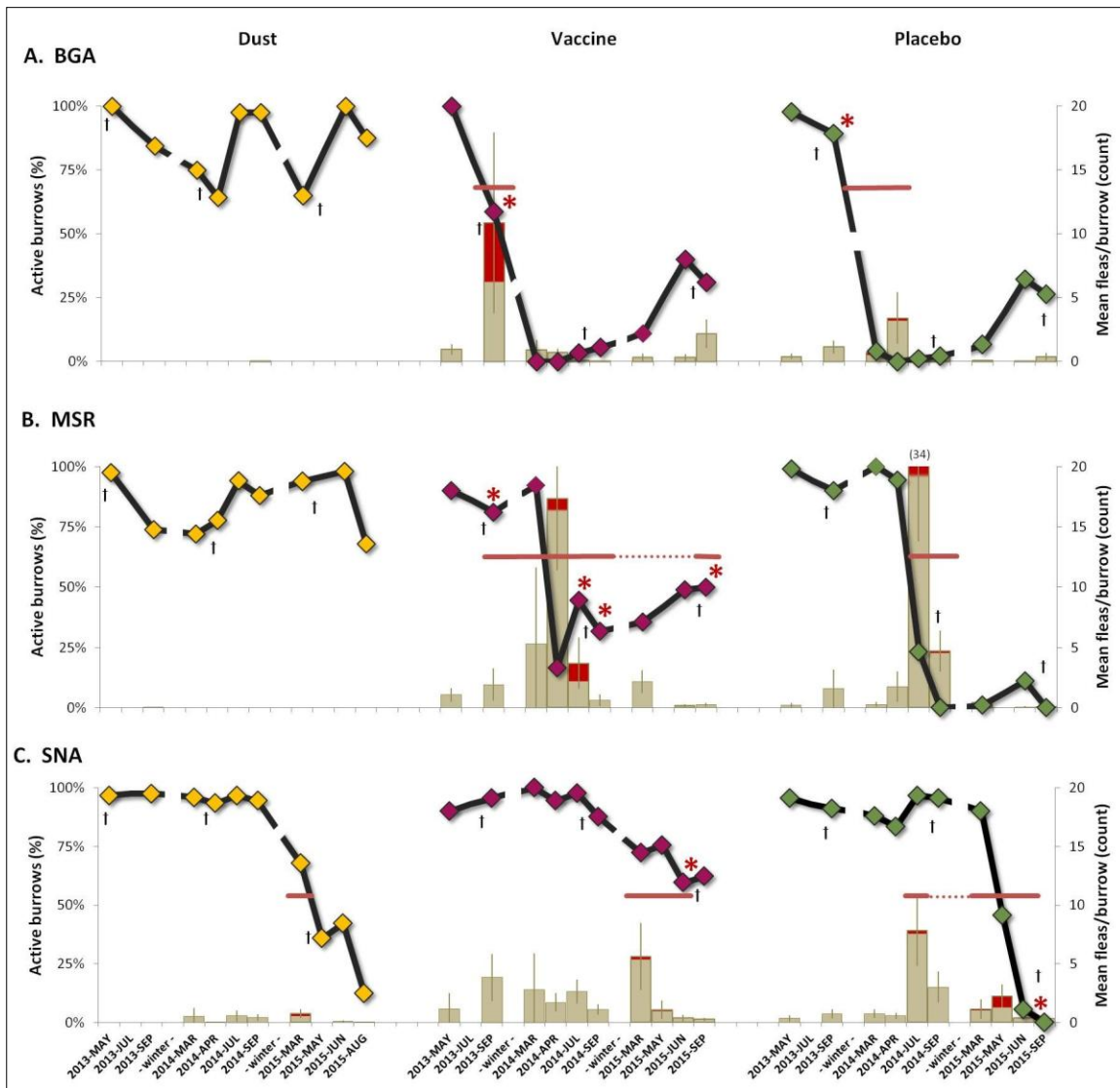


Figure 2. Black-tailed prairie dog (*Cynomys ludovicianus*) burrow activity (active burrows/total burrows scored) on the individual study areas (A. BGA, B. MSR, C. SNA; black lines, left axis). Fleas were collected from prairie dog burrows in May, July, and September 2013-2015 in areas that received deltamethrin dust, vaccine or placebo baits. Breaks in the lines represent overwinter periods when sampling was not conducted. Mean flea abundance in prairie dog burrows is shown (bars, right axis); the portion of each bar shaded in red represents the overall proportion of flea pools (from prairie dog burrows) positive for *Yersinia pestis* DNA at each plot-sampling point, shown as an index of relative plague activity. The duration of sustained plague activity (red horizontal bars) and sporadic plague activity (red dotted red lines) is shown. Sampling periods in which *Yersinia pestis* positive carcasses or on-host fleas were collected are shown as red asterisks. † represents the month of treatment application. All error bars are 95% confidence intervals. Figure from: Tripp et al. 2017, Annual burrow dusting or oral vaccination prevents plague-associated black-tailed prairie dog colony collapse. *EcoHealth*. 14:451-462.

Dusting is a superior plague management option when immediate flea suppression to impede plague transmission is necessary (Tripp et al. 2016, 2017). The plague management benefits of dusting are immediate and do not depend on additional applications to achieve maximum (or cumulative) efficacy. However, the efficacy of dust to suppress fleas begins to wane 8-10 months after application, and annual application is necessary for continued flea control (Tripp et al. 2016, 2017). Additionally, a once per year dusting schedule will likely result in a 2-4 month period when suppression wanes and fleas begin to rebound. If a seasonal peak in flea abundance and plague transmission (Tripp et al. 2009) coincides with a gap in effective flea control, an epizootic of plague is possible (Tripp et al. 2017).

The target dust application rate with DeltaDust® dust is 5g/burrow to achieve minimally effective flea control. However, users often overlook monitoring of the dusting equipment and the dust application. But systematic monitoring is important because many commercially available “dusters” become mechanically unreliable and their output rates are functionally unknown without continuous maintenance, calibration and output monitoring.

Vast acreages are often dusted to achieve effective plague mitigation (Griebel 2012; Tripp et al. 2016). The relatively short timeframe available to conduct dusting campaigns, combined with the tedious nature of the fieldwork and unpredictable weather can undermine the efficacy of flea control. Additionally, a <5 g/burrow dust output may not effectively control fleas. This incomplete flea control may be perceived as “resistance” to the insecticide and may contribute to conditions conducive to the potential development of such insecticide resistance.

Since 2008, CPW has dusted 500 to >2,900 acres of Gunnison’s prairie dog habitat annually with beneficial effects on colony persistence and growth in treated areas. Prairie dog populations have been stabilized and occupancy was maintained on dusted colonies, even in colonies where plague activity was not completely suppressed. The majority of dusted colonies have shown stable to modest expansion since dusting began (CPW unpublished data). These outcomes played a significant role in the US Fish & Wildlife Service’s recent decision to refrain from federal listing of the Gunnison’s prairie dog.

Plague Vaccine

Vaccination can reduce the impacts of epizootic plague on prairie dog apparent survival and abundance and prevent colony collapse when compared to untreated control plots (Figure 2; Tripp et al 2017). Although vaccination can have beneficial effects, complete protection from plague transmission and mortality has not been demonstrated.

Experimental and field data demonstrate incomplete protection from plague challenge in prairie dogs vaccinated only 30 days earlier (Rocke et al. 2014; Tripp et al. 2017). Therefore, a single application of oral vaccine during or in the days before a plague epizootic should not be expected to suppress plague activity or prevent widespread prairie dog mortality.

In one field experiment, some survivors of a plague epizootic had dispersed (1.3-km) away from the location of their original capture after two annual vaccine treatments, suggesting that as the colony and its underlying social structure collapsed, the survivors dispersed from their home coterries (Tripp et al. 2017). Dispersal or emigration of survivors also may partially account for the relatively low apparent survival observed at some study plots (Tripp et al. 2017).

Although protection was incomplete, recovery of this fragmented plague vaccine-treated colony was already underway one year later, while the adjacent control plot has remained unoccupied since collapsing. This was probably due to a small number of vaccinated prairie dogs surviving the epizootic on treated plots but not on control plots.

On one 40.5 hectare (ha) experimental plot in northern Colorado, two annual vaccinations preceding a plague epizootic appeared relatively effective in protecting adult prairie dogs. Activity, density and survival of adult prairie dogs remained stable during this time, but unvaccinated juveniles did not survive (Figure 2; Tripp et al. 2017).

Applying vaccine baits at rates of ≥ 65 /ha or ≥ 1 bait/active burrow in late summer and autumn, when uptake is likely to be higher (Tripp et al. 2014; Figure 3) and juvenile prairie dogs are more likely to become vaccinated (Tripp et al. 2014, 2015; Rocke et al. 2015), may also increase effectiveness of oral vaccination as a plague management tool.

Oral vaccination can protect some prairie dogs from plague and suppress epizootics provided application occurs well in advance of a plague outbreak. The benefits of annual vaccination, although less immediate than those of burrow dusting, accrue over time as multiple generations of prairie dogs are vaccinated. As the proportion of the population that is vaccinated increases, the potential protective effects of “herd immunity” may also accrue (Rocke et al. 2017). Taken together, these field experiments demonstrate that repeated annual vaccination of larger occupied areas may provide broader protection to prairie dog colony complexes.

In 2016 and 2017 plague vaccine was applied to larger areas (2,600–5,500 acres) of Gunnison’s and black-tailed prairie dog habitat as part of management and research programs. The expansion in treated acres builds upon the area experimentally vaccinated as part of the field evaluation of vaccine efficacy conducted in 2013–2015. While vaccination has not been shown to provide complete protection from plague, it does suppress transmission and facilitate more rapid post-epizootic recovery (Tripp et al. 2017). No adverse effects of vaccination have been observed and plague-impacted sites continue to recover while the vaccination program has grown (Tripp et al. 2017). Continued use,

monitoring and refinement of vaccination as a plague management tool is likely to suppress plague activity and stabilize prairie dog populations while helping to maintain colony occupancy.

Plague Management Strategies

Regardless of the specific strategies adopted (e.g., dusting or plague vaccine individually or in combination), treating small plots within larger colonies or small colonies within larger complexes appears unlikely to be effective in suppressing plague. As untreated colonies succumb to plague, infected fleas concentrate on remaining animals, which may overwhelm protection afforded by either vaccine or dust.

Annual management to mitigate plague and stabilize selected prairie dog populations for conservation purposes will be needed for the foreseeable future. Consequently, we encourage modifying plague management approaches to experimentally incorporate plague vaccine, dusting, streamline monitoring and compare preventive treatments in order to develop a sustainable adaptive framework for plague management in selected prairie dog habitats and black-footed ferret recovery areas.

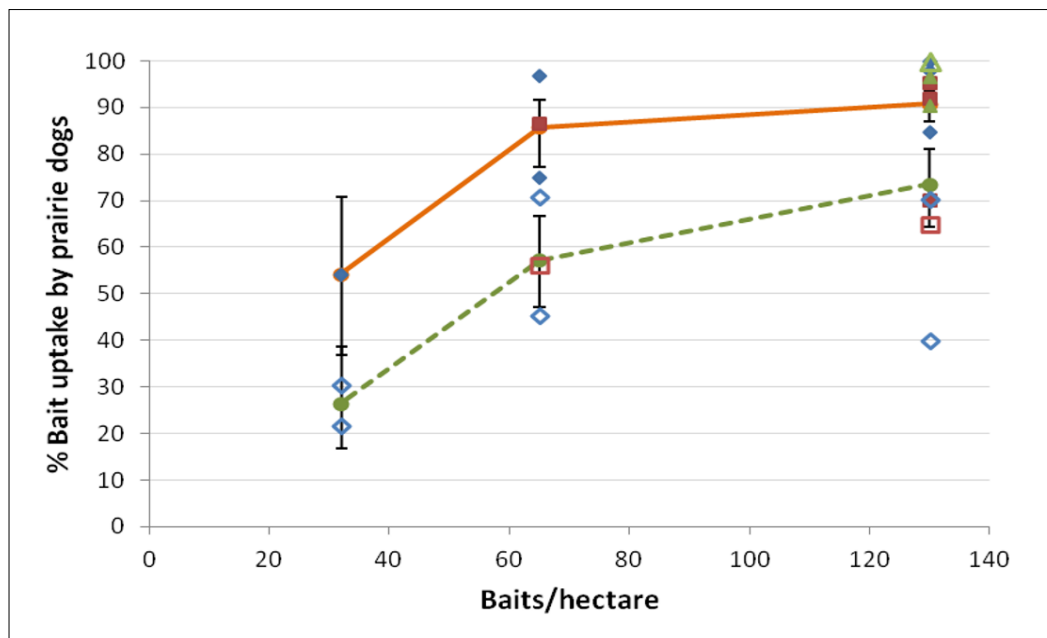


Figure 3. Bait uptake by three species of prairie dogs during spring and autumn and at three bait densities (32, 65,130 baits/ha). The trend lines (solid orange = fall, dashed green = spring) show the mean uptake at each bait density when trials are pooled across species. Error bars are 95% confidence intervals. Bait uptake during each trial is shown as symbols (black-tailed prairie dogs (*Cynomys ludovicianus*) = red squares, Gunnison’s (*C. gunnisoni*) = blue diamonds, and white-tailed (*C. leucurus*) = green triangles). Solid symbols represent trials conducted in the autumn; open symbols represent trials conducted in the spring. Figure from: Tripp et al. 2014, Season and application rates affect vaccine bait consumption by prairie dogs. *Journal of Wildlife Diseases* 50:224-234

Plague Vaccine Bait Manufacture

Plague vaccine bait is manufactured at Colorado Parks and Wildlife laboratories from a mixture of a bio-polymer matrix (Incortrix, FoodSource Lure Corporation, Birmingham, Alabama, USA), distilled water and an attractant (peanut butter).

This bait formulation allows: 1) live vaccine (standard dose = 5×10^7 plaque-forming units (pfu) of RCN-F1/V307) to be incorporated into the mix at a temperature that assures viability; 2) the vaccine bait mixture remains malleable; and 3) the vaccine remains viable through drying. Vaccine-laden bait made with this matrix can be shaped and sized according to target species because vaccine is distributed throughout the bait material (Corro et al. 2017).

This modified formulation substitutes FD&C Blue #1 food dye for rhodamine B biomarker (Fernandez and Rocke 2011) to decrease bait size and maintain palatability. This change sacrificed ability to bio-mark individual animals consuming bait. However, observation of blue-stained feces can confirm vaccine uptake by target species (Corro et al. 2017).

Experimental modifications to the bait formulation allowed creation of a mass production system for bait manufacture. A “carp bait” production machine (BoilieLab Machine, Midland Carp and UAB Topas LT, Lithuania) was adapted to make vaccine baits. These machines produce round, uniform baits of user-specified diameter, e.g., 14-mm diameter wet baits in 8-kg batches (Corro et al. 2017).

Manufacturing vaccine bait with BoilieLab machines is relatively efficient. It is anticipated that 100,000 baits/machine-week can be achieved (Corro et al. 2017). Labor savings and formulation modifications cut estimated per bait production costs by ~50%. Bait made as described have already been distributed in the field using all-terrain vehicle-mounted dispensers (“3-shooter”) and by the USFWS in an unmanned aerial distribution system (USFWS 2016). Mechanized distribution of bait in the field reduces costs by replacing ground delivery on foot in short-grass prairie habitats.

Plague Management Field Schedule and Guidelines

Six months prior to conducting plague management

Determine which plague management strategy will be employed in each Individual Population Area (IPA) or colony complex. Distribution of plague vaccine and insecticidal dusting for vector control are the primary management techniques currently available.

Review the list of potential plague management sites in the IPA that have been treated in the past or need to be treated and determine distribution across the landscape as well as land ownership status (Federal, State, State Land Board or Private). It is important to have a strong understanding of colony distribution and status in the IPA or colony complex for efficient scheduling and determination of which plague management technique to employ.

Contact federal, state and private landowner partners and determine the status of permissions for plague management activities at each site. Check with federal partners that all NEPA assessments, Pesticide Use Proposals (PUP) and Pesticide Applicator Licenses are current (if needed).

Three months prior to conducting plague management

Conduct a site visit to check the status of colonies. If a colony has changed significantly in size or if a colony has never received plague management before, generate an accurate colony perimeter to guide plague management activities.

If a colony has not changed in size, the dusting perimeters and bait distribution transects from the previous year may be used. Bait distribution transects and dusting perimeters have been created for all colonies, dusted or vaccinated, and can be provided upon request. If a new colony is identified or a new colony perimeter has been mapped, new bait transects will need to be created using ArcGIS.

Area Biologists should consult with Species Conservation Coordinators, Wildlife Health staff and Area Wildlife Managers to outline a draft schedule for plague management. It would be prudent to use fieldwork schedules from the previous year as a guideline when creating a draft schedule. For example, in the Gunnison Basin IPA in 2017, bait distribution occurred on 495 acres (17 colonies) over four days (8- to 10-hour days) with seven technicians. In the San Luis

Valley IPA in 2017, bait distribution occurred on 614 acres (15 colonies) over six days (8- to 10-hour days) with an average of four technicians.

It is important to keep land management agencies informed of the schedule as they may be able to provide assistance in the field and can advise if there are times when plague management fieldwork should be avoided (e.g., during heavy grazing or public use). Equipment and supplies needed for dusting are transported in a cargo trailer and can be difficult to organize quickly. Arrangements for the transport of dust and equipment will need to be made >1 month prior to the start of dusting. Plague vaccine bait needs to remain **frozen at all times** during transport, storage and until the time of distribution. It is important to establish access to freezer space near field sites when developing the schedule.

Two weeks prior to conducting plague management

Finalize all colony perimeters and create bait transects with the most current colony perimeters (if the perimeter has changed from the previous year). As new transects are created in ArcGIS they are given a default (non-sequential) name. If desired, rename new transects with BaseCamp® or MapSource® to maximize efficiency and eliminate potential confusion in the field.

Coordinate the transfer of equipment, dust and bait from the Wildlife Health Laboratory in Fort Collins to desired field sites. The Wildlife Health Laboratory will need a minimum of 10 days to package frozen bait and prepare it for field distribution. Keep the Wildlife Health Laboratory informed of any changes to the schedule or colony acres to be treated.

Using the GPX file with bait transects, copy and paste all transects into an excel file. Create an excel sheet outlining transect identification and the length of each transect (Figure 4). Use this file to divide walking distances among technicians and volunteers. The number of baits prescribed to the colony is determined by either multiplying the colony area, in acres, by 40 baits (GUPD/WTPD) or 50 baits (BTPD). Sum the length of all transects. Be aware that some transects may be longer than a kilometer, convert these numbers manually to meters. Divide the total transect distance (m) by the total number of baits to calculate the distance between which baits should be distributed.

Example #1: “Miller Ranch” = 35.8 acres × 40 baits/acre = 1,432 baits.

18,497 meters of total transect length ÷ 1,432 baits = 12.92 m between baits.

Example #2: “Powerline” = 66.7 acres × 40 baits/acre = 2,668 baits.

27,182 meters of total transect length ÷ 2,668 baits = 10.19 m between baits.

On the day before bait distribution

Divide the individual bait transects or dust perimeters among the available technicians and volunteers performing fieldwork. This should be divided using length of transects, some technicians may be given many short transects and some may be given fewer long transects.

In the INDIVIDUAL TRANSECT DISTANCE column in the excel sheet, divide “DISTANCE OF TRANSECT (m)” by DISTANCE BETWEEN BAITS. The result will be the number of baits distributed along each individual transect (Figure 4). After assigning a set of transects to each technician, sum and highlight the number of baits in each set to determine the number of baits each technician should be allocated. Example: BLM-18 = 26,171 total transect length in meters ÷ 7 technicians = 3,740 m and 370 baits per technician. Technician 1: Tracks 1-45, technician 2: 46-66, technician 3: 67-88, etc.

Some colony perimeters are divided into multiple subunits. It may be advantageous to print a map or display bait transects or dusting perimeters on a laptop to aid technicians in orientation. Upload all GPX files to as many GPS units as there are technicians. Always bring extra GPS units for unexpected volunteers. Also, bring a laptop with the upload GPX file as an additional back up. The Garmin GPS (model 62 or 64) units can only hold 200 named transects at a time. Depending on the colony size and number of colonies scheduled for bait distribution or dusting in a given day, downloading and reloading new transects from the laptop in the field may be required. Always have a supply of replacement AA batteries for GPS units. Always have nitrile gloves available for technicians and always wash hands after handling vaccine bait.

Field distribution of bait can begin when the GPS files and units are ready and bait is in a nearby freezer. Prior to bait distribution or dusting verify that the following tasks have been completed:


- Land management agencies and partners have been informed that vaccine bait is going to be distributed or dusting will occur in specific areas.
- All gate keys and lock combinations are available.
- Bait to be distributed that day should be removed from the freezer and placed in a cooler filled with ice packs on the morning of bait distribution. If dusting, all equipment should be organized and air tanks filled.

<i>Transect Name</i>	<i>Distance of Transect (m)</i>	<i>Baits per Transect</i>	Powerline		
001A	15	1	# Acres	# baits	total transect distances (m)
002A	140	14	66.7	2668	27182
003A	11	1			
004A	161	16		distance between baits	check (sum #baits)
005A	213	21		10.18815592	2668
006A	13	1			
007A	219	21		distance per person	
008A	270	27		3397.75	
009A	57	6			
010A	14	1			
011A	441	43			
012A	814	80			
013A	291	29			
014A	195	19			
015A	170	17			
016A	122	12			
017A	73	7			
					316

Figure 4. Example bait distribution Excel sheet.

- Technicians are scheduled to arrive at the first site as the sun is rising. All bait needs to be distributed before 2pm. The earlier the better
- Check weather conditions for the day. Do not distribute bait on days when the above ground activity of prairie dogs will be limited. Do not distribute bait on days when persistent precipitation, high wind (>25 mph), extreme heat (>30°C (86°F)) or cold (<7°C (45°F)) are forecasted. **Prairie dogs must be active and above ground on the day of bait distribution.**
- Dusting may occur in a broader range of weather conditions. Avoid dusting on days with persistent precipitation or in muddy conditions to limit potential damage to equipment and roads.
- Distribute GPS units and bait (or dusting equipment) that have been assigned to technicians and volunteers.
- Commence bait distribution or dusting.

The following data must be recorded and transferred to the Wildlife Health Laboratory:

- Track logs from all GPS units combined in a single file named with the date and colony location of bait distribution.
 (Example: 8.23.2017.Carpenter. Cimmeron.Gate)
- Site Visit data sheet
- Bait Distribution or Dust Output Log data sheet

Field Safety

Field Technician Plague Safety Precautions

General Precautions

Excerpted from www.CDC.gov/plague:

Workers who suddenly develop serious illness with fever should immediately seek medical attention. The attending physician should be informed that the investigator works with/around animals that might harbor plague (*Yersinia pestis*) or other zoonotic disease agents. The physician should contact local public health authorities promptly if *Yersinia pestis* associated illness is suspected. A blood sample should be obtained and forwarded to the state health department for *Yersinia pestis* antibody testing (CDC 2018).

Bubonic plague

Initial signs and symptoms of plague can be nonspecific and include fever, chills, headache, malaise, myalgia, nausea and prostration. Bubonic plague, the most common clinical form of the disease, usually presents with painful, swollen lymph nodes (buboes) that develop in the lymph nodes draining the site of the fleabite or other site where the bacteria initially gained entrance to the body. Illness onset for bubonic cases usually occurs 2-6 days after exposure to infectious fleas or other sources (CDC 2018).

Septicemic plague

Expect fever, chills, prostration, abdominal pain, shock and bleeding into skin and other organs. Virtually always fatal unless treated promptly with appropriate antimicrobials. Illness is likely to occur in 2-6 days (CDC 2018).

Pneumonic plague:

Expect fever, chills, cough and difficulty breathing, rapid shock and death if not treated early. Virtually always fatal unless treated promptly with appropriate antimicrobials. Illness onset for pneumonic plague usually occurs 1-3 days after exposure (CDC 2018).

Plague is normally maintained in certain rodent species and their fleas. Plague transmission to humans usually occurs through the bite of infectious rodent flea (Figure 5). Some cases also occur as a result of direct contact with infected animals (Figure 7) or inhalation of infectious respiratory droplets spread by persons or cats with plague pneumonia and cough (CDC 2018).



Figure 5. Prairie dog flea.

Safety Precautions for Reducing Risks of Fleabites

Wear appropriate clothing. For the best protection, wear long-sleeved shirts, full-length pants and boots. The less skin exposed, the lower the risk of a fleabite. Fleas that find themselves on the wrong hosts often will abandon such hosts relatively quickly. If fleas do not have ready access to host skin, they are more likely to jump off before biting. Treat clothing and exposed skin with an insect repellent containing *N,N*-Diethyl-meta-toluamide

(DEET, Figure 6). If used appropriately, DEET is a safe and effective repellent for rodent fleas.

When sampling rodent burrows with flannel attached to the end of a plumber's snake, place all collecting flannels (swabs) quickly into plastic bags for later processing in the laboratory. This will reduce the risk that fleas will hop from these materials onto workers. Zip top bags work very well for collecting swabs because they can be quickly sealed and are made of relatively tough plastic. Workers should notify their supervisors if they believe they have been bitten by fleas or have otherwise experienced a potential exposure to plague. Do not carry potentially flea-infested collecting material in the passenger compartment of field vehicles. (Note: This might include used flannel, flags, rodent traps, etc.)

Collection of Dead Animals

Follow appropriate precautions for reducing risk of fleabite, including use of repellents (Figure 6). A carcass should be collected as follows: Pick up the carcass with rubber or nitrile gloves and place it in a plastic bag contained within another plastic bag ("double-bag" the carcass). Seal the bag for transport to the laboratory.

Alternatively, the carcass can be collected as follows: Grasp the outside of a sturdy plastic bag at the middle point of the bag's bottom end (opposite of the opening). Roll the grasped bag up over the forearm so that it turns inside out (This forms a protective "sleeve" around the arm with the hand and forearm inside the inverted bag). Pick up the dead animal using the hand inside the inverted bag. Roll the bag back down the forearm and around the carcass until the hand is fully exposed and the bag is "right side in" (The bag will now contain the dead rodent, Figure 8). Tie a knot or otherwise seal the opening of the bag. Place the bag within another bag ("double-bag" the carcass) and seal that bag prior to transport of the specimen to the laboratory. Fill out a carcass submission form (in Appendix 1) and make sure to collect GPS waypoint (UTM coordinates). A full Carcass Collection protocol with more detailed instructions is below.



Figure 6. DEET spray provides topical protection from fleas and ticks.

Collecting Prairie Dog and Small Mammal Carcasses

Purpose

If a prairie dog or other small mammal (mouse, vole, chipmunk, ground squirrel or rabbit) carcass is found in the field, it should be collected for necropsy.

Materials

- GPS
- 2 large (gallon) zip top bags
- Submission form
- Cooler/ice packs/freezer
- Gloves

Methods

When conducting plague management and/or prairie dog surveys, zip top bags and all relevant forms should be kept in your vehicle and in your pack.

When a dead Prairie dog or other small mammal is found, (Figure 7), use a GPS to mark the location and write down the exact UTM coordinates on the submission form (in Appendix 1) as well as the collection bag. Fill out the submission form as completely as possible, being sure to note all important details. If a submission form is unavailable, write down the date, GPS location, name of collector and any relevant notes on the zip top bag. The laboratory cannot accept carcasses without this information.

With the wind to your back, use gloves to place the carcass in a zip top bag, then put the bag in a second zip top bag to double-bag it. If gloves are not available, fold a zip top bag inside out, use one of the corners to pick up the carcass before folding the bag right-side in over the carcass, then double-bag it. If zip top bags are not available, using a trash bag or other leak proof bag is acceptable. Perform the procedure quickly and use caution to avoid contact with fleas or bodily fluids (Figure 8).

If the carcass will be returned for necropsy the same day as collection, a cooler with ice packs will be sufficient to keep the carcass cool. If a cooler is unavailable, keep the carcass in the shade and as cool as possible until a cooler, freezer or other transport becomes available. If it is unknown when necropsy will take place or if it will be more than 24 hours, place the double-bagged carcass in a freezer and notify Wildlife Health Program staff that a carcass has been collected.



Figure 7. Prairie dog carcass.



Figure 8. Collection of a prairie dog carcass in the field.

Garmin GPS and ArcGIS Instructions

How to Use a Garmin GPS Unit

Purpose

Demonstrate useful functions and operations of Garmin GPS units in plague management.

Materials

- Garmin GPS unit (model 62 or 64)

Methods

To contact Garmin technical support staff call (913) 397-8200 or visit: <https://support.garmin.com/en-US/>

Understanding Buttons

The POWER button on the right side of the unit will turn the unit on and off and must be held down for several seconds. If the screen is too dark, adjust the backlight by clicking the POWER button one or more times until the desired illumination is reached, the battery life will also be displayed on this screen.



Figure 9. Garmin 64s GPS unit. A. Front of unit turned on and displaying the MAP window. B. Back of unit with USB port exposed.

This GPS unit (Figure 9) is displaying the MAP window. When in this window, the IN and OUT buttons will decrease or increase the map scale, respectively (zoom in and out). The PAGE button will cycle through the various available menus. Based on the window the user is in, the MENU button will allow the user to adjust various settings.

The ENTER button acts as a SELECT button or an OK button. The QUIT button will exit out of the current window. The QUIT button also cycles through the

menus in the opposite order as the PAGE button. The MARK button marks the current location of the GPS unit and a CREATE WAYPOINT prompt will appear. The FIND button allows the user to search and locate tracks, waypoints and routes that are saved to the GPS unit memory. The center toggle button allows the user to move left, right, up, or down through a menu page.

On the back of the unit under the dust guard, there are two ports. The port in the center is for an external antenna and will not be used. The other port is a micro USB port that is used when transferring data to or from the unit.

Turning on the GPS

Press and hold the power button on the side of the GPS unit for several seconds until the display lights up. The map screen should begin to display. It may take several seconds for the GPS to load. All tracks are listed by proximity, not alphabetically.

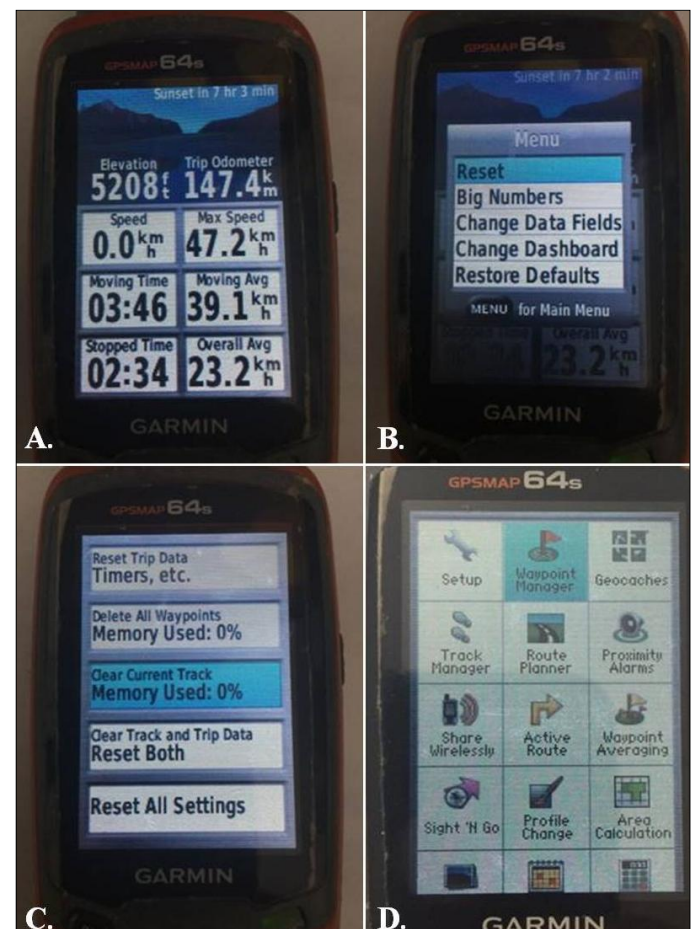


Figure 10. A. Trip computer window screen. B. Menu within trip computer. C. Reset menu to clear GPS data. D. Main menu with waypoint manager highlighted.

Turning tracks on

To turn tracks on (recording the path of the user), select the PAGE button until the main menu page is displayed, (Figure 10). Navigate to the Setup icon and click the ENTER button. Select the Track Log dropdown menu and then select RECORD, SHOW ON MAP (Figure 11). Tracks are now being recorded and the track will be displayed on the map window. To get back to the map window, select QUIT until the map window is displayed.

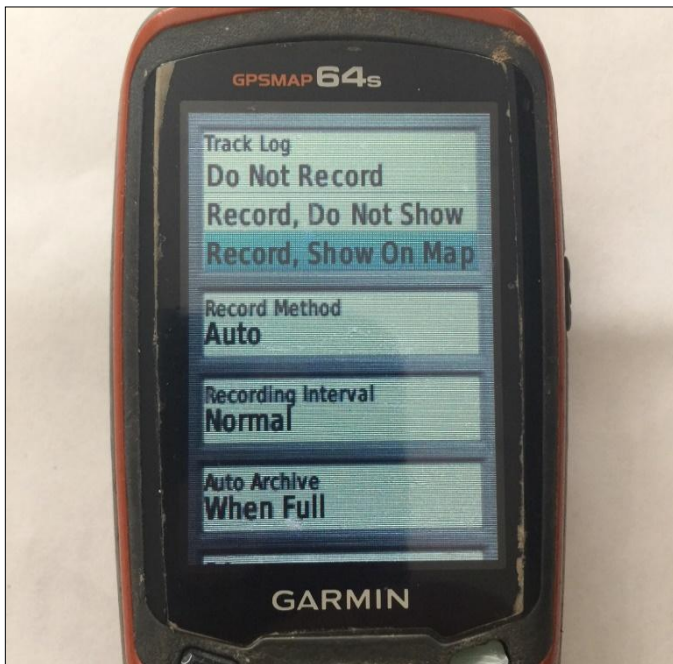


Figure 11. Recording Track Logs on Garmin 64s GPS.

Saving current tracks

To save and clear tracks navigate to the MAIN MENU window and select TRACK MANAGER. Select CURRENT TRACK and select SAVE TRACK. A default date and time will be displayed in the name field. Either use that default name or change the name to something descriptive. Select DONE and select YES when asked to clear current tracks and associated trip data.

Turning loaded transects on

Tracks loaded onto a GPS unit will not automatically display on the map window. To display tracks, navigate to the MAIN MENU page. Select the TRACK MANAGER icon and scroll down to the desired track. Select the ENTER button and scroll down to the SHOW ON MAP button and select ENTER again. The track will now display on the map window. Repeat these steps for all tracks that need to be displayed.

Clearing GPS Data

If all data has been copied and saved from a GPS unit to a computer, the data on the unit may be removed. To remove tracks first, navigate to the trip computer page, (Figure 10). Press the MENU button and select RESET using the ENTER button. Select ENTER and YES for delete all waypoints and clear current tracks (Figure 10). Now navigate to the Track Manager icon on the Main Menu page. Select the ENTER button and then select the MENU button. The option to Delete All Saved will be displayed. Select ENTER and select YES. All tracks will be deleted from the Track Manager page.

GPS Data Uploading from Computer

Purpose

GPX files may need to be uploaded from a laptop to one or more GPS units. When performing bait distribution, site visits, burrow swabbing, dusting, burrow counting and perimeter mapping, pre-loaded GPS data are used for orienting and organizing crews in the field.

Materials

- GPS units (model 62 or 64)
- Computer with MapSource® or BaseCamp® application
- Garmin cable

Methods

Start by uploading the desired GPX file in desired map software. This can be done in one of three ways. Drag the GPX file into the map window of MapSource® or BaseCamp®. The tracks and waypoints will load automatically. Alternatively, GPX files can be loaded in MapSource® by selecting the File dropdown menu, then clicking Open to navigate to the GPX file of interest. In BaseCamp® select FILE and IMPORT INTO and navigate to the GPX file. Ctrl + O will open the Windows Explorer where files can be selected in MapSource® only.

Once the file is imported into MapSource® or BaseCamp®, connect the GPS unit to the computer using a Garmin USB cord. The GPS unit does not have to be turned on. In MapSource® select the Transfer dropdown menu and select SEND TO DEVICE. The computer will scan for any GPS units connected to the computer. A window labeled “SEND TO DEVICE” will appear. If the Device text field is blank, then click FIND DEVICE (Figure 12). The computer will scan for any GPS units connected to the computer. The GPS unit will

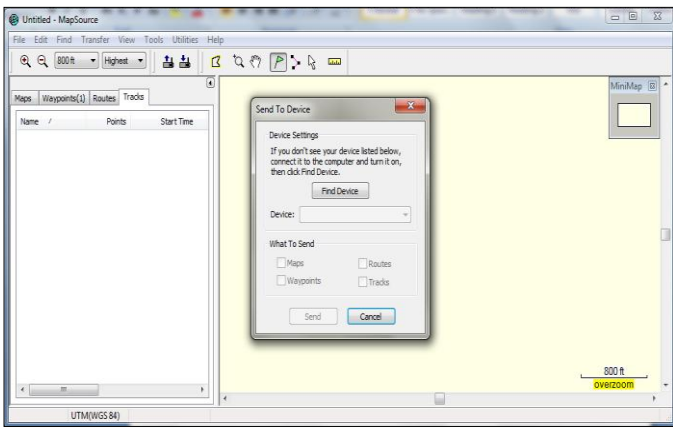


Figure 12. Send to Device Prompt window without GPS device recognition.

appear in the DEVICES: text field (Figure 13). If the GPS unit does not appear in the DEVICES: text field, unplug the GPS unit from the computer and reconnect the GPS unit. This should solve any connection issues. Connecting to a GPS unit may take several minutes and may depend on USB port configuration. In BaseCamp®, send the GPX file by selecting DEVICE then SEND TO DEVICE. Similar to MapSource®, BaseCamp® will take several seconds to load a GPS unit. When the GPS unit loads to BaseCamp®, select SEND TO DEVICE. Open a windows explorer folder and right click and EJECT the GPS unit.

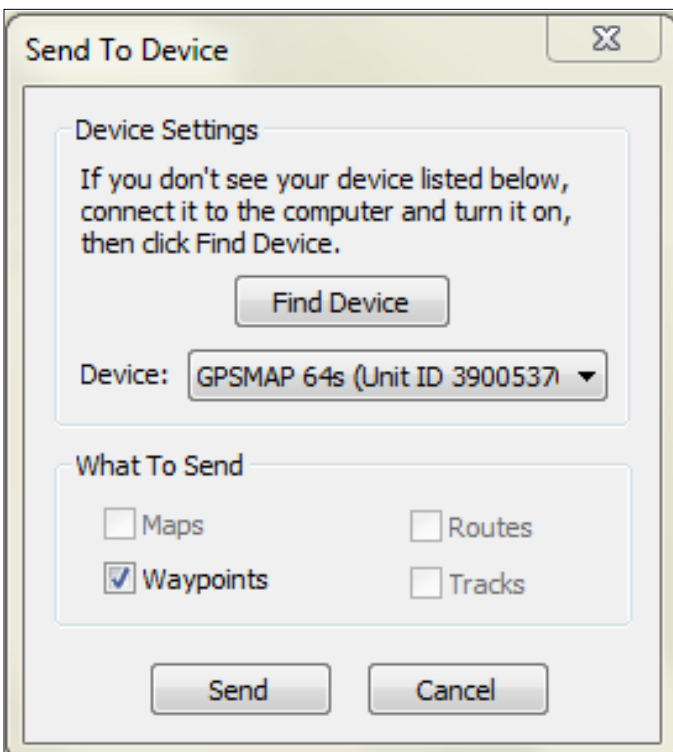


Figure 13. Send to Device prompt window with GPS device recognition.

When MapSource® finds a GPS unit; it will be displayed in the DEVICE: text box and look similar to Figure 13. Before clicking SEND, make sure the Waypoints and Tracks checkboxes are selected. Click send. A message that says “The data was successfully sent” should appear. The GPS unit must be properly ejected before unplugging it from the computer. Open the Windows Explorer folder and right click on the device on the left-hand side of the folder window and select Eject. Once successfully ejected, unplug the GPS unit and repeat the process for the remaining GPS units.

GPS Data Downloading to Computer

Purpose

Upon completion of a field task, it is important to copy and save all spatial data collected on the GPS units (waypoints & tracks). All data should be saved to a computer hard drive and to another back-up location such as an external drive or a Google Drive. The data must be copied to the correct location (folder) and labeled correctly.

Materials

- GPS units
- Computer
- Garmin cable

Methods

Collect all GPS units that need to be downloaded, identifying which unit each technician used that day. On the computer, navigate to the folder where the file will be saved. Example: “Google Drive/2016/Files From GPS”. Navigate to the Google Drive, the file for the correct year, then to the “Files From GPS” folder.

Make a new folder within the “Files From GPS” folder, (Figure 14). Name it with the date (April 7th 2016 would be entered as: 4.7.16), where the data was collected, the task(s) performed and the area(s) work was conducted.

In the folder created for the work completed that day, create a new folder for each technician with their initials and the GPS unit number (Figure 15).

To extract the data from the GPS unit, plug the GPS into the computer and navigate to the following folder Computer/LocalDisk(:C)/GarminGPSMAP/Garmin/GPX. The GPS unit acts as a standalone hard drive and does not need to be turned on before plugging it in. Copy and paste all the data files from the GPX folder into the folder for the corresponding technician and GPS unit (Figure 16).

Double check to make sure the data made it onto the computer. Then eject the GPS from the computer by clicking the “Safely Remove Hardware and Eject Media” icon and select the GPS unit and wait until a message saying “Safe to Remove Hardware” pops up before unplugging the GPS unit. Do not unplug the GPS without *EJECTING* it.

Once the GPS is safely ejected, the data can be deleted on the GPS so it is ready for new tracks, waypoints, etc. The GPS must be cleared in all of the following three ways:

- 1) Navigate to the ‘Trip Computer’ page in the GPS unit, then press MENU, select RESET, DELETE ALL WAYPOINTS
- 2) Navigate to the ‘Trip Computer’ page in the GPS unit, then press MENU, select RESET, CLEAR CURRENT TRACK
- 3) Navigate to the Main menu in the GPS Unit (hit MENU twice), go to TRACK MANAGER, select MENU, DELETE ALL SAVED

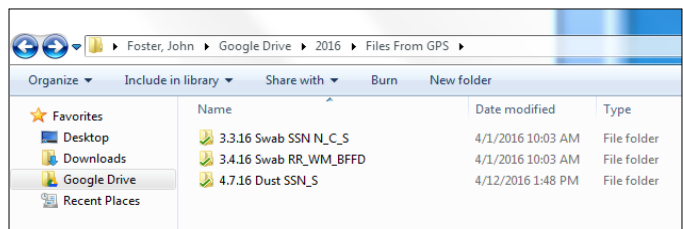


Figure 14. Example of file names and file saving locations.

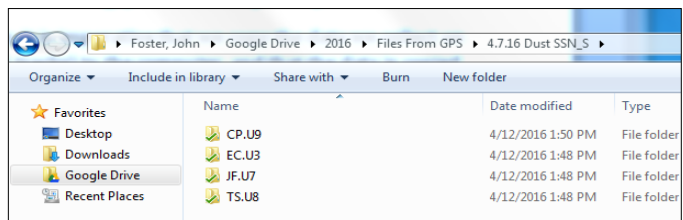


Figure 15. Example of individual folder set up for each technician’s GPS unit data.

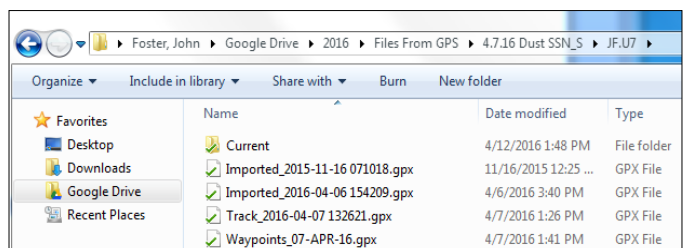


Figure 16. Example of downloaded GPX files from Garmin 64s GPS unit.

GPS Burrow Marking

Purpose

To mark burrow activity at colonies on GPS units for later analysis.

Materials

- GPS

Methods

Every burrow that is dusted needs to be marked on a GPS. Burrow classifications are placed in the ‘Note’ section of the waypoint page in the GPS. Please DO NOT RENAME THE WAYPOINT NUMBER. The waypoint numbers are used to know how many burrows each person dusts at a colony. To edit the ‘Note’ section is as follows: Select MARK->down arrow->down arrow->ENTER. Classify the burrow with A, B, C, 1, or K. Press Done (the screen will then be back at the main waypoint page). Press “Done” again (to mark and save the burrow waypoint). If a burrow was classified incorrectly: MENU->MENU->Waypoint Manager->[find the waypoint needing to be edited] -> ENTER->Note-> [change classification] ->DONE->QUIT. Navigate back to the ‘Map’ page on the GPS.

There are 5 ways to classify a burrow:

A: for an obviously active prairie dog burrow. Mark burrows with an ‘A’ if there is fresh scat (within 2 days; still dark in color and soft or moist - not sun bleached, brittle or crumbly when compressed), obvious fresh digging, prairie dog seen or heard at the burrow.

B: for an obviously inactive prairie dog burrow. Mark burrows with a ‘B’ if the opening is obstructed by vegetation or spider webs to the point where prairie dogs can’t freely move in or out, or the burrow is collapsing or obviously unusable.

C: for an unclassified prairie dog burrow. Mark burrows with a ‘C’ if activity cannot be determined

1: for ground squirrel burrows with a narrow diameter burrow opening and no raised mound.

K: for ‘other’ burrows, such as predators (coyote, fox, badger) or anything that cannot be classified as above.

Converting GPX to Shapefile or Shapefile to GPX using DNRGPS

Purpose

To convert shapefiles to GPX file format or GPX file formats to shapefiles.

Materials

- Computer with DNRGPS software application

Methods

DNRGPS is a software application that converts GPX file types (points, tracks, routes, etc.) into a shapefiles or KML file. DNRGPS can be downloaded from Minnesota’s Department of Natural Resources webpage (<http://www.dnr.state.mn.us/mis/gis/DNRGPS/DNRGPS.html>). Start by uploading the desired file into the DNRGPS application. Select the FILE drop down and navigate to LOAD FROM then select FILE (Figure 17).

Navigate to the desired file folder and select the file to be converted. If the file is not displayed, the file type may need to be changed. To do this, select the dropdown menu to the right of the FILE NAME text field (Figure 18). Select the file type for the file being converted from the dropdown menu, this should allow the file to appear in the window. Select the correct file and click OPEN. The tracks, waypoints and routes will load into the DNRGPS window.

To change the file type and location, select FILE, then SAVE TO, then FILE. This window prompts the user to specify the output location that the file will be directed to (Figure 19). Navigate to the desired location for the file and name the file descriptively. Change the SAVE AS TYPE dropdown to the desired file type and click SAVE. The newly created file will be saved in the above specified output location. Navigate to that folder to ensure the file has been created.

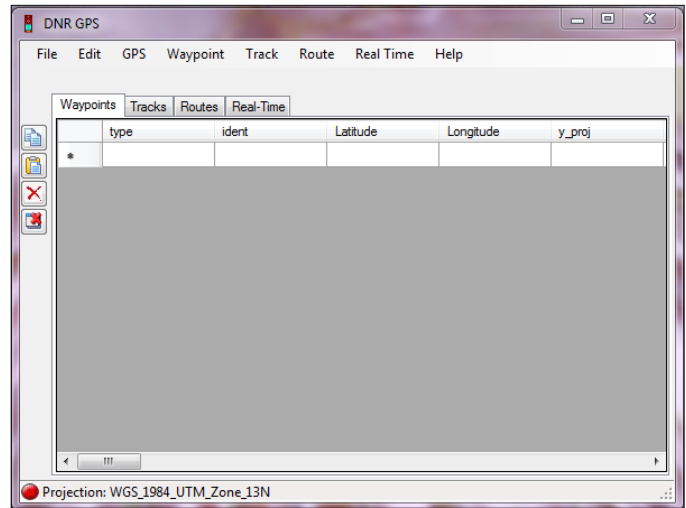


Figure 17. DNR GPS main window prompt.

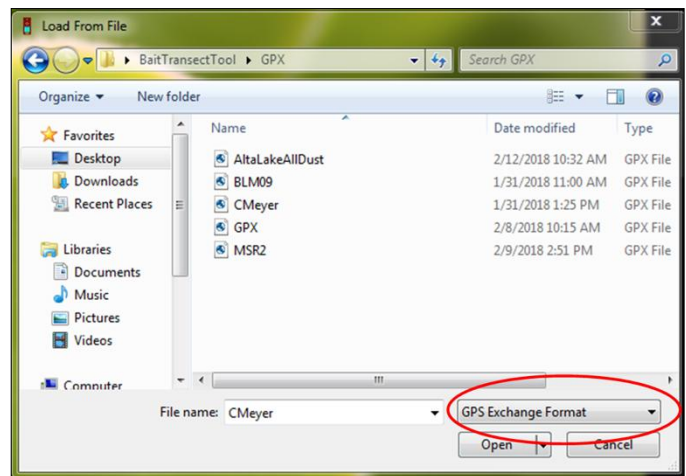


Figure 18. Load From File window. Red circle indicates where file formats can be changed.

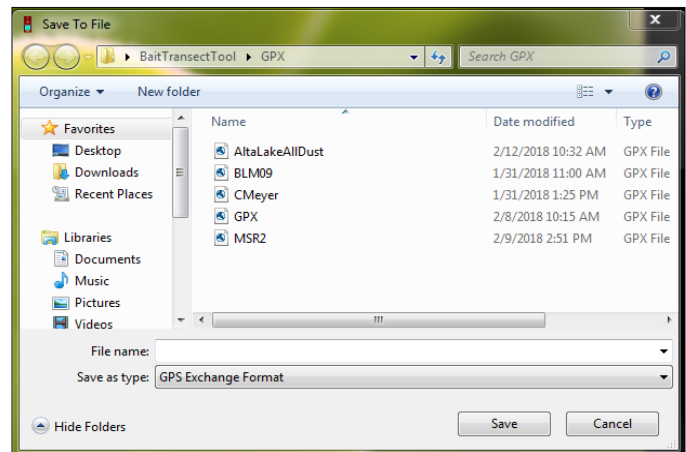


Figure 19. Save a file to a location and change Save as Type.

Creating Transects with ArcGIS Toolbox

Purpose

To automatically produce bait transects, grids, random points and perimeters; as well as to create shapefiles and GPX files.

Materials

- Computer with an advanced user ArcGIS license
- Zipped folder containing the bait transect toolbox

Methods

In order for this tool to execute, the user must have an advanced ArcGIS license. This tool does not function if the user does not have an advanced license. To check the status of the ArcGIS account, search for ArcGIS administrator within the Microsoft START search field and select ArcGIS Administrator. Click the DESKTOP folder icon and be sure the Advanced (ArcInfo) Concurrent Use license is selected.

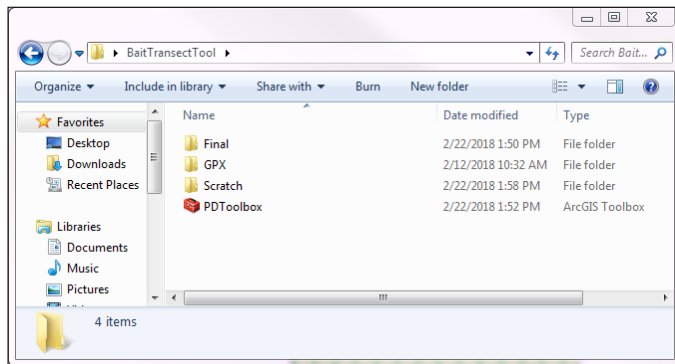


Figure 20. Contents of the BaitTransectTool file folder.

Unzip the BaitTransectTool folder to a convenient location on the computer. In the file, there are three folders and an ArcGIS Toolbox, Figure 20. It is recommended that this folder be unzipped into a hard drive location and not a temporary drive (such as a Cloud Drive, flash drive, etc.).

Copy all GPX or shapefiles of interest and paste them into the BaitTransectTool folder on the computer. Copy and paste GPX files into the GPX folder. If starting with a shapefile, copy and paste that file into the Scratch folder. All output files will be directed to the Final folder. These models have preset pathways and organizing files this way makes the management of files and input and output file locations easier.

Now, open ArcMap and select a blank map template. ArcMap must first connect to the unzipped BaitTransectTool folder. To connect to this folder, select the CATALOG tab on the right-hand side of the ArcMap window. Select the CONNECT TO FOLDER icon (📁) and navigate to the location where the BaitTransectTool is unzipped and click OK. The folder will appear under FOLDER CONNECTIONS in the catalog tab. View the folder contents by selecting the EXPAND icon (⊕) to the left of the folder name. Select that EXPAND icon to the left of the PDTToolbox.tbx folder. The twelve models should now be displayed (Figure 21).

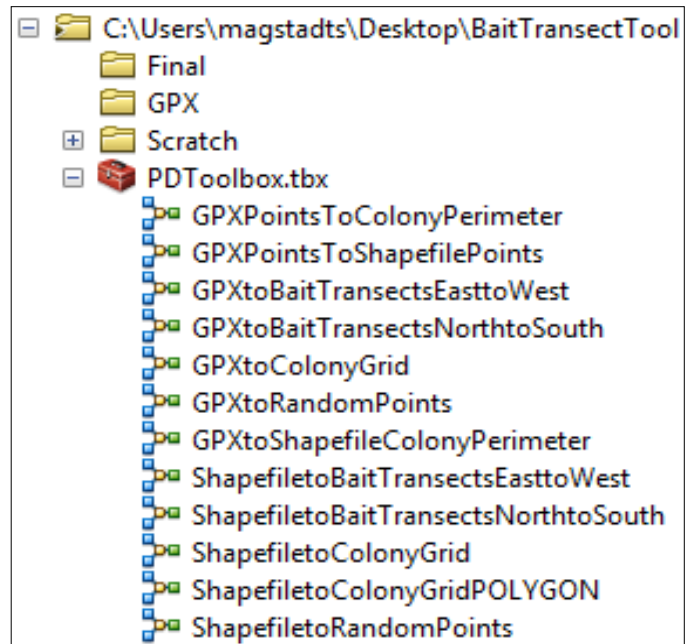


Figure 21. BaitTransectTool displayed in the Catalog window.

This toolbox contains models that convert input GPX files or shapefiles by outputting shapefiles. These tools can output grids, bait transects, colony perimeters, random waypoints and perimeter shapefiles from input GPX files or shapefiles. Decide which tool to use by referring to the table in (Figure 22).

Starting File	Ending Process	Recommended Tool
GPX file of a colony perimeter	Shapefile of colony perimeter	GPXtoShapefileColonyPerimeter
GPX file of a colony perimeter	Shapefile of an overlaid grid	GPXtoColonyGrid
GPX file of a colony perimeter	Shapefile of bait transects	GPXtoBaitTransectsEasttoWest
GPX file of a colony perimeter	Shapefile of bait transects	GPXtoBaitTransectsNorthtoSouth
GPX file of dusting points	Shapefile of colony perimeter	GPXPointsToColonyPerimeter
GPX file of dusting points	Point shapefile of dust points	GPXPointsToShapefilePoints
GPX file of a colony perimeter	Shapefile of Random Points	GPXtoRandomPoints
Shapefile of colony perimeter	Shapefile of an overlaid grid	ShapefiletoColonyGrid
Shapefile of colony perimeter	Shapefile of bait transects	ShapefiletoBaitTransectsEasttoWest
Shapefile of colony perimeter	Shapefile of bait transects	ShapefiletoBaitTransectsNorthtoSouth
Shapefile of colony perimeter	Shapefile of an overlaid grid	ShapefiletoColonyGridPOLYGON
Shapefile of colony perimeter	Shapefile of random points	ShapefiletoRandomPoints

Figure 22. Table of ArcGIS Tools, desired ending, processes and tool recommendations.

This toolbox contains several models that may not be useful. If creating bait transects for plague vaccine bait distribution, use either one of four models. If starting with a GPX file and creating bait transects that run east to west, then use the GPX to Bait Transects East to West model. If starting with a GPX file and creating bait transects that run north to south, use the GPXtoBaitTransectsNorthtoSouth model. If starting with a shapefile and creating bait transects that run east to west, then use the Shapefile to Bait Transects East to West model. If starting with a shapefile file and creating bait transects that run north to south, use the Shapefile to Bait Transects North to South model.

The GPXPointsToColonyPerimeter model is used to convert GPX points to a colony perimeter shapefile. When this model is run, the PointtoPolygon_Tbl.dbf will be created in the Scratch folder. To reuse this model, the PointtoPolygon_Tbl.dbf file must be deleted by right clicking and deleting the file in the ArcMap CATALOG pane.

Once the appropriate tool has been selected, open the tool by double clicking it. After several seconds a prompt window will appear (Figure 23). Directions for the tool can be viewed in the tool prompt window (Figure 23). Read the initial prompt. Instructions for each specific data field can be viewed by clicking on the desired data field. All fields must be completed for the tool to execute properly. All fields must be filled by selecting the FOLDER icon (📁) or by typing integers into the text fields that require a number. If there is confusion about specific data fields, refer to the specific data field directions, on the right side of the prompt window.

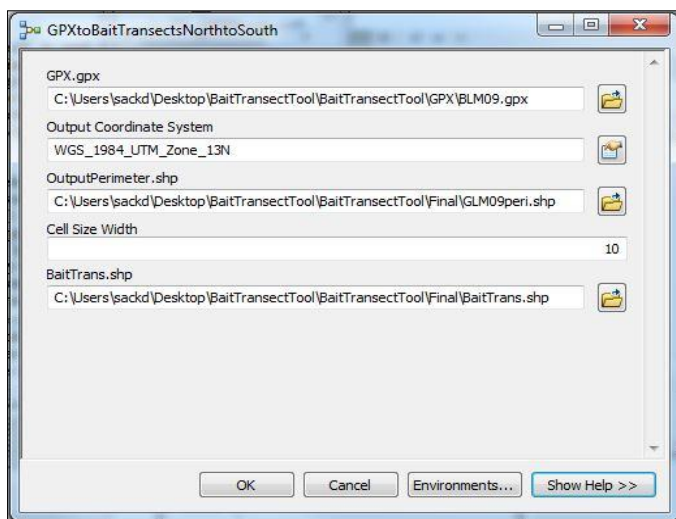


Figure 23. An example of an ArcGIS tool prompt window.

When all required data fields are filled, select OK and wait for the tool to process. If the tool does not automatically display the output files, navigate to the output file location in the Catalog tab and manually drag and drop the files into the ArcMap data frame window. When the output shapefiles have been created, they can be converted to GPX files by using DNRGPS.

Creating Transects Manually

Purpose

To create bait distribution transects to be used in the field, using a GPX file or shapefile of a colony perimeter.

Materials

- Computer with ArcMap and an advanced user ArcGIS license

Methods

Start by saving the GPX or shapefile to a convenient folder. It is recommended that this folder be unzipped into a hard drive location and not a temporary drive (such as a Cloud Drive, flash drive, etc.). If starting with a GPX file convert it to a shapefile. This is done using the program DNRGPS.

If starting with a shapefile, then the conversion using DNRGPS is not necessary. When opening up ArcMap, the window (Figure 24) will open.

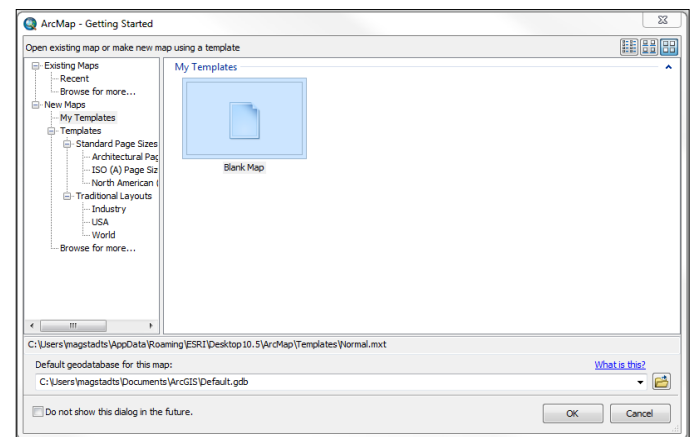


Figure 24. ArcGIS window to select a Blank Map Template.

Select MY TEMPLATES and select BLANK MAP and click OK. In the top left corner of the ArcMap window select FILE then click MAP DOCUMENT PROPERTIES (Figure 25). Make sure the STORE RELATIVE PATHNAMES TO DATA SOURCES box is checked. Click APPLY and then OK. Now view the shapefile.

To view the shapefile, select the CONNECT TO FOLDER ICON (). Navigate to the folder containing the colony perimeter shapefile and select it to view its contents. Open the CATALOG tab on the right hand side of the ArcMap window and navigate to the folder with the desired shapefile. Once connected to the folder, its contents can be viewed. Click and drag the shapefile to the TABLE OF CONTENTS pane on the left side of the ArcMap window (Figure 26).

The shapefile in the TABLE OF CONTENTS pane should be visible and the colony perimeter should appear in the data frame window. Click the SEARCH tab and

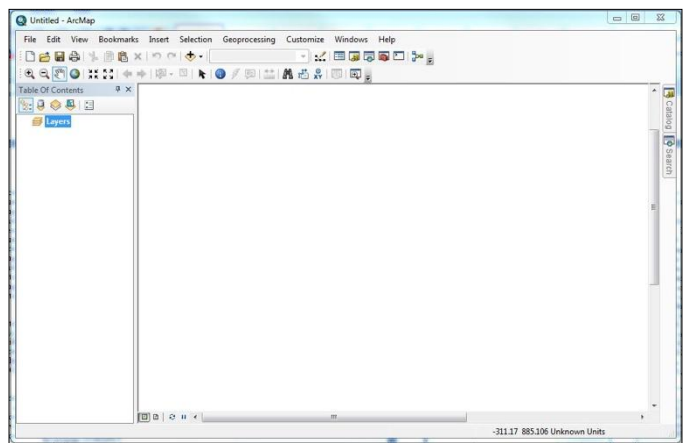


Figure 25. Blank ArcMap template window.

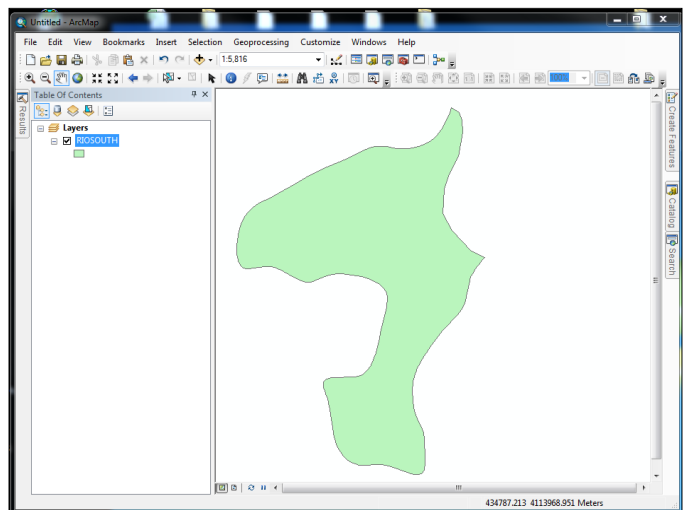


Figure 26. ArcMap window with only a colony perimeter shapefile.

search for a tool called Fishnet and click on that tool. The window should be set up similar to the (Figure 27).

Click the FOLDER icon () and save the OUTPUT FEATURE CLASS to a recognizable location. The TEMPLATE EXTENT should be set to the colony perimeter shapefile being used. Both the CELL SIZE WIDTH and CELL SIZE HEIGHT should be specified to 10, if the desired distance between transects is 10 meters. Make sure CREATE LABEL POINTS is unchecked and click OK. A FISHNET layer is then created and will appear covering the extent of the colony perimeter. Start editing the fishnet layer and delete either the vertical or horizontal lines. Under the TABLE OF CONTENTS, right click on the fishnet layer that was created and select EDIT FEATURES and select START EDITING. Right click again on the fishnet layer and select OPEN ATTRIBUTES TABLE. Select either horizontal or vertical lines. Items selected will be deleted (Figure 28).

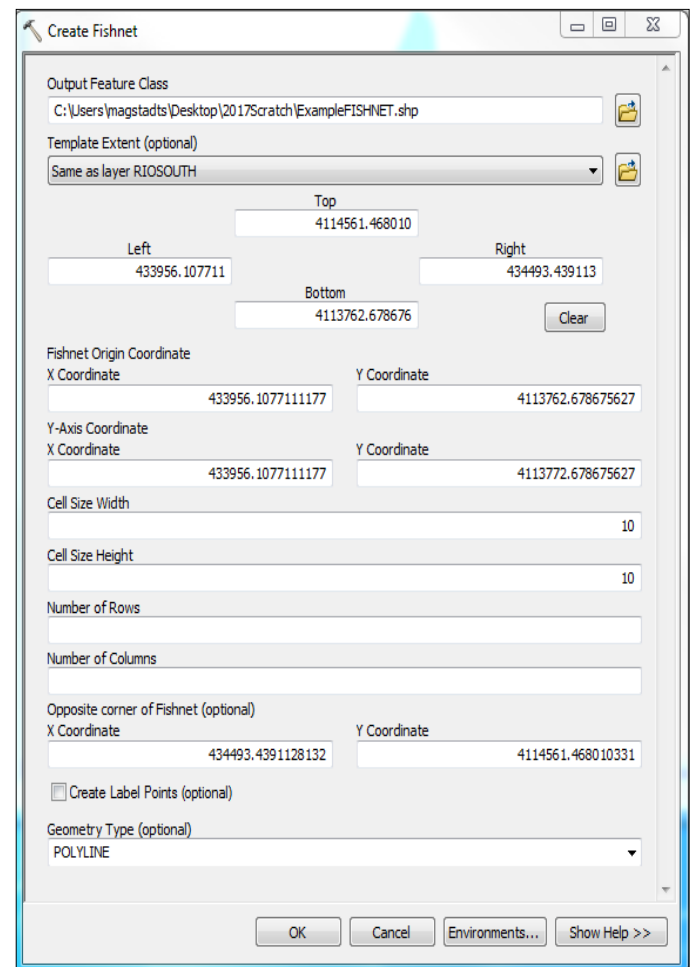


Figure 27. Create fishnet tool. Leave Number of Rows and Number of Columns blank and uncheck Create Label Points. Geometry Type must be polyline.

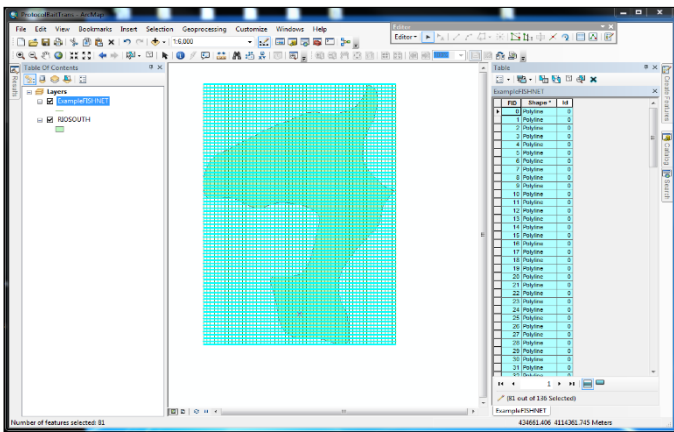


Figure 28. Fishnet with transects running in both x, y directions.

In the example used, all horizontal lines were selected by clicking and holding the box next to the FID column and dragging down until all horizontal lines were highlighted blue. To delete the blue lines that were selected, click the X symbol and observe that the lines selected were deleted. Now only vertical lines will be displayed. Click the EDITOR dropdown menu and select SAVE EDITS. Click the EDITOR dropdown menu and select STOP EDITING (Figure 29). Now clip the fishnet to the perimeter layer. To do this, select the GEOPROCESSING tab and click CLIP (Figure 30).

The INPUT FEATURES should be specified to the clipped fishnet layer that was created in the previous step, Figure 30. The CLIP FEATURES should be specified as the colony perimeter shapefile. The OUTPUT FEATURE CLASS specifies the name and file location for the newly created clipped layer. Select the FOLDER icon (📁) and save the file to the same recognizable working folder. Make sure to deselect the original fishnet layer in the table of contents.

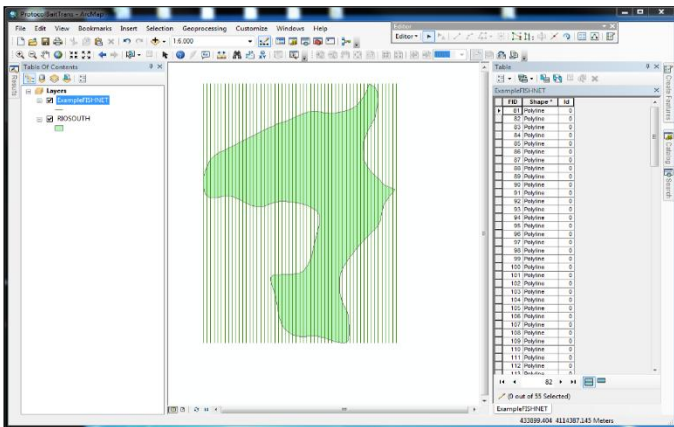


Figure 29. Unclipped fishnet over colony shapefile. Transects running east to west are deleted.

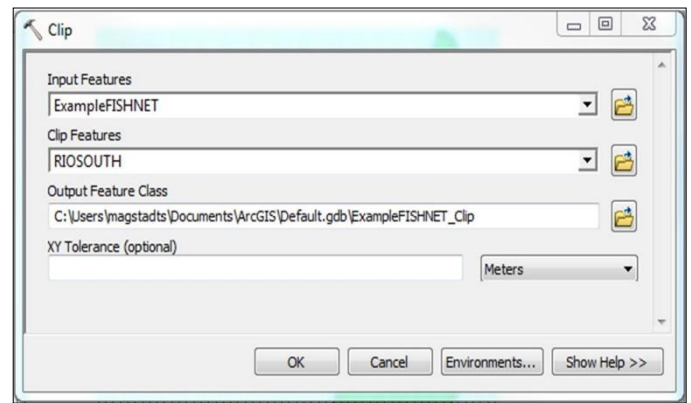


Figure 30. Clip tool menu. INPUT FEATURES is the bait transect shapefile and the clip feature is the colony perimeter shapefile. OUTPUT FEATURE CLASS is the location that the clip feature will be saved to.

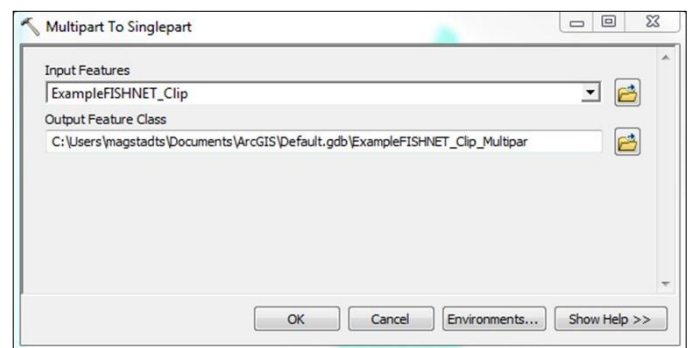


Figure 31. Multipart to Singlepart window prompt. Input and output file locations can be selected by clicking the folder icon.

In the SEARCH tab, type “Multipart to Singlepart” and select that tool. The INPUT FEATURES should be set to the clipped fishnet layer just created (Figure 31). The OUTPUT FEATURE CLASS is the final shapefile layer that will be created and used for bait transects (Figure 32). Click the FOLDER icon (📁) to name and save the file. The shapefile generated is the final layer used to

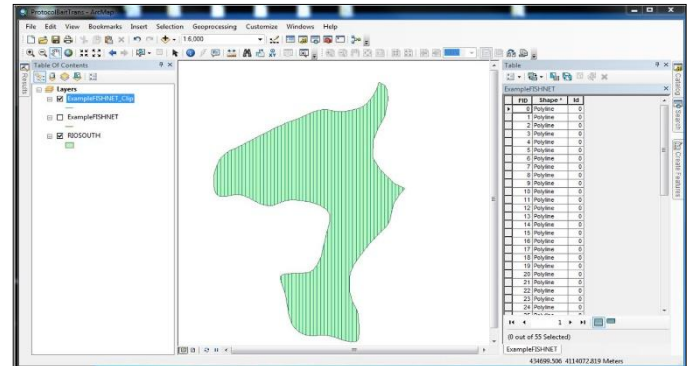


Figure 32. ArcMap window displaying completed bait distribution transect shapefiles and a colony perimeter. These files will be imported into DNRGPS to be converted into GPX files for use on a GPS.

create a GPX file for use on a GPS. The final layer should be converted to a GPX file format using DNRGPS software application.

When a new set of transects is created in ArcMap, the transect names are generated in a non-sequential manner. This default naming system can be spatially confusing when many (>2) technicians are working on the same colony. To limit this confusion, transects are renamed in sequential order to organize and simplify work in the field. Renaming primarily occurs when bait is scheduled to be manually distributed (on-foot) or when burrow counts will be conducted along transects. The default naming system is generally retained for ATV bait distribution or for small colonies with <10 transects.

The Garmin 64s GPS units can hold up to 200 uniquely named transects. To work around this limitation, the “join” tool may be used in MapSource® to connect several transects into a single long transect with a unique name. This function is particularly helpful for ATV distribution on large acreages with many transects. Joining transects also simplifies operation by eliminating the need to “turn on” multiple transects on the GPS unit. The most efficient way to rename transects is to open the transect GPX file with MapSource® or BaseCamp® to view the colony perimeter and all transects. Start at one side of the colony and rename all transects sequentially (Example, East to West), manually entering a new name for each transect. Colonies are not typically uniform in shape.

Be careful while working through the transect list to ensure that no transect is missed. Each new transect name should be unique (Example, A167, A168, A169, A170... etc.). Unique transect names help avoid conflict when colony files are combined for fieldwork. Although renaming transects is time consuming the benefits are more organized and efficient bait distribution in the field.

Prairie Dog Colony and Burrow Monitoring

Prairie Dog Colony Perimeter Mapping

Purpose

To create or update a colony perimeter map that outlines the active area of a prairie dog colony.

Materials

- GPS units (Garmin model 62 or 64)
- Computer with MapSource® or BaseCamp® application and ArcMap
- Garmin cable
- Pin-flags
- Binoculars

Methods

The goal is to create a perimeter map that outlines the active colony area. Perimeter maps are used to track changes in colony area and are necessary to guide the prescription and application of plague management. The colony perimeter is defined by tracing an outline around the outermost active burrows of a colony. The vegetation clip line (where taller vegetation has been clipped by prairie dogs) can serve as a visual guide to locate current prairie dog activity and to aid the mapping process. However, only active burrows should be used to determine and map the colony perimeter.

Active burrows have fresh prairie dog scat within 1 meter of the opening and/or signs of fresh digging. Active burrows are generally not overgrown with vegetation or occluded with debris or spider webs (see Figure 33 and Figure 34 in Prairie Dog Colony Site Visit section).

Prairie dog colony perimeter mapping may be conducted from an ATV or on foot. Mapping should be conducted with a quality GPS unit (Garmin 64). Create a new “track log” to record the travel path around the outline of active burrows. While creating a new “track log”, record GPS waypoints at active burrows around the perimeter as they are encountered. These points are used during post processing to correct and finalize polygons. Marking waypoints at active burrows allows the mapper to explore and investigate the area and burrows along the perimeter. Avoid including single outlier burrows that are ≥ 50 meters from the mapped perimeter or that are obviously not part to the main colony. Including outlier burrows will inflate the mapped colony area. Also, avoid mapping areas of unoccupied habitat (dense shrubs,

rocky areas, drainages, hardscape) within the boundary of the colony. If necessary, map these unoccupied areas separately for later exclusion during data processing. Mapping should be conducted when the prairie dog species is active (not hibernating) and when tall vegetation will not obstruct the view of the colony. Ideally, mapping will occur during the same time (season) each year.

A previous out-of-date colony perimeter may be used as a guide while checking or updating perimeters. Mapping can be especially difficult in the 1-2 years following a plague epizootic when prairie dog colonies can be fragmented and patchy with burrow activity that is difficult to determine accurately. When mapping a new colony or updating a perimeter after a plague epizootic it is often necessary to conduct a site visit (see Prairie Dog Colony Site Visit) to observe and mark areas of activity. If the colony perimeter is difficult to determine use pin-flags or GPS waypoints to mark active burrows on the outer edge of the colony. After conducting a site visit and marking active burrows, a “track log” can then be created while traveling along the outline of these marked burrows. After mapping the data will be exported to ArcGIS for processing, correction and area calculation (see Garmin GPS and ArcGIS Instructions section). Additional colony mapping guidelines and details are available if needed (Biggins et al. 1993; 2006; Plumb et al. 2000; USFWS 2016).

Prairie Dog Colony Site Visit

Purpose

To quickly monitor colony status through visual observation of prairie dog relative abundance, distribution and activity in an efficient manner and provide a consistent and accurate method to record repeated observations of colony status, colony perimeter and occupancy.

Materials

- GPS with colony boundary loaded
- Binoculars
- Datasheets, clipboard and pencil
- Kestrel or other temperature and wind recorder

Methods

Observation

Site visits may be conducted during any portion of the day as long as the weather is appropriate for observation of prairie dog activity. Avoid conducting surveys between December – February in black-tailed prairie dog habitat and October – April in Gunnison’s and white-tailed prairie dog habitat. Observations conducted during poor weather (high winds, excessive heat/cold and precipitation) can artificially affect the assessment of colony activity. Vegetation (tall grass or shrubs) may also negatively influence the visibility of prairie dogs. However, the observation data is still valuable as a record of occupancy and they provide a colony status report, which can help to inform management decisions.

Once the colonies that will be visited have been determined, use a GPS with preloaded perimeters to navigate to the colony. Use the site’s natural topography when choosing where to begin the visit. Take advantage of the ridgelines and areas of higher elevation to observe prairie dog activity with minimal disturbance due to your presence.

While approaching the colony in a vehicle or on foot, move slowly and stop before you alert prairie dogs to your presence. Use a GPS unit to monitor your position and to remain undetected. Ideally, an initial assessment of above ground activity can be conducted before startling the animals. It is important to remain quiet and have the windows open (if in a vehicle) to help hear prairie dog calls. Without leaving the vehicle (if not on foot), begin conducting a thorough observation of the entire site. Use binoculars to scan the entire site counting all the animals observed.

Move slowly around and throughout the site (on foot or in a vehicle depending on site-specific circumstances) observing prairie dogs at regular intervals (every 100 m depending on the colony area). As the prairie dogs become aware of your presence, they will begin to quickly move to their burrows and begin alarm calling. The most productive time to observe and count is usually when the prairie dogs run to their burrows. Counts will quickly diminish as the prairie dogs move below ground. Conducting site visit observations from a vehicle is often more productive than on foot. The vehicle is less startling than a person on foot and the prairie dogs will remain alert but remain aboveground longer when observed from a vehicle. Prairie dogs on colonies where shooting is common are likely to move below ground more quickly. In these cases, observers must be ready to conduct counts before all prairie dogs disappear below ground.

After completion of the above observation method, the colony may now be entered on foot for a more precise observation of burrow activity. Begin walking through the colony investigating areas where animals were observed or areas where activity was reduced. Bring binoculars and use the GPS to aid in finding areas that may have been obstructed from view during the initial observation. It is also beneficial to use the GPS to record your tracks and take waypoints at areas of interest to guide follow up site visits. While on foot, observe the area around the burrow entrance for signs of activity (fresh scat, fresh digging, spider webs, etc.), listen for prairie dog calls and watch for visual confirmation of occupancy.

Record Data

After completing a thorough observation and walk through of the colony, return to the vehicle to begin recording observations on the provided data sheet in Appendix 1. Date, start time, end time, observers’ initials and contact are important to note at this time. In addition to noting the site/colony ID, it is also necessary to provide a UTM coordinate of the locations where the observations were conducted. Different agencies may use different colony names so this information will help to ensure that the correct area was assessed. Accurate weather information is also needed. Recordings should include temperature, wind, cloud cover (estimated percentage of the sky with clouds) and if there was any kind of precipitation within the past 24 hours. The next step is to provide as much detail as possible about what was observed while on the colony. If there were multiple crewmembers in the area, this section should be completed jointly. Most importantly, were prairie dogs

observed above ground? Were prairie dog calls heard? Depending on the time of year, there could be juveniles present. If young can be distinguished from adults, the number of juveniles observed should be differentiated.

Visual cues may not always lead to an accurate assessment of the colony status when prairie dog density is low. Therefore, burrow appearance should also be assessed. Signs of activity at the burrow can include fresh digging and fresh scat (Figure 33 and Figure 34).

Subjective measures for burrow density can be scored on a scale from 0 to 4. Density can vary throughout the colony and consist of very dense pockets of burrows scattered around a vast area of unoccupied grassland. Make the best subjective assessment possible. A short description of observations with your ranking is required.



Figure 33. Fresh digging and fresh scat near a prairie dog burrow.



Figure 34. Comparison of fresh and old scat outside an active prairie dog burrow.

- 0 indicates that 0-10% of the surveyed area has visible prairie dog active or inactive burrows (e.g., burrows have collapsed or are indistinguishable from surrounding unoccupied habitat).
- 1 indicates that 25% of the area surveyed has visible prairie dog burrows.
- 2 indicates that 50% of the area surveyed has visible prairie dog burrows.
- 3 indicates that 75% of the area surveyed has visible prairie dog burrows.
- 4 indicates that 90% to 100% of the area surveyed has visible active or inactive prairie dog burrows that are located throughout the colony and at high density (e.g., burrows fill the colony area with few unoccupied patches).

Overall colony activity uses a similar grading scale. Colony activity can vary throughout the colony and consist of very dense pockets of activity scattered around a vast area of unoccupied grassland. Make the best subjective assessment possible. A short description of observations with your ranking is required.

- 0 indicates that 0-10% of the burrows appear to be active or the site is believed to be unoccupied (e.g., no prairie dogs seen or heard with no burrows appearing active).
- 1 indicates that 25% of the area surveyed has visible prairie dogs or active burrows.
- 2 indicates that 50% of the area surveyed has visible prairie dogs or active burrows.
- 3 indicates that 75% of the area surveyed has visible prairie dogs or active burrows.
- 4 indicates that 90% to 100% of the area surveyed has visible prairie dogs or active burrows located throughout the colony (e.g., colony is thriving and prairie dogs are seen at nearly every burrow and nearly all burrows appear active).

Additional comments and a short, legible, description should also be recorded to aid with the overall assessment of the colony. Completing a site visit should not require a lot of time. From start to finish this whole process should not take more than 30-60 minutes and should be completed every time the colony is visited. The site visit data sheet should also be filled out when visiting the colony for other purposes such as burrow swabbing, bait distribution, or other purposes.

Burrow Activity Survey

Purpose

To efficiently monitor colony status through visual observation of prairie dog burrows providing a consistent and accurate method to record repeated observations of colony status and occupancy.

Materials

- GPS with colony information loaded
- Data sheet, clipboard and pencil

Methods

After conducting visual observations, a more detailed walk-thru assessment of burrow activity should be conducted. A GPS pre-loaded with the colony perimeter and 5-10 spatially distributed random points located within the perimeter should guide the burrow activity assessment. The area of the colony will dictate the number of points. Divide the points among the available crew.

Navigate to the designated random points and select 10 individual and visually prominent burrows to score for activity. After scoring a burrow, visually select the next burrow (from a distance >5 meters away and before observing the “condition” of the burrow). Try to limit bias toward “active burrows”.

Navigating in a “rough circle” or in random directions around the random point (within 50 meters) are efficient methods. Mark a waypoint on the GPS at the location of each selected burrow. Record each waypoint ID on the data sheet. Each of the selected burrows must be within 50 meters of the random starting point. If 10 burrows cannot be located within 50 m record the distance on the data sheet and note which burrows are beyond the 50 m distance.

Score the activity of each selected burrow (active, inactive, likely active, likely inactive). Detailed training will be provided to surveyors to correctly and consistently identify active and inactive burrows (see scoring instructions below).

Also record information about burrow type (multiple or single entrance) and the signs of prairie dog activity observed, (fresh digging, fresh scat (still soft), prairie dog audio, prairie dog visuals, spider webs, vegetation or debris in burrow entrance, collapsing burrow entrance). The data sheet provides a key and description of the above classifications. The time spent at each burrow should be no more than 30 seconds.

After each set of 10 burrows have been observed and scored, write a brief summary of observations regarding the current occupancy and activity in that area. Upon completion the survey (all points), also write a brief summary of observations and overall impressions of the current occupancy and activity on the entire colony.

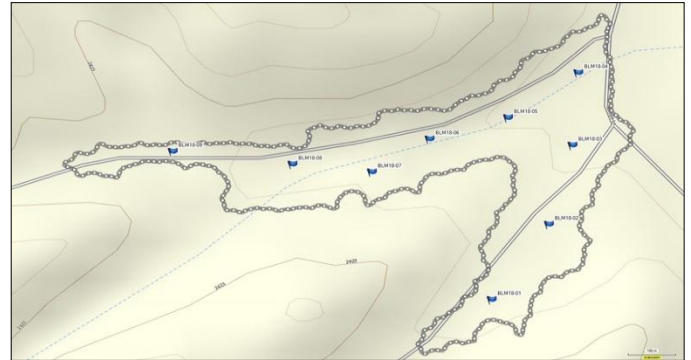


Figure 35. Example of colony with designated random sample points.

Active burrows must have: obvious prairie dog activity within 1 meter of the burrow entrance, fresh scat (within 2 days; still dark in color and soft or moist - not sun-bleached, brittle or crumbly when compressed), obvious fresh digging, and prairie dogs seen or heard at the burrow.

Inactive burrows must have: obvious signs of inactivity, the burrow opening is obstructed by vegetation or heavy spider webs to the point where prairie dogs cannot move freely, or the burrow is collapsing or obviously unusable.

Likely active burrows must have: no obvious fresh prairie activity, no fresh scat, no obvious fresh digging, prairie dogs are not seen or heard at the burrow. Use all available observations of the area immediately surrounding the burrow in question to assign this score. In many cases, active burrows will lack signs of fresh digging and scat.

Likely inactive burrows must have: no obvious signs of inactivity. Burrow opening is not obstructed by vegetation or heavy spider webs, and the burrow is not collapsing or obviously unusable. Use all available observations of the area immediately surrounding the burrow in question to assign this score. In areas recently impacted by plague, many inactive burrows may lack clear signs of inactivity.

Note (‘GS’) if scored as a ground squirrel burrow with a narrow diameter burrow opening and no raised mound or the presence of ground squirrel scat (considerably smaller than prairie dog scat), or ground squirrels are seen or heard at the burrow.

Prairie Dog Count and Observation

Purpose

To monitor colony status by observing and counting the number of prairie dogs and proportion of adults and juveniles in an established observation plot or perimeter.

Materials

- GPS with observation points and boundary loaded
- Camp chairs
- Binoculars
- Datasheets, clipboard and pencil
- Radios (optional)
- Kestrel or other temperature and wind recorder
- Timer or stopwatch
- Warm clothing, gloves

Methods

Observation Points and Plots

Counts are generally conducted in the early morning (between sunrise and 10 AM). Counts should not be conducted in poor weather. High winds, excessively hot or cold temperatures and precipitation can negatively influence counts. Vegetation (tall grass or shrubs) may also negatively influence the visibility of prairie dogs. However, the count data is still valuable as a population index, which can help to inform management decisions. Generally, this method is useful for generating data to determine the minimum number known alive, occupancy and/or changes ($\geq 25\%$) in prairie dog abundance.

Observation points should be located in areas that take advantage of the sites natural topography. Ridgelines or areas of high elevation that overlook the plot make ideal observation points. Observation points within the plot or colony should be avoided. Points on the periphery or adjacent to the observation plot are preferred.

In the days before observations begin, the plot boundaries of a colony or count area must be determined and marked. The marking method will depend on the size and shape of the plot. Small square plots (i.e., ~2 ha) within larger prairie dog colonies may be marked by placing steel t-posts or pin flags at each corner. Straight-line boundaries are marked with flags that are spaced so that the boundary-line is visible with the optics used to conduct the count. At least one boundary marker flag should be visible in the optics field of view at all times (~10-m spacing). Large gaps between flags on boundary lines can make it difficult to determine if a prairie dog on the boundary line is in the plot.

Marking plot boundaries is especially important if a large prairie dog colony is subdivided into smaller plots for simultaneous observation by two or more observers. The boundaries between these plots need to be carefully determined and marked to prevent double counting. In many cases, a plot or colony boundary will be a road, fence or a ridgeline. Clearly visible boundary lines such as these rarely need to be marked. For small colonies or colonies bounded by topography that limits visibility, counting all prairie dogs visible from an observation point is often the best and most repeatable approach.

Counts

When traveling to the colony, allow enough time to arrive at the pre-set observation point, replace any missing flags or markers (cattle and pronghorn may disperse pin flags if given the opportunity). Once at the observation point get setup and seated in a camp chair for 15 minutes before the first count (Figure 36). Once seated, limit body movements and noise, only stand if absolutely necessary.

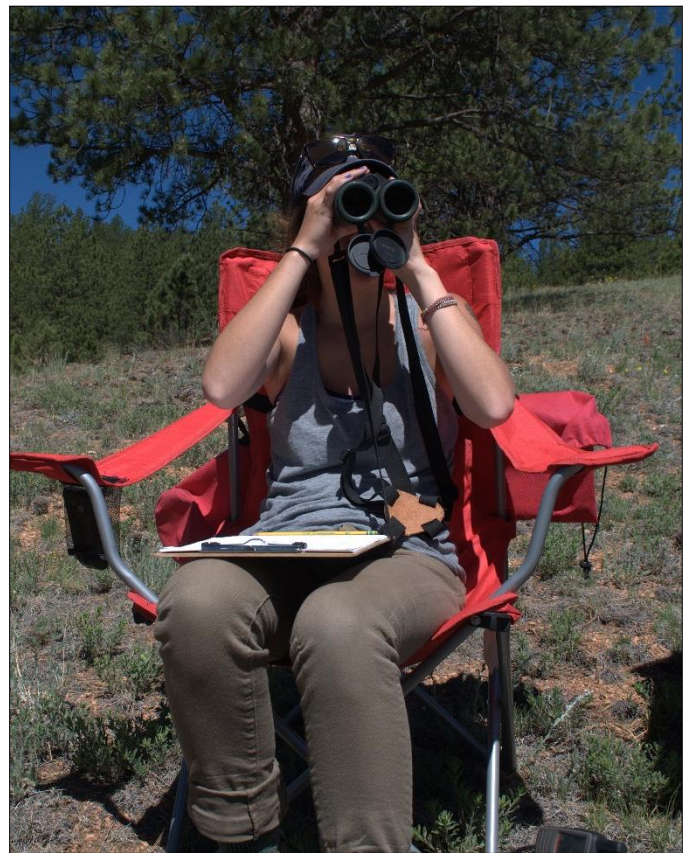


Figure 36. Technician seated with data sheet and optics for prairie dog observation.

Use a GPS unit to locate the pre-set observation points. Make sure all boundary flags are visible. Record the UTM coordinates of the observation location, the temperature, the wind and weather conditions, the optics used and other information as listed on the data sheet. A designated timekeeper may radio technicians at 15-minute intervals to begin counts, if observations are being completed by two or more observers. Alternately, all technicians should have a reliable time keeping device and conduct all counts at the predetermined times.



Figure 37. Black-tailed prairie dog eating bait.
Photo by Bruce Gill.

Begin and end each count in the same area of the colony/plot specified. Focus the binoculars on the area of the colony/plot specified and scan that area with a consistent method. Use the binoculars field of view to divide the plot into vertical or horizontal strips equal to the width of the field of view.

Example: start in the “bottom left corner” of a colony. Count all of the prairie dogs visible in a swath moving towards the “upper left corner”. At the end of the swath, note the right-hand extent of the field of view and move binoculars to the right until this point is now the left-hand extent of the view (allow some overlap). Move the binoculars “downward” to count all of the prairie dogs visible in this second swath. Repeat this method until the entire plot has been observed. Use plot markers and boundary flags as well as any landmarks (rocks, shrubs, horizon features) to maintain visual orientation and prevent gaps in coverage or double counting. A count should represent a “snapshot” of all individuals above ground during the count.

When the count is completed, record the total number of prairie dogs observed. Wait until the next count (every 15 minutes). If juvenile prairie dogs are distinguishable from adults (approximately May-July), record the number of juveniles separately. Repeat counts at each 15-minute interval. Only stand at the agreed upon end time and be sure that all other observers are done with their counts before standing. The final count should be conducted while standing. Often the highest count of a session will be observed while standing or when a predator or other disturbance has alarmed the prairie dogs and they begin moving to their burrow entrances.

Record Data

Double check that the observation point, UTM coordinates, temperature, wind, optics type and other information as listed on the data sheet have been recorded. Also, record raptor or other predator species seen on or near the colony along with the time and prairie dog behavior exhibited in the comments section. Record any other events that could influence count estimates (tall vegetation or other visual barriers, vehicle traffic, other wildlife sightings, changes in temperature or wind). Also, note if prairie dogs were observed interacting with vaccine (Figure 37), outside the plot or if prairie dogs were seen or heard while traveling to and from the observation point (i.e. on adjacent plots/colonies).

Burrow Counts (Grid Method)

Purpose

To create a detailed map of how many active and inactive burrows are on a prairie dog colony or plot.

Materials

- GPS with grids loaded
- Counters/Clickers
- Pencils
- Flour
- Printed colony maps with grid overlay

Methods



Figure 38. Burrow count grids (50 m x 50 m) over a colony.

Using Garmin MapSource® or BaseCamp® tracks or transect lines in ArcGIS create a series of grid squares (30 to 50 m²) overlaid on the colony perimeter (Figure 38). Number and assign the grid squares to technicians. Navigate to the corner of the square grid and turn on the tracks on the GPS. Start walking in a zigzag pattern throughout the assigned grid. Each time a burrow is encountered, mark a waypoint on the GPS.

Burrow locations are recorded on a single GPS unit with the active or inactive classification recorded in the comments section while marking the waypoint. Use a pinch of flour to visibly mark burrows as they are counted. The flour marking technique helps ensure every burrow is counted while also preventing double counting along grid boundaries. When grids are completed, write the number of active and inactive burrows on the map and move to the next square. When finished, the data keeper will collect all maps and collate all data onto a single data sheet. Be sure to download and save all the marked burrow waypoints onto the computer at the end of the day.

Burrow Counts (Belt Transect Method)

Purpose

To create a detailed map of how many active and inactive burrows are on a prairie dog colony or plot.

Materials

- GPS with transects loaded
- Datasheet
- Clipboard
- 3-meter pole (PVC or plastic conduit)
- Backpacks

Methods

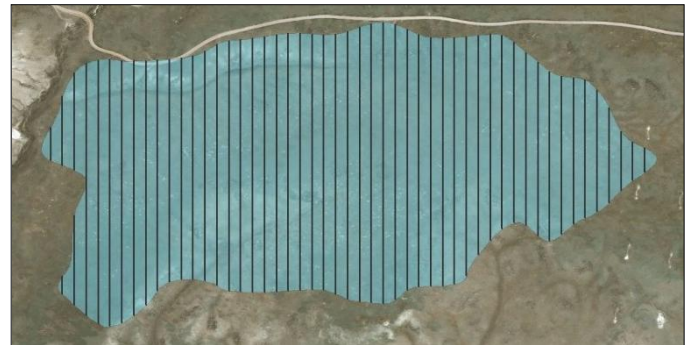


Figure 39. Burrow count transects (20 m spacing) over a colony.

Create transects with ~20 m spacing overlaid on the colony perimeter and load transects to GPS units (Figure 39). The transect spacing and number of transects selected will depend on the proportion of the colony area needed for the survey (ex. 10, 15, 25%). The total length of transects multiplied by 3 (width of the belt transect) will provide the area (m²) surveyed. At the colony, attach the center of the 3m pole to the frame backpack with wire. Attach a twine streamer to both ends of the pole that will touch the ground and define the edges of the 3m belt transect at ground level. Navigate to one end of the assigned transect.

Clear the track information from the GPS unit (Page to Trip Computer, -> menu -> reset -> trip data and odometer). Turn track log feature on and take a waypoint. Record the date and time, beginning waypoint, GPS Unit, Initials, site ID and transect ID on the data sheet. Follow the track to reach the opposite end of the transect.

Every time the 3 meter pole passes over a burrow opening, mark the burrow on the data sheet and check burrow classification. The following burrow metrics should be identified:

Active- the burrow is actively used by prairie dogs (based on scat, digging, or other signs of activity); reasons for activity should be indicated on most burrows

Fresh Scat- scat is within 1m of burrow, is dark in color and looks to be no more than 2 days old

Fresh digging- digging around burrow has occurred within last 2 days: dark earth, disturbed ground

Blue scat- scat dyed blue (or other color) from bait consumption is within 1m of burrow entrance (Figure 40)

Inactive- the burrow is not actively used by prairie dogs (based on webs or vegetation obstructing entrance)

Webs- burrow entrance is covered with webs, indicating a prairie dog has not used the burrow for several days

Vegetation- plant matter is growing or has collected in burrow, obstructing entrance (small ground cover does not qualify)

Once on the transect line, look for a point on the horizon to aid in orientation and check the GPS regularly while making necessary course corrections. Follow the transect line accurately enough to ensure that adjacent transect lines are not crossed. Do not actively aim towards or away from burrows while walking. Do not walk transects covering an area that has already been covered.

Mark data that are related to the burrow activity on the data sheet. When each transect is completed, also mark the end with a new waypoint and turn the track log feature off. Record the end waypoint, distance covered from GPS and end time on the data sheet.

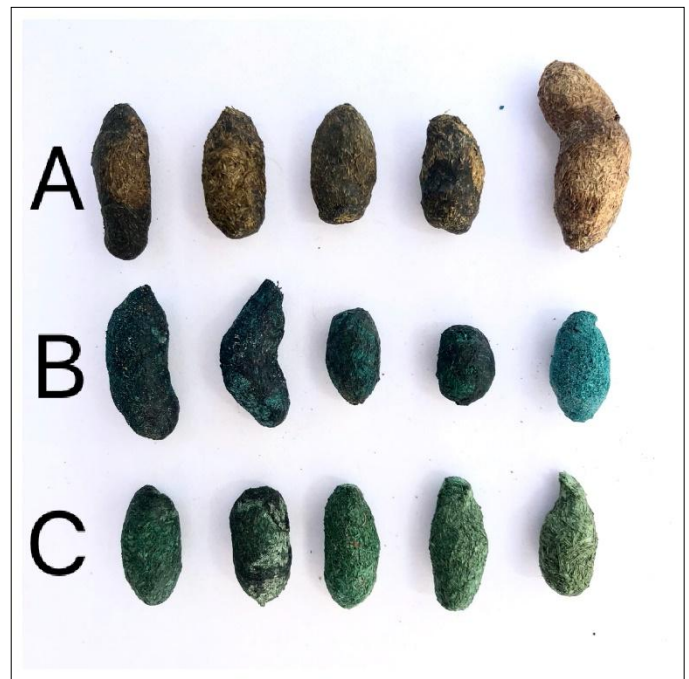


Figure 40. Examples of prairie dog scat collected 6 days post vaccine bait distribution. A) Normal, no bait consumed; brown, black or dark green. B) Heavily stained; very apparent blue hues. C) Moderately stained; apparent blue or bright green in color.

Burrow Flea Monitoring

Burrow Swabbing

Purpose

To collect fleas from prairie dog burrows for plague surveillance and to gather data on flea vector species diversity and abundance. At each colony/plot, 90 burrows are swabbed. The goal is to obtain an even distribution of samples across the colony by visiting 9 spatially distributed sampling points. To further distribute sampling points, 10 randomly selected burrows within 100 meters of each sampling point will be swabbed.

Materials

- GPS with points and boundary loaded
- Extra AA batteries
- Radios (optional)
- 11 x 11-inch white cotton flannel swabs
- Swab bundles for all points (one bundle of 20 swabs covers 10 burrows or 1 sampling point)
- Zip top bags to cover all points (one bundle covers 10 burrows)
- Backpacks
- Burrow condition and labelling guide
- Nitrile gloves
- DEET spray (in separate container, not near swab material)
- Permanent markers
- ¼ inch plumbers snake (a flexible coiled wire with a clip at one end) marked at 0.5 m intervals
- Trash bags and bag labels
- Cooler

Methods

Before burrows can be swabbed for flea samples, swabs must be cut from a bolt of white flannel fabric. First position tables together so that lengths of fabric can be laid out. Next, cut down the center of the folded side of fabric using a pair of sharp scissors. With a template square (11 by 11 inches) and a permanent marker, mark down the length of the fabric at 11-inch intervals. Cut the fabric at each marked interval. The swabs will now be in stacks of two pieces of cloth (each \approx 11 by 22 inches). When the cutting is complete, stack five strips (ten total pieces of cloth) on top of each other and cut down the center of the stack. In each stack, there should be twenty square pieces of cloth. Each bundle of twenty individual square swabs is enough for one swab point in the field. For a typical site, nine of these bundles will be needed (90 swabs total).

Technicians will apply a DEET spray to their clothing prior to swabbing to decrease the potential for contact with fleas (Figure 6). Wear nitrile gloves while handling DEET spray and discard them prior to swabbing. **TAKE CARE TO KEEP DEET AND SWABBING SUPPLIES SEPARATE. DO NOT CONTAMINATE SWABS WITH DEET OR DELTADUST®.**

Pack a backpack with a plumbers snake, burrow condition/ labeling guide, multiple permanent markers and the appropriate number of swab bundles and bags (Figure 41). Divide the points among the crew and head to the first point.

Find a burrow in the area near the swabbing point and mark it on the GPS. Attach a swab to the clip end of the snake (Figure 42). Snake it down the burrow as far as it will go. Flicking, shaking or twisting the snake will get the swab to go farther down. The snake is marked at 0.5m intervals. The goal is to have the swab sample as far into the burrow as possible. If the swab cannot reach a minimum depth of 0.5 m, a different burrow should be selected.



Figure 41. Burrow swabbing backpack with two bundles of swabs and plumbers snake.

Keep the swab and snake in the burrow for 30 seconds. While waiting, label a zip top bag with the required information, this is explained on the burrow condition and labeling guide. Remember to mark the burrow location on the GPS and record that waypoint ID on the zip top.

Remove the snake from the burrow. Take the swab and quickly zip it in the zip top bag (Figure 43). Snake the second swab into the burrow and wait 30 seconds. If the distance is different, record the distance on the zip top. Retract the second swab and put it in the bag with the first swab and record all burrow condition and sampling information, (Figure 44). If fleas are observed on the swabs write “fleas” on the bag.

Move on to another burrow and repeat the process, trying to get a representative sample of all burrows in the colony area. Distance between burrows should be relative to the size of the colony; small colonies will lead to closer spaced samples, large colonies will lead to samples being farther apart. Burrows should be selected at random and inactive looking burrows can still be sampled if the burrow entrance is open and a minimum depth of 0.5 m is achieved.

On the last burrow at each sampling site (1-9), write some notes about general impressions/observations of the sample area. Include the perceived health of the colony, proportion of active and inactive burrows, how many fleas were seen, etc. When all sampling points have been swabbed, place the zip top bags into a white (13-gallon) trash bag. Fill out and attach a bag label or label the trash bag with the date, colony site name and initials of technicians. Place the trash bag in a cooler for transport. Do not keep swabs in a hot vehicle. Fill out a basic site-visit form.

Place the trash bag in an appropriate freezer location after returning from the field. On a master swabbing data sheet or freezer inventory log, record the colony/site ID, initials of technicians, date swabbing was conducted and date frozen. Download GPS waypoints to the appropriate folder (refer to GPS Downloading Protocol). Fleas need to be submitted to the Wildlife Health Laboratory as soon as possible for analysis.



Figure 42. Burrow swab attached to plumbers snake prior to sampling.

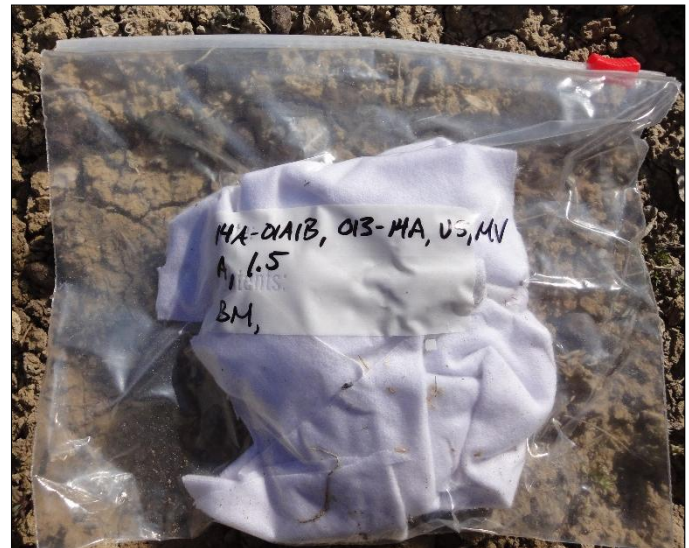


Figure 43. Two swabs secured in correctly labeled zip-top bag.

<u>Key For Burrow Descriptions:</u>	
BS:	Burrow is a single
BM:	Burrow is one of several in a mound
D:	Fresh digging present
FS:	Fresh scat present
W:	Webs in burrow entrance
Veg:	Vegetation at burrow entrance
V:	(Visual) Dogs seen in immediate area- distance and direction
A:	(Audio) Dogs heard in immediate area- distance and direction

Figure 44. Burrow condition and sampling key.

Flea Processing

Purpose

Burrows are swabbed to collect fleas for plague surveillance. The fleas collected on swabs will be picked from the swabs and stored for later PCR laboratory diagnostics. The goal of flea picking is to find all fleas on a swab and put them in correctly labeled vials in preparation for identification and PCR.

Materials

- Picking data sheets on clipboard
- Tweezers
- Isopropyl alcohol
- Torch to flame sterilize tweezers
- Numbered vials with saline
- White paper laboratory mats
- Spare vial rack
- Nitrile gloves
- Daily flea picking log sheet

Methods

Preparing Vials

The vials with fleas from swabs will be labeled with a numerical sequence starting with “1” at the beginning of the year and increasing numerically. Do not skip numbers and do not use the same number twice. Keep everything organized and do not botch the vial number sequence. Pre-label the vials prior to picking, especially if more than one person is picking to prevent vial numbers from being repeated or skipped. If a labeling error occurs note it on the log sheet and provide the number sequence of vials that may be affected.

The vials with ticks will be labeled with a separate “T-#” sequence starting with “T-1” and continuing on in numerical order (Do not mix up the flea and tick number sequence – Check the work as fleas and ticks are being collected). Errors are easily corrected if caught early. Consult the daily flea picking log sheet for the vial number to start each day. Have numbered saline vials ready and filled with approximately 1 mL of saline solution.

Sorting Swabs

Swabs will be picked according to a site priority list that will be provided. Each site will have 9 swab points with 10 burrows (zip top) at each point, totaling 90 individual bags. Each zip top bag will have a colony site, swab point number and waypoint number labeled. Sort bags

by swab point as well as by waypoint number to help organize information written on the picking data sheet. If a zip top bag is missing, leave room on the data sheet for it in case it is found.

It is important to always finish picking one colony before starting a new one. Sort the swabs from one large trash bag into the 9 smaller groups by point (usually 10 bags at each site, there may be some exceptions). Separate swabs from each site into different bags (old grocery bag) and place in a cooler with ice packs, back in the necropsy freezer, or in the small chest freezer in the supply trailer. Never put unpicked swabs in a -70°C freezer. Pick the entire trash bag the same day it is removed from the freezer. Keep freeze-thaw cycles to a minimum. If picking a partial bag, make note in the picking log.

Picking

Lay out zip top bags from a swab point in order of increasing waypoint numbers (e.g., 051, 052, 053). Transfer data written on the bag to a data sheet. Write any extra information in the comments, noting if any swabs are missing. Place a clean paper laboratory mat on the tray in the fume hood and remove a swab from the bag onto the mat. Slowly scan the swab looking for fleas and ticks. Be sure to thoroughly check both sides of each swab and around the frayed edges. Fleas are usually a reddish color with a metallic shine to them, look for these characteristics as well as the overall shape of the flea (Figure 45). There are various sizes and colors of fleas. Be aware that the fleas often try to burrow into the fabric, so it is important to look for dark "slivers" in the cloth.

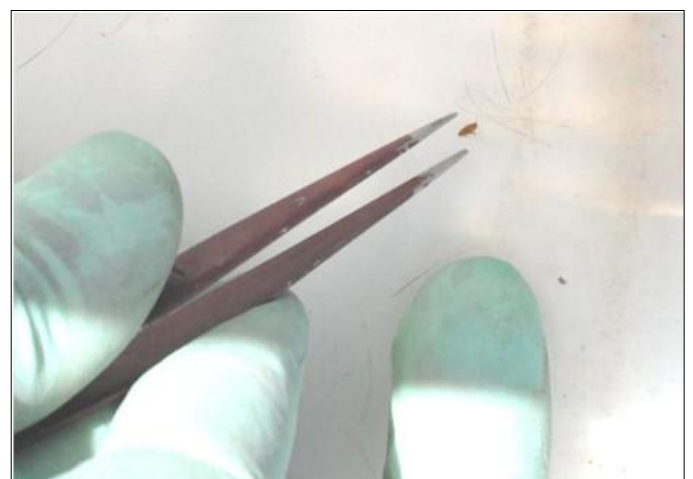


Figure 45. Removing fleas from a swab.

If a flea or tick is found, use tweezers to place it in a vial of saline (Figure 46). Put all fleas from the same zip top bag into the same vial. Fleas from the next zip top bag go into the next vial in the sequence. Ticks have their own number sequence and individuals from the same burrow go into the same vial. Repeat this scanning and picking method on the second swab. Check the empty bag for loose fleas.



Figure 46. Fleas in a vial of saline.

After scanning both swabs, record the vial number and number of fleas and ticks on the data sheet. If fleas or ticks were found on the swabs, dip tweezers in ethanol and torch the tips for a few seconds to sterilize. Dump the dirt and debris from the laboratory mat into the trash. Do this in between each bag. The laboratory mat should be replaced after each site or when the mat is too dirty to see fleas.

Place vials with fleas in numerical order in a rack and place tick-only vials in a separate rack. Place picked swabs in a trash bag to be washed. Keep the label tag associated with each site paper clipped to all the data sheets from the whole site. Enter flea picking data into computer on a regular basis.

Daily Flea Picking Log Sheet

The information from each trash bag of swabs that have been picked that day needs to be logged into the daily log sheet which is located in a binder in the laboratory drawer. Double check vial numbers and fix any errors.

Cleaning up

Place vials with fleas in one freezer box and tick-only vials in a separate freezer box. Make sure the freezer boxes are clearly labeled with the site ID and associated vial numbers. Keep vials in their numerical order. Leave an empty space in the vial box between the end of one

site and beginning of the next. Place boxes in a -70°C freezer. Keep fleas frozen and keep freeze-thaw cycles to a minimum. Wipe down fume hood and trays with alcohol and a paper towel to disinfect work area. Flea samples may now be processed in the laboratory for identification to species (Stark 1958, Hubbard 1968) and plague diagnostic tests via PCR (Griffin et al. 2010). Flea identification and PCR diagnostic protocols are available from the CPW Wildlife Health Program upon request.

Cleaning Swabs

Once picked, dirty swabs need to be washed before they are repurposed. Dirty swabs are kept in the white shed until they are washed. Label bags “dirty swabs”. To wash swabs, place loose swabs into washing machine. Make sure to unfold and shake out swabs prior to washing to ensure they are thoroughly washed (not balled up). Fill washer, below maximum capacity, with swabs and add clothing detergent. When done and dry, place swabs into a new trash bag and label bags “washed swabs”.

Plague Vaccine Bait Production

Bait Production Lab Set-up

Purpose

To efficiently organize and setup materials and equipment for the production of plague vaccine bait.

Prior to laboratory setup, all materials and ingredients must be stocked according to bait production needs. It is important to ensure that the laboratory spaces for drying and producing bait are both de-winterized and operational before laboratory organization can be done. This means that all air conditioning systems need to be

operational, hot water should be readily available, waste water holding tanks need to be emptied (where applicable) and all electrical outlets and lights are functional.

Thermostats for both laboratory spaces should be set to 20°C (69°F). Use the below list of formulation and manufacture equipment (Figure 48) as well as the laboratory space diagram below (Figure 47) to efficiently set-up and organize laboratory equipment and materials. For proper storage of materials and ingredients, see Figure 49. Proper BoilieLab Machine set up is shown in Figure 53.

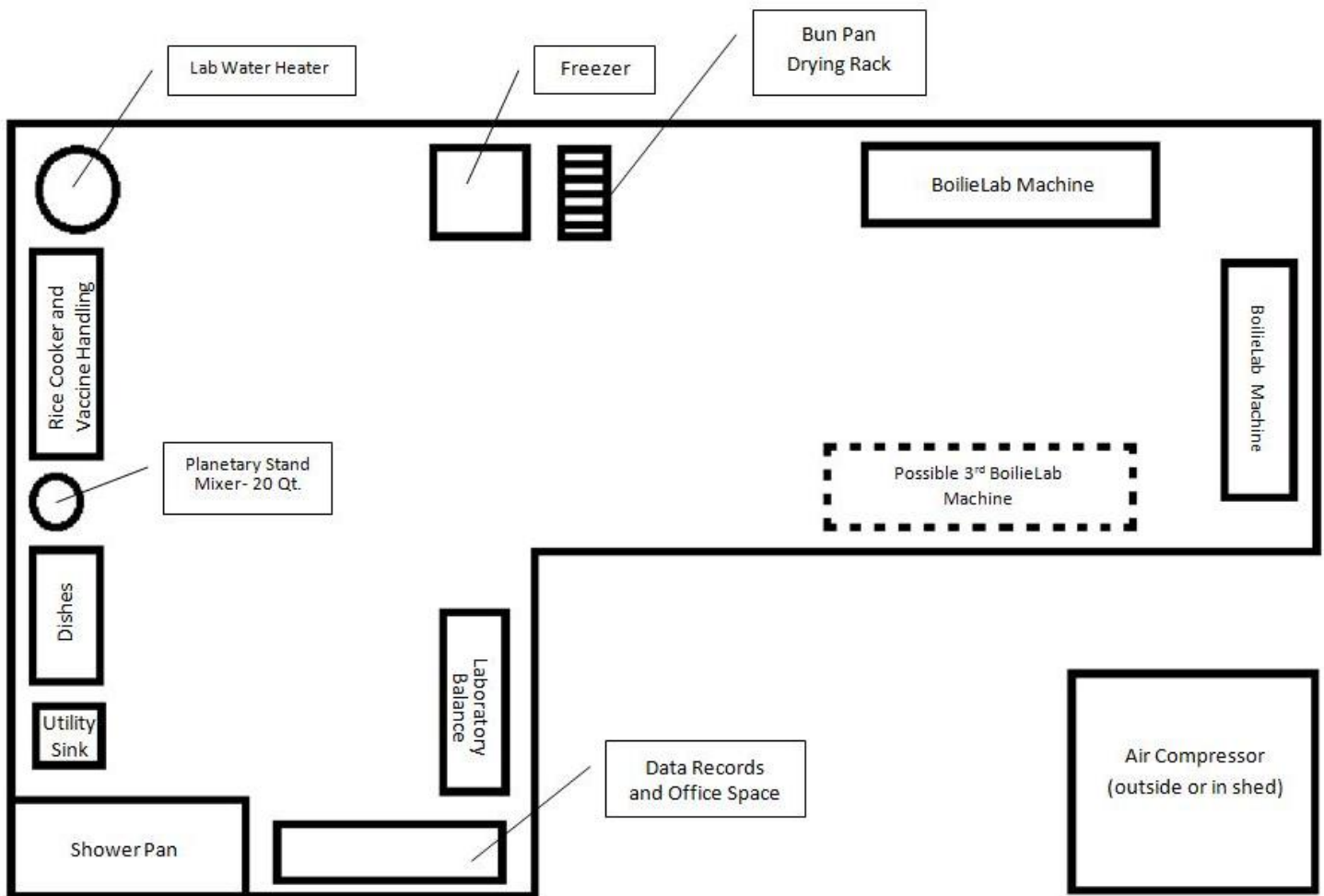


Figure 47. Plague vaccine bait production lab space diagram.

Formulation		
<i>Equipment</i>	<i>Quantity</i>	<i>Source</i>
Commercial Rice Cooker (capable of raising 5 liters of water to >40°C)	1	<i>Brand:</i> Aroma <i>Purchased:</i> Amazon.com
Planetary bench top mixer (20 Qt.)	1	Hospitality Supply, Inc 1833 E. Harmony Rd. Unit 15 Fort Collins, CO 80528 hospitalitysupplyonline.com
Large mixing bowl (20 Qt.)	1	
Planetary paddle mixer attachment	1	
Clear Plastic pans for measuring ingredients (various sizes)	3	
Spatulas (various)	2	
Vortex Mixer	1	<i>Brand:</i> Barnstead/Thermolyne <i>Model:</i> M37615
Graduated Cylinder (100 mL x 2 mL)	1	Thermo Fisher Scientific Inc.
FisherScientific Thermix® Stirring Hot Plate <i>Model</i> 210T	1	
Laboratory balance (5 kg x 1 g)	1	<i>Brand:</i> Ohaus ES6R <i>Purchased:</i> Amazon.com
Laboratory balance (2.00 kg x 0.01g)	1	VWR International LLC
Corning Sterile 220 mL specimen cups with lid	1	
Drummond Pipet-aid with 25 mL Stripette Serological Tips	1	Drummond Scientific Company
Instant Read Digital Thermometer (12 inch stem, +/- 1.0°C accuracy)	1	<i>Brand:</i> Thermco <i>Purchased:</i> Amazon.com
Walk-In-Freezer (-20°C)	1	N/A
Manufacture		
Automated “BoilieLab” Machine	2	UAB Topas LT Melioratoriu 6a, LT-72109 Taurage, Lithuania BoilieRoller.com
“BoilieLab” pneumatic extrusion tube	7	
“BoilieLab” extrusion tube end caps	2	
BoilieLab extrusion yellow nozzle #4	2	
COX 20-Ounce Sausage Applicator	2	<i>Brand:</i> COX <i>Purchased:</i> Amazon.com
Air compressor (5 gal. at least)	1	<i>Brand:</i> Porter Cable <i>Purchased:</i> Home Depot
Drying		
Bun pan racks (20 tier, 26”x20”x69”)	6	Hospitality Supply, Inc 1833 E. Harmony Rd. Unit 15 Fort Collins, CO 80528 hospitalitysupplyonline.com
Perforated bun pans (18’x26’x1’)	120	
Miscellaneous Laboratory		
Nitrile Exam Gloves	As needed	VWR International LLC
3M N95 Particulate Respirator		
Goggles		
Labeling tape	4 rolls	
Laboratory consumables	As needed	
Rubber bands	As needed	
Permanent markers	1 box	
Freezer gallon storage bags	As needed	
Plastic storage bins with lids	10	

Figure 48. Bait production laboratory equipment and materials information.

<i>Ingredient</i>	<i>Quantity</i>	<i>Source</i>	<i>Product Number</i>	<i>Storage Instructions</i>
Distilled Water	4522g (placebo) 4322g (treated)	Variable	N/A	Stored under rice cooker table on floor
Incortrix Bait Powder	2319g	Food Source Lures 201 39 th Street North Birmingham, AL 35222	Prairie Dog Bait Mix	Store in sealed plastic tubs or in shipping boxes in freezer
Peanut Butter	1159g	Golden Organics 4941 Allison Street Arvada, CO 80002	PNBC35 (peanut butter creamy organic)	Stored in lab at room temperature until mixed. Once mixed peanut butter must be refrigerated.
Vaccine Dye	40g (0.5% w/w)	IFC Solutions 1601 Linden Avenue Linden, NJ 07036	FD&C Blue 1 Sky Blue shade	Stored in a dry dark location. If aliquoted—then store in a sealable tub.
Placebo Dye	20g (0.25% w/w)	IFC Solutions 1601 Linden Avenue Linden, NJ 07036	FD&C Red 40	Stored in a dry dark location. If aliquoted—then store in a sealable tub
SPV Vaccine	See "Calculating Vaccine Quantities"	Colorado Serum Company 4950 York Street Denver, CO 80216	18051 Yersinia Pestis Vaccine	Stored at -70°C until thawed for use in formulation. Once aliquoted—store in refrigerator until needed.

Figure 49. Sources, product numbers and storage instructions for ingredients and materials for bait formulation.

Collecting and Managing Bait Production Data

Purpose

To make an accurate record of all plague vaccine used, all bait produced and where all bait was distributed, as well as materials and equipment used in laboratories.

Methods

It is important to create and update a laboratory inventory of all equipment that is being utilized in the laboratory. It is also necessary to have a dynamic inventory of bait production ingredients. Always utilize log sheets (Appendix 1) and fill out all sections. All log sheets should be recorded into electronic Excel spreadsheets. All vaccine and bait production data sheets are available in Appendix 1.

Calculating Plague Vaccine Quantities

Purpose

Properly calculate usable, lot-sized, quantities of sylvatic plague vaccine.

*The vaccine calculation worksheet may be provided upon request. Vaccine breakdown and dilution will not be necessary when vaccine is packaged according conditional licensure, after which vaccine will be provided in 4,000 standard dose volumes.

In order to accurately calculate the quantity of vaccine needed per lot, a vaccine dilution worksheet has been developed using Microsoft Excel. To begin, all vaccine serial concentrations are listed from earlier derivations, which will then be utilized in the vaccine dilution worksheet. Instructions detailing how to use the vaccine dilution worksheet are below.

To identify the volume of vaccine and TRIS buffer necessary per extrusion tube, the desired wet kg of bait material being formulated should be entered into the red box at cell P18. The grams of bait material being formulated will be determined. This process is done automatically (by multiplying cell P18 by 1000) and does not require action. The vaccine dilution worksheet then determines the number of baits being manufactured from the above determined number of grams of bait material in each extrusion tube (assuming each wet bait = 2.13 grams).

Using the already derived vaccine concentrations of each serial, the volume of vaccine liquid amounting to one dose (5×10^7 pfu) was derived using the total available volume of the serial divided by the total available doses (column P). Based on the number of baits per extrusion tube, assuming the goal is to provide one dose of vaccine per bait, the volume of vaccine required is found (column U).

The volume of TRIS needed is found in column U. Assuming the extrusion tube is filled with an 8-kg lot (full tube), the vaccine volume will need to be buffered to 200 mL to ensure the vaccine is thoroughly mixed through the bait material. The total number of extrusion tubes that can be produced per serial is also provided in column G (this information can be helpful when aliquoting vaccine).

Handling and Diluting Plague Vaccine

Purpose

To accurately and safely measure and store lot-sized volumes of plague vaccine for future plague vaccine bait production.

Methods

WARNING

Vaccine contains live virus and PPE (gloves, safety glasses or face shield and N95 particulate mask) should always be used when handling. Always wash hands after

handling. Visually inspect all frozen vaccine stock containers to ensure there are no cracks or areas where vaccine may leak once thawed. The vaccine contains live virus that can be easily denatured and should be handled with care (never exposed to heat or sunlight).

Thermostats for laboratory spaces should be set to 20°C (69°F) and checked daily. The Drummond portable pipet-aid should be fully charged. Sterile storage containers should be stocked (220-mL sterile Specimen Container and smaller centrifuge tubes). Corning 25-mL sterile Stripettes should be stocked. Instructions for the autoclave can be found on the top of the autoclave machine. Each large 1000-mL flask or 2000-mL beaker with large stir bar should be autoclaved at 134°C (273°F) for 60 minutes prior to use. Each 1000-mL glass bottle, with blue lid, should be autoclaved at 134°C (273°F) for 60 minutes prior to use.

To appropriately measure and dilute plague vaccine into useable lot-sized quantities, use the set up in Figure 50.

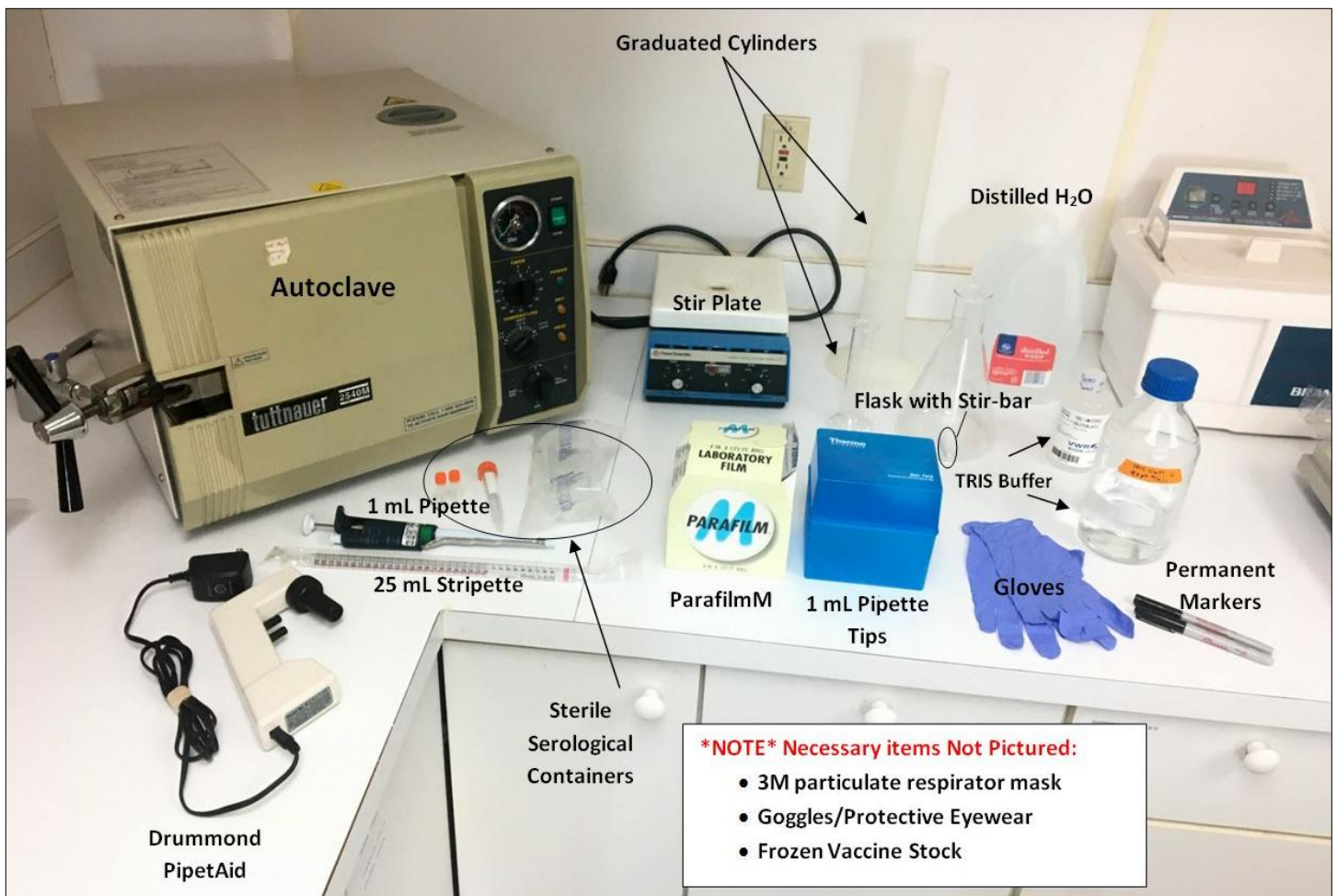


Figure 50. Materials needed for vaccine handling and dilution.

For virus dilution, begin by thawing the frozen stock of vaccine (Figure 51) at room temperature or in water at $\leq 25^{\circ}\text{C}$. Determine how much stock is needed to achieve 5×10^7 pfu/bait using the above directions for the vaccine calculator. Empty the thawed vaccine stock into the sterile flask or beaker of appropriate size (with stir bar inside). Place the beaker onto the stir-plate. Slowly turn on the stir function to create a vortex without creating bubbles. Stir stock for approximately 5 minutes or until particulate suspension is no longer visible. The vaccine upon thawing will have a flocculent appearance with small particulate suspended in the solution. This is characterized by small floating particles of a light color. All particulate suspended must be fully incorporated before aliquots are measured.



Figure 51. Bottles of Yersinia Pestis Vaccine.

Once the vaccine is thoroughly mixed, use the Drummond portable pipet-aid to measure the volume of vaccine needed for an 8-kg tube by pipetting, while continuing to stir with stir-bar, from the flask or beaker into a sterile 220-mL container. The container should then be closed tightly and wrapped with Parafilm M. Record aliquot information onto an Avery aliquot label and attach label onto vaccine container. Before all vaccine has been aliquoted, two 1-mL liquid archive samples must be taken and each should be pipetted into 1-mL sterile tubes. Record all aliquots and volumes on the Vaccine Aliquot Log Sheet (in Appendix 1) and note any volumes that are not as expected.

To prepare 10 mM TRIS, measure 990 mL of distilled water and pour into an autoclave sterilized liter bottle (with blue lid). Measure 10 mL of 1.0 M 9.0 pH TRIS buffer and pour into the same liter bottle. Then close the bottle lid tightly and tip the bottle a few times to fully homogenate. Label the bottle as follows: 10 mM TRIS Buffer, Produced MM/DD/YY and Technician Initials. Place TRIS buffer in the refrigerator until used for bait formulation. All labeled vaccine aliquots should be stored in the -70°C freezer.

Formulating Plague Vaccine Bait

WARNING

Vaccine contains live virus and PPE (gloves, safety glasses or face shield and N95 particulate mask) should always be used when handling. Always wash hands after handling vaccine. Visually inspect all vaccine stock containers to ensure there are no cracks or areas where vaccine may leak once thawed. The vaccine contains live virus that can easily be easily denatured and should be handled with care (never exposed to high heat or sunlight). Blue dye is a fine particulate and PPE (gloves, safety glasses or face shield and N95 particulate mask) should always be used when handling.

Purpose

To formulate plague vaccine bait that is viable for field use.

Materials

Ingredients for full 8-kg extrusion tube:

- 4522 g Distilled H_2O for placebo bait; 4322 g distilled H_2O for vaccine bait (vaccine will be diluted to 200 mL total volume)
- 2319 g Incortrix bait powder
- 1159 g Golden Organics peanut butter (+ 5 g for waste residue on spatula and bowl)
- 40 g Blue Dye Powder
- Vaccine aliquot (mL) and TRIS buffer (mL) (amount as calculated in vaccine worksheet)

Methods

Recommendations:

- Turn rice cooker to the “cook” (red solid light, not flashing) setting at the beginning of production to decrease wait time. Change the setting to “Warm” after 10 minutes. Thermostats for laboratory spaces should be set to 20°C (69°F) and checked daily.
- Have ingredients stocked and ready (i.e., have a tub of peanut butter opened and stirred completely with all excess oil mixed in—this is done by using a power drill and a paint mixer attachment). Once mixed, peanut butter should be refrigerated. Bait powder (Incortrix) should be stored in the upright freezer when possible or in a plastic sealable tub or bucket if freezer space is unavailable. Always start with stand mixer settings at the lowest possible speed setting (Speed 1). TIP: to prevent splashing, pulse before fully turning on the mixer.

- Record onto a production log sheet all volumes and weights of ingredients used in each lot of bait being formulated.

Begin by recording all measurements and weights of ingredients and materials into the Bait Production Data Log Sheet (in Appendix 1). Also, record all tube identification numbers for each lot being poured into large aluminum extrusion tubes.

Fill rice cooker water bath to the 2.5-L mark with distilled water and heat water to 70°C. Place mixer bowl on stand mixer and attach paddle mixing attachment. When water has reached 70°C, remove water from heat and pour into tall stove pot (or other appropriate vessel) on scale and weigh water, once weighed, add water to stand mixer bowl. Then, immediately stir in blue dye powder (also measured on scale to desired quantity) until well dissolved (no more purple specs or powder on top of water).

Once the blue dye is completely dissolved, immediately mix in Incortrix bait powder (use the pulse on/off method to prevent splashing) and continue to mix until all particles of the bait matrix have been hydrated (no more white particles). Then mix in 1159 grams of Golden Organics peanut butter. Stir on 2nd speed setting until mix reaches 40°C, 10-15 minutes. If formulating vaccine bait; turn mixer off once the mix reaches 40°C and add the vaccine. If formulating placebo bait, omit the addition of vaccine.

To incorporate the vaccine, vortex the aliquot of vaccine for 3-5 minutes, with lid and parafilm still securely in place. Measure TRIS buffer needed to bring volume of vaccine/TRIS mixture to 2.5% of the total volume. Slowly pour TRIS buffer into vaccine aliquot container. (Example 1: If we have a full 8000-gram batch of bait mixing and an aliquot of 80 mL of vaccine, we will need the volume of the vaccine-TRIS mixture to be 200 mL. We will then need to add 120 mL of TRIS to the 80 mL of vaccine to achieve a 200 mL volume of vaccine and TRIS.) (Example 2: If we have a 4000-gram batch of bait mixing and an aliquot of 35 mL of vaccine, we will need the volume of the vaccine-TRIS mixture to be 100 mL. We will now add 65 mL of TRIS to the 35 mL of vaccine to achieve a 100 mL volume of vaccine and TRIS.) Gently add half of the vaccine mixture to the blue bait material in stand mixer and stir on low for 2-3 minutes, pulse a few times to begin. Stop the mixer and add the other half of vaccine mixture and stir on low for 3-5 minutes. Stop the mixer and scrape the sides of the bowl into the slurry then continue to mix on low for an

additional 2 minutes. Do not over mix the bait slurry. The mixture should be poured between 32°C to 40°C.

Pour the entire bait mixture into the labeled (lot number) aluminum bait extrusion tube with the white plunger flush with the end of the tube (Figure 52). If there is surplus, pour extra bait material into the vaccine aliquot container or additional sterile 220 mL containers. Cover with gallon plastic storage bag and secure with rubber bands to limit dehydration.

After overnight storage (~12-30 hours), the bait extrusion tube can be processed through the BoilieLab Machine. The next lot of bait can now be formulated. It is necessary to wash the mixer bowl between lots to prevent potential soap contamination.



Figure 52. Pouring bait slurry into extrusion tube.

Plague Vaccine Bait Formulation Cleanup

When cleaning, PPE (gloves, safety glasses or face shield and N95 particulate mask) should be worn at all times. The only soap that should be used is AJAX bleach alternative. All items should be rinsed thoroughly to ensure that soap will not contaminate the formulation and potentially harm the vaccine. When cleaning extrusion tubes it may be necessary to tap out the plunger to ensure the tube is fully clean (a 1-m long wood 2x4 can be used). All equipment, other than the air conditioning unit, should be powered off for the day (it is also recommended that the planetary stand mixer be unplugged).

Bait Manufacturing Procedure Utilizing BoilieLab Machine

WARNING

Vaccine bait contains live virus and PPE (gloves, safety glasses or face shield and N95 particulate mask) should always be used when handling. Always wash hands after handling vaccine bait. The vaccine contains live virus that can be denatured and should be handled with care (never exposed to high heat or sunlight).

Purpose

To manufacture plague vaccine baits that are uniform and viable for field use.

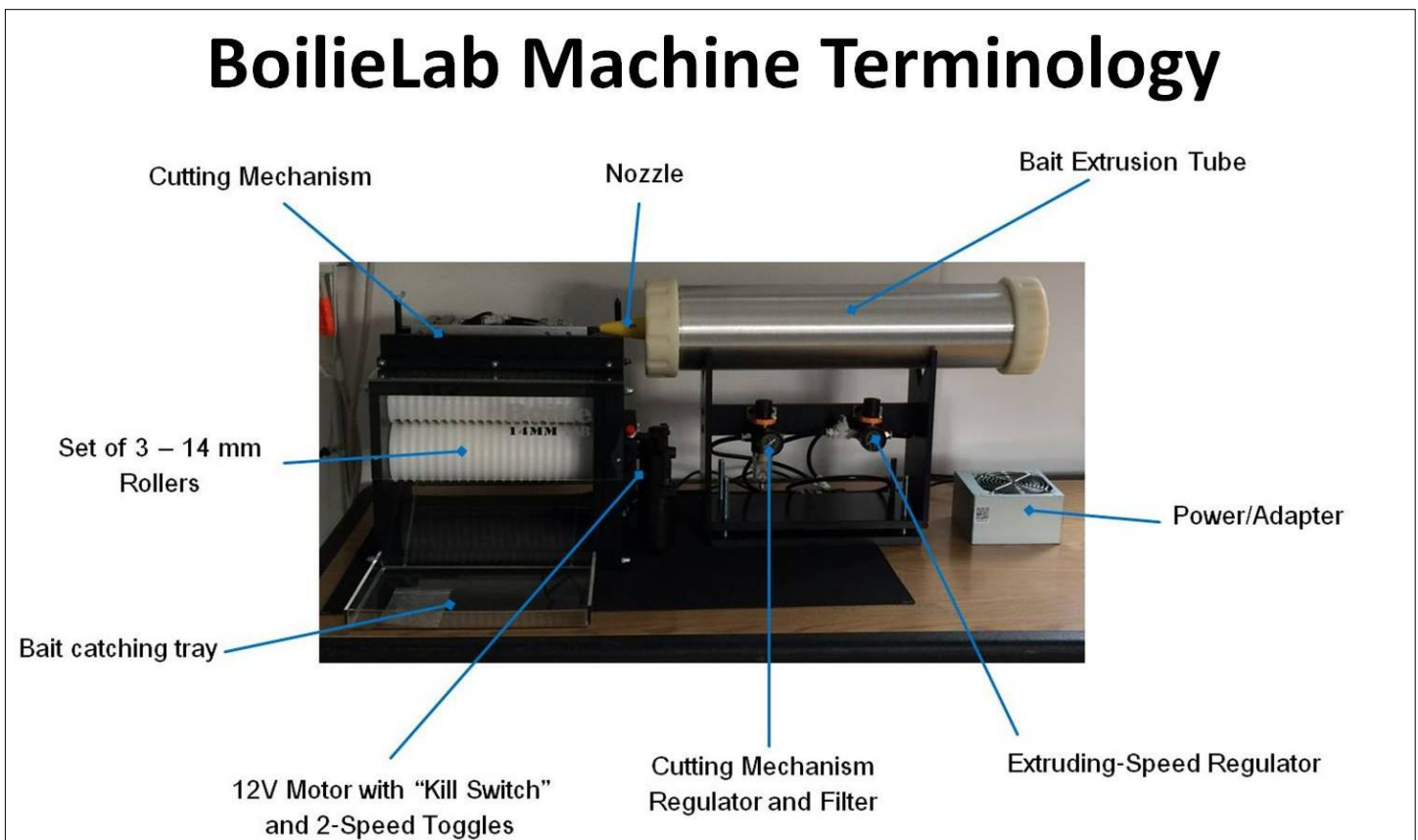


Figure 53. Overview of BoilieLab Machine parts and mechanisms.

Methods

Thermostats for laboratory spaces should be set to 20°C (69°F) and checked daily. Never remove/detach quick coupler pieces until air pressure in the line has been released. Uncoupling a pressurized line may cause injury or damage equipment. Always start with air pressure settings at the lowest possible setting and increase pressures slowly. When using a new batch/shipment of Incortrix make only 1 lot as a test batch to confirm that the bait will extrude and form properly. After 1 successful test, full production (multiple lots/day) may continue.

WARNING

The rollers of the BoilieLab only go in one direction. Use extreme caution when inserting wooden skewers in the roller to prevent clogs. There is an emergency stop button located on the right-hand side of the machine. Be aware of any loose clothing, fingers, and use hair ties when looking above the rollers.

Extrusion Methods and Quality Control

Plug in power transformer/adaptor as shown in the overview image of BoilieLab Machine in Figure 53. Ensure motor and trigger mechanism are plugged into the transformer, and make sure motor functions correctly and is securely in place on machine. Plug in air compressor and make sure hoses are connected properly. Connect air compressor to BoilieLab coupler. Prior to the next step, take and record a temperature of the bait material in the tube on the bait production log. Bait material should be between 17°C and 24°C. If too warm or too cold, check room temperature and set tube aside and allow the material to reach the appropriate temperature.

Select an extrusion tube that was prepared the previous day. Firmly screw end caps on the extrusion tube. One end cap will receive a stainless steel nozzle modified to an internal diameter of 11.40 mm. The other end cap has the coupler for attachment to compressed air and should be attached tightly to prevent pressure from escaping. Place the extrusion tube on the black stand (Figure 54). Align the tip of the nozzle to the edge of the “push bar”. Attach BoilieLab quick coupler to the extrusion tube end cap (be sure the coupler is flush with the cap). At this time, before beginning extrusion, make sure all components are working (i.e., trigger, motor, rollers). In order to properly operate “Cutting Mechanism Regulator”, the left pressure gauge should be set to 30 psi. While the right gauge, “Extrusion Speed Regulator”,

starting pressure should begin around 15 psi. Make sure perforated sheet pan is placed under rollers and labeled with the correct lot number.

In order to start the BoilieLab, engage air flow using the pneumatic line valve. As pressure builds, the plunger in the back end of the tube will begin pushing bait material through the nozzle (Figure 55). Material will exit the nozzle and move along the rollers in a cylinder towards the triggering mechanism. The material triggers the “push bar” that pushes the bait cylinder into the rollers, forming round baits. Be sure to pay attention to bait that may become stuck between the rollers. Use a clean cloth to gently wipe rollers from the back of the machine to prevent sticking. When baits become stuck between the rollers, a small wooden skewer can be placed between the rollers (from the top or front) to dislodge excess bait. Only use wood skewers and use caution when inserting anything in or near the rollers to prevent damage. In case of an emergency, there is a red “emergency stop” push button on the machine. Collect all crumbled, deformed or abnormal baits from the tray and rollers. This scrap material will be placed inside a resealable plastic bag to prevent the bait material from drying out while the rest of the tube is extruded. One 8-kg tube should fill five perforated drying pans.

Once the tube has been fully extruded, flip the pneumatic line valve into the off position and begin depressurizing the tube. With the pressure no longer in the tube, unscrew the end cap containing the nozzle. Firmly push the plunger down towards the other end with a gloved hand creating enough space for the collected scrap material. This material will be reextruded using the methods detailed above. At the end of the

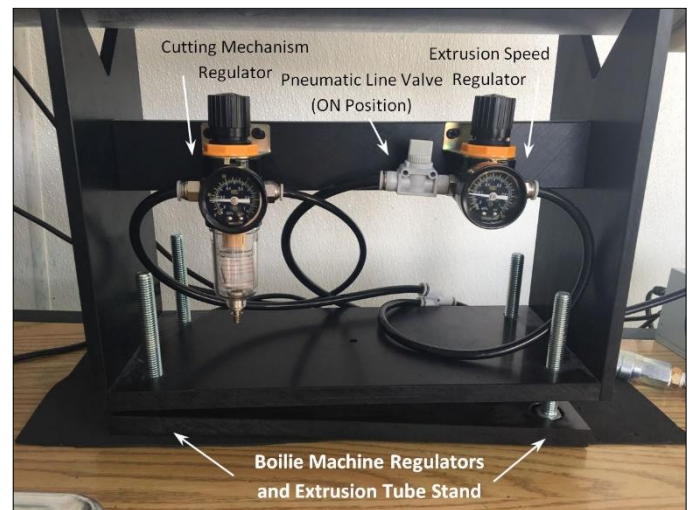


Figure 54. BoilieLab Machine regulators and pneumatic line valve on extrusion tube stand.

process there may still be some leftover material. This will be manually formed by “hand rolling” material into a cylinder and placing it into the rollers. Virtually no material should be wasted. Plastic tray covers should be used when transporting full trays into the proper drying locations before bait can be packaged.

Plague Vaccine Bait Manufacturing Clean-up

When cleaning, PPE (gloves, safety glasses or face shield and N95 particulate mask) should be worn at all times. The only soap that should be used is AJAX bleach alternative and should only be used when cleaning floors and sinks. However, when cleaning BoilieLab machines and formulation equipment, NO soap should be used. In order to clean BoilieLab machines, simply wipe clean any soiled areas with distilled water. When cleaning extrusion tubes, utilize hot water and a scrub brush only. It is also necessary, in order to clean extrusion tubes thoroughly, to tap the plunger from one end of the tube to the other. All equipment, other than the air conditioning unit, should be powered off for the day (it is also recommended that the planetary stand mixer be unplugged).

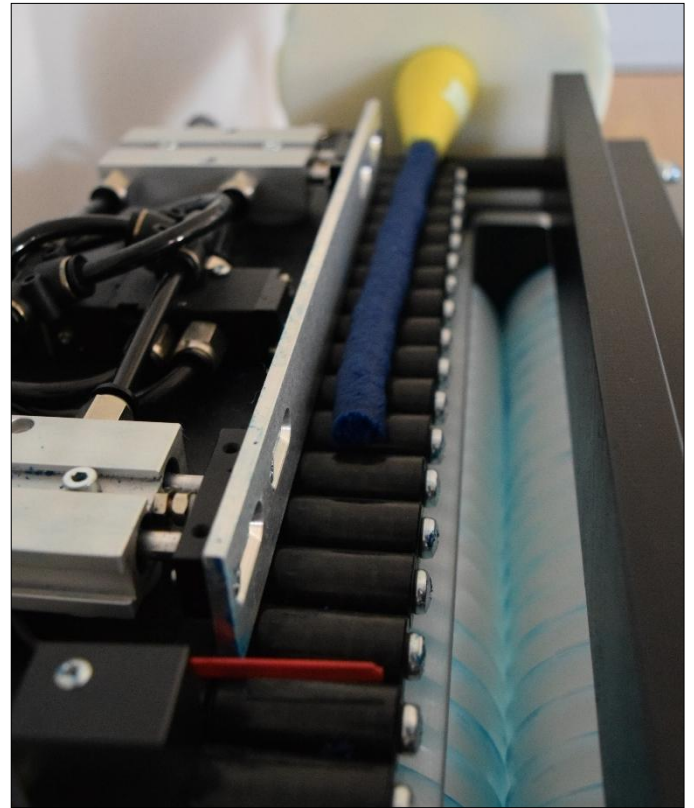


Figure 55. Bait extrusion along rollers.

Packaging and Labeling

WARNING

Vaccine bait contains live virus and PPE (gloves, safety glasses or face shield and N95 particulate mask) should always be used when handling. Always wash hands after handling vaccine. Thermostats for laboratory spaces should be set to 20°C (69°F) and checked daily. Prior to the packaging and labeling process, turn on all scales. Avery large vaccine and placebo bait labels should be printed and stocked in the drying laboratory.

Purpose

To create a detailed record of plague vaccine bait produced and packaged

Materials

- Nitrile Gloves
- N95 particulate mask
- Safety glasses or face shield
- Bait packaging log data sheet
- Permanent marker
- Zip top freezer bags (2-gallon sizes both)
- Large, clear weighing container
- Bait packaging pre-made labels
- 6 kg bench scale
- 2 kg bench scale

Methods

Begin by identifying the bait production lots that have completely dried and are ready for packaging. See whiteboard calendar and weigh 40 baits to get an estimate of individual bait weights. Each individual bait, when properly dried, should weigh between 0.8 and 1.1 grams. Each lot will be packaged into one 2-gallon freezer bag, or four 1-gallon freezer bags, with a pre-made stick on label (Figure 56). Record the lot numbers that will be weighed that day, what type of bait (P or V), staff initials and the date of packing onto the “Bait Packaging Log--2018”.

Be sure all scales are on and zeroed. Place the large, clear weighing container on the 6 kg scale and zero the scale. Slowly pour each tray of bait for one individual lot in the weighing container (Figure 57). (Usually there are

5-6 trays per lot and each tray should be labeled with the lot number.) DO NOT mix lots.

Once all of the bait from one lot is placed into the large weighing container, record the total weight in grams on the Bait Packaging Log in the column labeled “Total Dry Weight (g)”, for the appropriate lot number being weighed. Remove 40 individual baits from the large weighing container. Weigh these 40 baits on the small bench scale. Wait until the scale is done weighing and record the total weight, to the hundredth of a gram, of the 40 baits in the column labeled “40 Baits Weight (g)”.

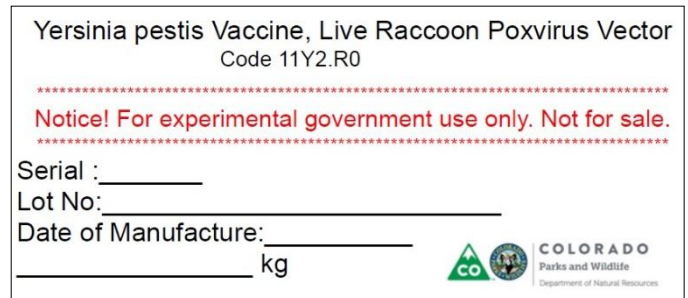


Figure 56. Packaging label for vaccine bait.

Next remove 5 baits for archival storage. To do this, remove 5 baits and place them in a Whirl-Pak®, labeled with the lot number and serial of vaccine (Once all bait has been weighed and archive baits have been bagged, place all archival baits in the right-side -70°C freezer in laboratory.) To bag and label the lot of bait before storage, start by removing the large weighing container of bait from the scale and replace the 40 baits from the previous measurement into the large weighing container.

Place a freezer 2-gallon bag onto the large scale (four 1-gallon bags may also be used). Zero the scale with the bag on the scale to get an accurate weight of bait that is being bagged. Once the scale is zeroed with the bag on the platform, start to slowly scoop bait into the freezer bag. An entire lot will fit into one 2-gallon bag. Record the weight of bait on the bag on label and on data sheet. Double bag the bait and place into the large chest freezer for storage.

Repeat the above steps for each lot of bait needing to be bagged that day. Place all bait into the large chest freezer in the appropriate section (as labeled in freezer).

Storing and Allocating Bait

Storing

Purpose

To appropriately store plague vaccine bait in a way that successfully protects the live viral contents until the time of field distribution.

Methods

All bait must be stored at -20°C (chest freezer inside the bait packaging laboratory space, Figure 58) until the time of field distribution. Bait is stored double bagged in zip bags labeled with lot numbers, serial, date manufactured and total weight.

Allocation

Purpose

To accurately measure plague vaccine bait for field distribution and to make an accurate record of all lots and serials used in the field and at which locations they were used.

Methods

Begin with an accurate list of colonies where bait distribution will occur and precise acreages to be treated. Prepare an Excel MS file with a colony list and accurate acreages. There should also be a cell to enter serials and lot numbers allocated to each colony.

Prior to allocating bait, all bait within each lot number need to be sieved. Sieving helps to remove irregular baits that could cause the 3-shooter to jam. Using the sieve with round holes first and the sieve with rectangular holes second, sieve all bait through the two sieves. Remove all baits that do not fall through the round sieve and remove all baits that fall through the rectangular sieve. All bait removed should be separated and labeled with the correct lot number followed by the letter R to indicate that the bag of bait was rejected from the sieving process.

When allocating, try to utilize the fewest number of vaccine serials and production lots for each site. Do not use multiple serials and several lots for a small site. Try to accommodate allocation with 1 serial and 1 lot for sites <80 acres). Be sure to record all lots used and to which site or colony they were allocated to.



Figure 57. Weighing vaccine bait.



Figure 58. Chest freezer with packaged bait.

Plague Vaccine Use Overview

Availability and logistical considerations

- vaccine and vaccine-laden bait (vaccine baits) are manufactured “as needed”, so allow ample lead time (≥ 6 months recommended) for acquisition
- vaccine manufactured by Colorado Serum Company (Denver, Colorado, USA)
- presently no private commercial source of vaccine bait
- vaccine bait manufacturing details (Corro et al. 2017) are included in the below Plague Vaccine Bait Production section

Necessary approvals

- conditional license requires state veterinary and wildlife management agency authorizations for vaccine purchase/use
- additional federal approvals (e.g., NEPA) may be needed in some locations or situations
- other rules or restrictions on acquisition or use of vaccine/vaccine bait may apply

Vaccine bait storage, shelf life, etc.

- store frozen (-4°C); do not store in a “self-defrosting” freezer
- vacuum-sealed bags preferred (especially for long-term [>1 year] storage)
- recommended shelf life: at least 4 years (may affect palatability)
- OK to refreeze unused bait but minimize repeated freeze-thaw.
- begin thawing immediately before use; bait can be distributed frozen
- avoid prolonged exposure to UV light prior to distribution
- avoid distributing at temperatures $>30^{\circ}\text{C}$ (86°F)

Vaccine bait handling

- avoid exposure to or direct contact with the vaccine virus
- wear disposable gloves when handling vaccine bait.
- wash hands and equipment after bait distribution
- properly dispose of “trash” (i.e., bag up discarded materials)
- handle bait gently to avoid fragmenting (especially important with mechanized delivery systems)

Vaccine application rate

- dose/acre; assumes 1 dose = 1 bait = 1 gram
- for black-footed ferret sites, vaccinate prairie dogs at least 3 months before first planned release; multiple vaccine applications over 1 or more years before release strongly recommended
- apply at least once per year
- for typical prairie dog densities, distribute at 40-50 doses/acre (Tripp et al. 2014; Rocke et al. 2017; Tripp et al. 2017)
- recommended: 40 doses/acre for white-tailed and Gunnison’s prairie dogs; 50 doses/acre for black-tailed prairie dogs
- higher application rates may be needed if prairie dog density is exceptionally high
- minimum recommended application rate is 25 doses/acre

Timing of application

- seasonal and within day; weather constraints, etc.; Tripp et al. 2014, Abbott et al. 2017
- time application to maximize uptake by prairie dogs.
- relatively dry forage conditions favor uptake
- uptake may be higher in autumn in many locations
- for hibernating species, distribute ≥ 3 weeks before onset
- begin bait distribution in early morning; avoid late afternoon/evening applications
- avoid distribution when weather may reduce above-ground prairie dog activity

Spatial footprint (minimum acreage; minimum proportion of acreage; Rocke et al. 2017; Tripp et al. 2017)

- for operational black-footed ferret recovery sites $<1,500$ acres, the entire colony complex should be treated
- for sites $\geq 1,500$ acres, a contiguous core of at least 1,500 acres should be treated
- Whole complexes, whole colonies and large area treatments are likely more effective
- for experimental assessments, smaller subdivisions of sites may be justifiable
- for other conservation purposes, different criteria or considerations may apply

Spatial distribution of vaccine within plots (Tripp et al. 2014; Rocke et al. 2017; Tripp et al. 2017)

- distribute bait systematically and uniformly to assure optimal uptake by prairie dogs
 - recommended: distribute every ~10 meters (~30 feet) along parallel transects ~10 meters (~30 feet) apart.
 - option: distribute 4 baits within 1 meter (3 feet) of opening of each active prairie dog burrow (not efficient on large sites ≥ 40 acre)

Record keeping

- minimum: record vaccine lot and bait lot number, date received; date, time completed; amount, location, ambient temperature
- site/system-dependent: distribution rate and method used, track logs/spatial GIS data, etc.

Monitoring and assessment

- minimum: annual assessment of prairie dog activity/occupancy and spatial footprint/area occupied
- enhanced: prairie dog abundance or density
 - methods: indexes (count active burrows, animals); mark-resight or recapture
- optimum:
 - black-footed ferret occupancy, abundance, or density (applies to ferret recovery sites only)
 - methods: indexes (e.g., spotlight count); mark-resight or recapture; survival (e.g., vaccinated vs. unvaccinated ferrets)
 - plague surveillance
 - methods: opportunistic (e.g., carcass testing); systematic flea surveys (burrow swab, serology (prairie dogs, ferrets, other carnivores))
 - vaccine uptake
 - methods: dyed scat (qualitative, quantitative); bait removal; serology
- enhanced and optimum approaches could be annual or at intervals (e.g., every 3–5 years)
- adaptive management experiments and controlled field experiments encouraged wherever feasible to help advance understanding of vaccination specifically and plague control in general (Rocke et al. 2017; Tripp et al. 2017)

Plague Vaccine Field Distribution, Data Management and Monitoring

Manual Bait Distribution

Purpose

To distribute plague vaccine bait on prairie dog colonies in a systematic and uniform manner with the intent of maximizing the number of prairie dogs that find and consume a bait, i.e., distribute bait along transects (10 m between transects), dropping baits at pre-set intervals, to achieve the desired bait density or baits/acre (~8 meter spacing for 50 baits/ac and ~10 meter spacing for 40 baits/ac).

Materials

- GPS loaded with transects and colony boundaries
- Frozen allocated bait in coolers
- List of transects and #baits/transect
- Nitrile gloves
- Bait measuring cup (1 cup-holds approx. 100 baits)
- Measuring tape
- Zip top bags
- Extra AA batteries
- Marking flags
- Permanent markers
- Laptop with transect files for backup
- Printed map of colony (helpful/optional)

Methods

Bait distribution should occur in the morning to encourage prairie dogs to consume bait on the day of distribution and prior to nocturnal small mammal

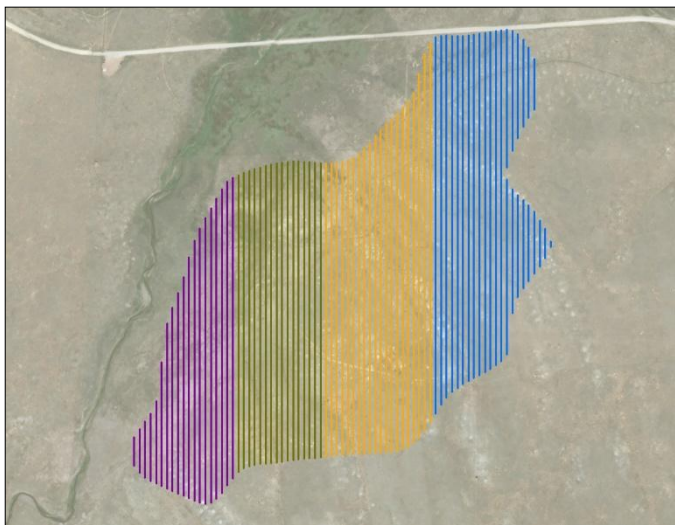


Figure 59. Visual of four groups of transects for four technicians grouped spatially.

activity. Bait distribution should not occur if inclement weather is forecast. Avoid bait distribution on days with warmer or colder than average temperatures. Bait must remain frozen and in coolers prior to distribution in the field.

Place a measuring tape on the ground and measure the correct number of steps each technician will need to take between baits (usually 8 or 10 meters). Each technician will walk the distance, counting the number of steps needed to cover the distance. The number of steps necessary to cover the distance will vary by each technician's gate and the terrain.

Assign transects to each crewmember using the total distance walked rather than by the number of transects (it is best to group transects spatially, (Figure 59). Turn assigned transects on one at a time in the GPS by selecting show on Map. This allows the technician to see his or her assigned transects and where bait will need to be distributed. Locate the bait that has been allocated to the colony (there may be several bags). Wearing nitrile gloves, collect the amount of bait needed to complete all assigned transects. Use a measuring cup (1 cup \approx 100 baits) or digital kitchen scale to measure grams of bait needed for each technician and place bait into a zip top bag to assist with accurate distribution.

Go to the first transect, turn the GPS "tracks on" and begin walking the assigned transect, distributing bait at the prescribed distance (usually every 8 or 10 meters). The transects are a guide to help with accurate and systematic bait distribution. It is not necessary to walk exactly on the transect line. Walk quickly and glance at the GPS unit regularly to make any necessary course corrections. It may be useful to locate a marker on the horizon that is in line with the transect and use the marker for rough visual orientation. If a spatial record of bait distribution locations is needed for mapping or bait removal monitoring it will be necessary to mark a waypoint at each bait distribution location (or at a designated subset of locations). Continue this process until all assigned transects are completed. All bait allocated to a colony should be distributed on that colony. If some bait is "leftover" after distribution (usually because a technician under-distributed while counting steps) the remaining bait may be distributed in high burrow density areas within the target colony. Communicate with other technicians. If one technician has "leftover" bait another may have had too few and will need the remaining bait to complete distribution on their assigned transects.

Some large sites will have too many transects (≥ 200) to load onto a single GPS unit. In this case, the transects will be divided spatially into groups and these groups will be loaded onto individual GPS units. Technicians need to ensure they have their assigned transects on their GPS prior to beginning.

In many cases, GPS data will need to be downloaded and saved on a field laptop when finished with a site. After GPS data is successfully downloaded, the GPS units can be cleared and a new set of assigned transects can be uploaded. When transects are completed for the day, fill out a bait distribution log sheet (in Appendix 1) that tracks the amount, type and lot number of bait distributed at every individual colony. Include any comments or notes and explain why bait was leftover or not distributed. Any leftover or unused bait must be weighed and entered on the log sheet, including lot numbers and dosage. Unused bait must be kept frozen to maintain vaccine viability. If bait is thawed, they will still be viable if kept cool and distributed in the field within 48 hrs. If distribution will occur >48 hours after bait is thawed it should be refrozen and reallocated. Also, fill out a site visit form (in Appendix 1) at each colony.

ATV Bait Distribution

Purpose

To distribute plague vaccine bait on prairie dog colonies in a systematic and uniform manner. Distribute bait along transects (30 m between transects) with a “3-shooter” mounted on an ATV. Bait is distributed at pre-set intervals, to achieve the desired bait density or baits/acre (~8 meter spacing for 50 baits per acre and ~10 meter spacing for 40 baits per acre). Drive transects, distributing baits every set number of meters, to achieve a certain number of baits per acre.

Materials

- GPS loaded with transects and colony boundaries
- Frozen sieved bait in coolers
- List of transects and #baits/transect (optional)
- Nitrile gloves
- ATV gas can
- Air compressor to fill ATV tires
- ATV handlebar GPS mount
- 3-Shooter
- Charged 3-shooter battery
- Plywood 3-shooter mounting platform
- Crescent wrenches and ratchet straps
- Medium cooler for bait storage and ratchet strap
- Bait measuring cup or scale
- Zip top bags
- Extra AA batteries
- Laptop with transect files (optional)

Methods

Randy Matchett (USFWS) and Kurt Kreiger (Model Avionics) designed the 3-shooter prototype and developed initial instructions for its field use.

Once at the site, strap the 3-shooter mount to the front of the ATV using ratchet straps or heavy-duty black rubber bungie cords. Ratchet the mount down tightly and anchor the straps to solid points on the ATV. To avoid driving over the ratchet straps, tie loose ends of the ratchet straps off (Figure 60). Load pre-sieved and allocated bags of frozen bait into a small cooler and attach to the ATV rack behind the driver with a ratchet strap. Do not overload the cooler and treat bait with care to prevent damaging or crushing the bait. Attach a GPS unit loaded with transects to the handle bar mount on the ATV. Attach the 3-shooter to the ATV mount using bolts; wing nuts and a crescent wrench (do not over tighten bolts). Be sure to orient the 3-shooter towards the driver correctly (Figure 61). The pause switch and counter should be facing the driver. Proper alignment

will result in the “center bait” contacting the outer edge of the left ATV fender and bouncing outward to the ground when distributed. This will create a visible event for the driver to see and hear.

Once everything is tightened down and secure. Shut the ATV off and plug in the 3-shooter battery to the power port on the side of the 3-shooter (Figure 62). The plug end of the battery is yellow and will only fit into the 3-shooter one way. When the battery is plugged in, the machine will start to find a GPS location. The wheels on



Figure 60. ATV ready for bait distribution. Cooler and three shooter strapped down securely and GPS mounted to handlebars.



Figure 61. Side view of 3-shooter. Baits will fall from the center and bounce off the fender of ATV.

the left and right of the machine will begin to rotate. The red and green LED lights will be flashing and eventually become solid when a GPS location is found. It is very important for the unit to be absolutely still (ATV motor turned off) and have a clear view of the sky until GPS fix is reliable, as indicated by the green LED light turning solid.

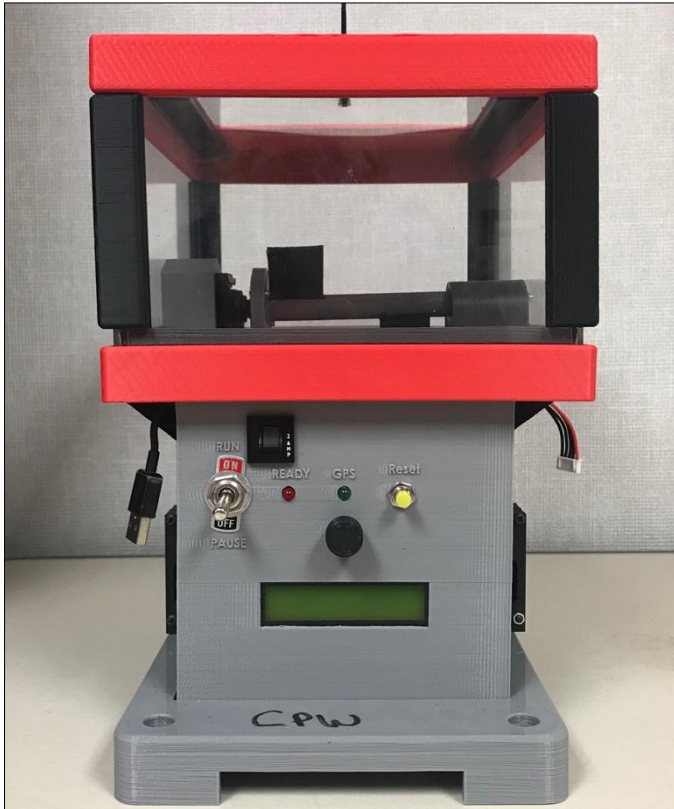


Figure 62. 3-shooter: large switch pauses the machine, black knob will cycle when bait is distributed, and yellow button resets the count which is displayed in the screen. The red and green LED lights indicate satellite reception and power.

When the lights turn solid, the 3-shooter has been located using satellite GPS and is ready for operation. This may take several minutes. While waiting, load approximately 1 kg of frozen sieved bait (1000 baits) into the hopper. Before starting, turn the Pause switch from OFF to ON.

Start driving and observe that the black knob protrudes outward and retracts back in. When driving, observe left and right that vaccine baits are being thrown out. Also observe that bait is being distributed out of the bottom of the 3-shooter. The counter on the 3-shooter displays the number of baits that have been dispensed through each of the three tubes. The counter should continuously tally the distributed baits and is the most visible indicator if there is a jam in any of the 3 tubes.

Operator vigilance is imperative. A jammed machine will quickly burn out an actuator motor and disable the machine. If at any point the black knob stops cycling, you detect 3 baits are not dropping at each trigger, or the counter stops counting, immediately stop driving and turn the pause switch to OFF and unplug battery. If it is jammed, try to manually pull the knob out. This method may release the jam but if it does not clear the bait, remove the dispenser from the mount and tip the 3-shooter over (removing all bait) and continue to push and pull at the knob. A jam occurs as a result of having large or misshapen baits attempt to go through the machine, or smaller fragments wedging in the tubes with a normal-sized bait. It is important to sieve all bait. After sieving, large or misshapen bait will be removed and should reduce machine-jamming.

When transects are completed, turn the pause switch to off and unplug the battery when not in use (Figure 62). The on-off switch only controls the trigger mechanism and stirring rod. If battery is left plugged in, it will lose its charge (permanently damaging the battery) and continue to collect location data. Charge batteries each night. **Never let the battery discharge below 13.5 V.**

All bait allocated to a colony should be distributed on that colony. If some bait is “leftover” after distribution (usually because a technician under-distributed while turning corners between transects) the remaining bait may be distributed in high burrow density areas within the target colony. Communicate with other technicians. If one technician has “leftover” bait another may have had too few and with need the remaining bait to complete distribution on their assigned transects.

Remove the 3-shooter mount from ATV before trailering and store for transport. When finished with bait distribution, fill out a bait distribution log sheet (Appendix 1) that tracks the amount, type and lot number of bait distributed at every individual colony. Include any comments or notes and explain why bait was leftover or not distributed. Also, fill out a site visit form (Appendix 1) at each colony. Any leftover or unused bait must be weighed and entered on the log sheet. Unused bait must be kept frozen to maintain vaccine viability.

3-Shooter Lithium Battery Care and Safety

Modern Lithium Polymer batteries (LiPo, Li-Poly) are used to store and deliver large amounts of energy from lightweight packs. Always treat LiPo batteries as fuel. Lithium Polymer cells must be handled with appropriate precautions and care. There is a risk of accidental fire if the battery is improperly stored or charged.

Always

- **LiPo battery fires cannot be extinguished with water.** If a fire occurs, cover it with either sand or salt. **NEVER use water to extinguish a LiPo battery fire.** Water acts like fuel on a LiPo battery fire.
- Only use a charger specifically designed to charge Lithium Polymer cells (imax B6AC, Figure 63). Failure to do so may cause fire, which may result in personal injury and/or property damage.
- Check the voltage of your LiPo pack before charging, only charge if it is 3.30V or greater per cell (e.g. 13.2V for a 4S pack).
- Only charge in area away from other flammable materials and on a non-conductive and non-flammable surface. Use a LiPo Guard bag or a non-conductive container like a glass dish with a lid as it may help contain any fire.
- If a cell ‘puffs up’ disconnect it immediately and cover it in sand or place it in a nonconductive container, outside and away from all flammables for at least 30 minutes.
- If charging indoors, only charge in a room with a smoke detector.
- Manually check the temperature of your LiPo battery pack during charging. LiPo cells should never get warm when being charged.
- If any of the individual cells become warm/hot disconnect it immediately and cover it in sand or place it in a nonconductive container, outside and away from all flammables for at least 30 minutes.
- Store working LiPo batteries at room temperature between 5°C and 27°C (40°F and 80°F). The lower the temperature the better.
- If you plan to store your LiPo cells for an extended period (over 1 month) then discharge them as you would normally. Then charge them to only 3.80-3.85 V per cell.
- Physically disconnect batteries from all equipment to prevent slow over-discharge. The 3- shooter on/off switch does not stop slow discharge.

- Do ensure connectors are insulated correctly to prevent short circuit during storage.
- Do always check that batteries are physically and electrically undamaged before charge or discharge.

Never

- **Do not charge cells that have been stored in cold conditions (below 10°C/50°F), let them warm up (above 10°C/50°F), for a minimum of 30 minutes.**
- Never to use cells that have been stored at or below 0°C (32°F). Warm them first for at least 60 minutes above 10°C (50°F) before charging or discharging.
- **Do not use a charger that is not specifically designed to charge Lithium Polymer cells.**
- Do not leave cells un-attended when charging. You should always monitor charging.
- Do not charge LiPo batteries while still in the 3-shooter.
- Do not charge LiPo batteries in a vehicle.
- Do not carry LiPo batteries in your pocket. They have been known to short against coins and keys and cause fires.
- Do not puncture the cells.
- Do not short the wires of your LiPo cells/battery. Shorting can cause a fire or explosion. If you accidentally short the wires of a LiPo battery cover it in sand or place it in a nonconductive container, outside and away from all flammables for at least 30 minutes.
- Do not “Top up” your cells even if you charged them a few days ago. LiPo cells loose less than 1% of their capacity per month. Just use and then re-charge after use.
- Over-charging LiPo batteries will damage them and possibly cause a fire.
- Do not store your cells (more than 1 hour) in a hot place (i.e. in your car on a hot day) as this may cause them to catch fire.
- Do not charge hot cells. Warm cells are OK, let hot cells cool before charging.
- Do not allow charging to continue above 4.25V per cell.

- Do not discharge LiPo cells below 3.00V per cell (e.g. 9.00 V for a 3-shooter pack). This will damage the cells and reduce the life of your battery. If you accidentally discharge a battery below 3.00 V per cell, leave it for 30 minutes, if the voltage recovers to over 3.30 V per cell it should be OK to use again. If it does not recover to at least 3.00 V per cell, you have damaged the battery and it should be disposed of.
- Do not charge dissimilar or un-matched (brand) packs together.
- Do not charge any pack containing damaged or swollen/puffed cells.

Disposing of Lithium Polymer Cells

Discharge the battery to 0.0 V/cell. Make sure any output wires are insulated and then recycle the battery at a local recycling facility.

Storage of LiPo Batteries

Store batteries at 40-50% capacity. A 3-shooter battery should be stored between 14.9 and 15.3 volts. Store the battery in a cool place to help with the life of the battery. Refrigerators work great. If a battery is new (never been charged) it is already at storage charge voltage. Kurt Kreiger (Model Avionics) designed the 3-shooter prototype and developed initial recommendations for battery care.



Figure 63. Imax B6AC Lithium Polymer (LiPo) 3-shooter battery charger.

Updating Driver Software Instructions

To detect and connect with the 3-shooter, the computer's driver software may need to be updated. Contact Wildlife Health Program staff for a copy of the file needed to perform the update. Instructions below detail updating process for Windows 7.

- Save file ("arduino.inz") and rename to "arduino.inf".
- Plug the 3-shooter into a USB port.
- Red and Green LEDs should light up
- Using control panel> System and security> System> device manager, look for "other devices" and under it "Arduino MEGA 2560". There will be a yellow exclamation point (!) overlaying the icon. The list is in alphabetical order. Look between Network adapters and Processors. It may also be under Com ports. Just look for the yellow exclamation.
- Right click the Arduino/Atmega icon and select "update driver software".
- Click "browse my computer"
- Click "Let me pick..."
- Select "Multifunction adapters" then click next
- Under manufacturer, click "Generic..."
- Under model, click "Generic..." Then click "Have disk"
- Browse to where you saved the attached Arduino.inf file
- You should now be back in the "Install from disk" dialog box. Click OK
- You should now be back in the "Update Driver software- arduino mega 2560" dialog. Click next.
- Microsoft will now try to stop you from installing this driver. You may click "Install anyway..."
- About halfway through the wait you will hear a USB plug in sound.
- Click close
- Open Mission Planner and check that the COM port is correctly set to AUTO or to the port in use.

Changing Triggering Distance on the 3-shooter

Purpose

To change the 3-shooter trigger distance depending on species and colony location, e.g., to achieve a bait density of 50 baits per acre, trigger distance must be set to 8 meters and for 40 baits per acre, trigger distance must be set to 10 meters.

Materials

- 3-shooter
- USB to USB cord
- Computer with Mission Planner and correct driver installed

Methods

Begin by opening Mission Planner (MP). Always select **NO** when prompted for updates or by the wizard. To change the distance between triggers, plug the 3-shooter in to the computer. Once the Red light stops flashing, select CONNECT in MP. A message will pop up that says, “a new version of firmware is available”. Do not click OK. Exit out of the prompt. Once the 3-shooter is connected, select the CONFIG/TUNING tab near the upper left corner of the MP window.

On the left side of the window, select FULL PARAMETER LIST. Scroll down to CAM_TRIGG_DIST which is under the COMMAND column (Figure 64). Change the value to the correct distance and then select WRITE PARAMS on the right-hand side of the window. This writes your selected values to the controller in the 3-shooter. The trigger distance is now set. Select DISCONNECT and unplug the 3-shooter from the computer. Once the trigger distance is changed, it will remain at that distance until the trigger distance is changed again.

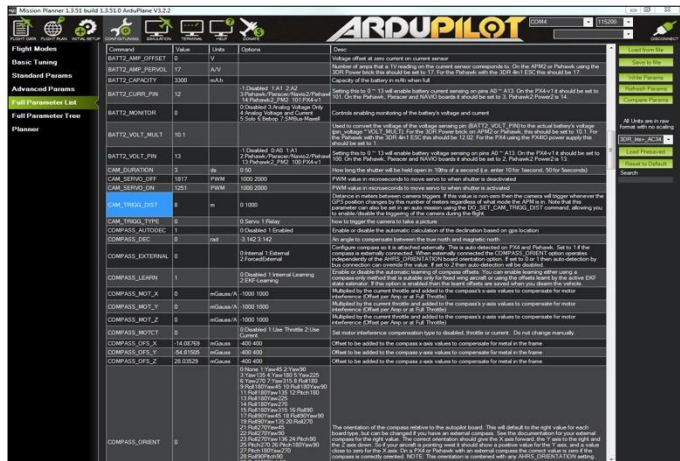


Figure 64. Mission Planner open to Full Parameter List and connected to 3-shooter.

Downloading and Clearing 3-shooter Data

Purpose

After distributing bait in the field using the 3-shooter, the data may be taken off and cleared.

Materials

- 3-shooter
- USB to USB cord
- Computer with Mission Planner and correct driver installed

Methods

After returning from the field, data on the 3-shooter should be cleared before the 3-shooter can be used again. Start by opening the Mission Planner (MP) program (Figure 65). Always select **NO** when prompted for updates or by the wizard.

Now, plug in the 3-shooter to the computer using the USB to USB cord. Green and red lights will begin to flash and wheels will spin slowly. Make sure the wheels are not obstructed. Possibly only the Red LED will become solid if you are in a structure or office performing download. Green may continue to flash and that is OK.

Click on the COM port selection and set to AUTO, then click the CONNECT button in the upper right corner of the MP screen. MP will now begin to connect to the 3-shooter device; this may take a few minutes. A message will pop up that says, “a new version of firmware is available”. Do not click OK. Exit out of the prompt.

Click on the Config/Tuning tab and near the bottom of the page, use the Log Path entry to browse to and select the folder/location on your computer for where you want the log files to be downloaded to. Click the scroll right arrow in the lower left window pane of Mission Planner under on the horizon/attitude display until the DATAFLASH LOGS tab is visible. Click the DATAFLASH LOGS tab then select DOWNLOAD DATAFLASH LOG VIA MAVLINK. A new window will pop up that reads “Getting list of log files...”, which means Mission Planner is now downloading the log files from the 3-shooter. Log files will eventually appear on the left-hand side of the window (Figure 66). Select DOWNLOAD ALL LOGS and wait until all logs are downloaded, this will take several minutes. The data is downloaded when the message “Download complete” is shown.

When the data is downloaded, it needs to be copied to another location. Navigate to the folder Local Disk (C:) > Program Files (x86) > Mission Planner > logs > FIXED_WING > 1. The folder named “1” contains the data downloaded from the 3-shooter. If another set of 3-shooter data are downloaded, the contents of the folder named “1” will be replaced; therefore, new data must be copied to a new location. Copy this folder and paste it into a new folder location such as a Google Drive to be processed at a later date.

Now the data must be cleared from the 3-shooter. In the LOG DOWNLOADER window, select CLEAR LOGS and then select OK. Wait a few minutes for the data to clear. To verify logs have been cleared, select download again. The MP response should be “no log files found”. Once the data is cleared, select the DISCONNECT button on the MP window. The data stored on the 3-shooter is cleared and is now ready to use again.

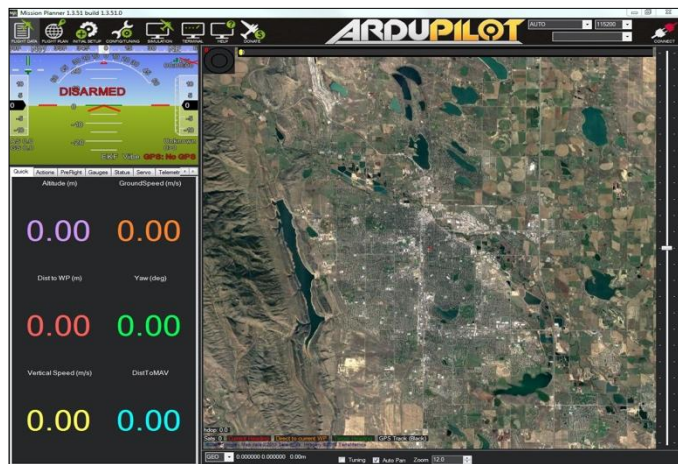


Figure 65. Mission Planner main window.

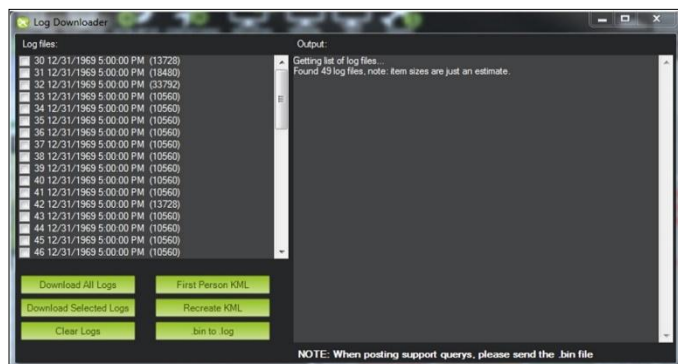


Figure 66. Log file download menu. Button to download all logs.

Manipulating and Creating Data from 3-shooter

Purpose

To convert downloaded text file data from the 3-shooter and create a shapefile in ArcMap. The overall goal is to create, X, Y, coordinates from trigger locations with a time stamp.

Materials

- Computer

Methods

Randy Matchett (USFWS) and Kurt Kreiger (Model Avionics) designed the 3-shooter prototype and developed initial instructions for its field use and data processing.

Navigate to the folder that the 3-shooter data resides in. Copy the entire folder and paste the folders contents into a new working folder. In the newly created folder containing the 3-shooter data, sort the files by TYPE to group the files into similar file types.

Observe the files with the format 2017-09-26 12-25-17 (file name will change based on the date of bait distribution), this is a text document with the file extension .log (this can be determined by right clicking any of these files and selecting PROPERTIES and observing the TYPE OF FILE, which should say “Text Document (.log)”). The .log files are the only files needed from the batch files Mission Planner provides.

This can also be done in windows Explorer, specify to do not hide file extensions and you will see the .log as part of the filename. The goal here is to have all downloaded files with the simple file extension of *.log, none with “bin” in the root name, in a folder by themselves.

Give the files with the format 2017-09-26 12-25-17 a new name. A new name is needed because the COMMAND PROMPT window will not recognize files with spaces in the file name. To do this, double click on the file name and change it to a desired name with no spaces. It is recommended that file names be changed to something as simple as 1, 2 and 3. Do this for all text files with the follow file name format and note the number of renamed files; 2017-09-26 12-25-17). The number of files renamed is necessary for later steps.

Select the newly named files by clicking the first file in the list. Hold CTRL key and click all remaining renamed files. When all files are highlighted, hold down the SHIFT key and right click one of the highlighted files and select COPY AS PATH. If the SHIFT key is not held down, only the COPY option will appear. The SHIFT key must be held down for the COPY AS PATH option to appear.

Paste all selected files into a new notepad file. Number the files with their pathways in a format similar that shown (Figure 67).

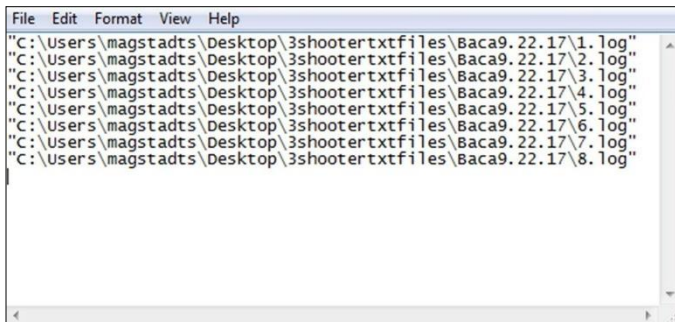


Figure 67. Notepad file with pathway to numbered log files.

In the notepad file, delete the beginning of the file pathway (ex. "C:\Users\magstads\Desktop\3shootertxtfiles\Baca9.22.17\") and the end quotation mark ("") of these pathways. In the notepad, click the EDIT tab and select the REPLACE... option. Copy the first part of the pathway ("C:\Users\magstads\Desktop\3shootertxtfiles\Baca9.22.17\") and paste it into the FIND WHAT: text field. Because this text is replaced and not deleted, leave the REPLACE WITH: text field blank. Click the REPLACE ALL button and observe that the beginning piece of the pathway is no longer there. At this point the text should look similar to (Figure 68):

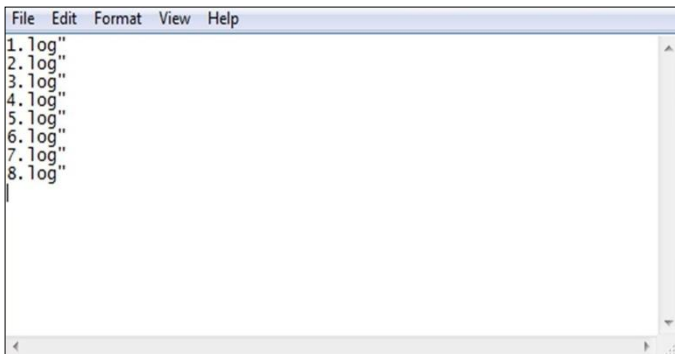


Figure 68. Notepad file of numbered log files with quotation marks.

The quotation mark at the end of each line needs to be replaced. To do this, click the EDIT tab and select the REPLACE... option. In the FIND WHAT: text field type "" and leave the REPLACE WITH: text field blank. Select REPLACE ALL and observe that the text file no longer contains any quotation marks. At this point the notepad file should look something similar to (Figure 69).

Now these files need to be prepared for the Command Prompt. The point of this next step is to combine each individual folder into one large text file so it is easier to work with and manipulate. In the Notepad window, set up the text files in the following format:

1.log + 2.log + 3.log + 4.log + 5.log + 6.log + 7.log + 8.log

Make sure there is a space between the file name and the plus sign (filename SPACE + SPACE nextfilename SPACE + SPACE nextfilename).

Open the Command Prompt window and setup the pathway the Command Prompt you will use. To do this, first open the Command Prompt window and make sure it is pinned to the computer's taskbar. To pin it to the taskbar at the bottom of the computer screen, right click on the program icon in the taskbar and select PIN TO TASKBAR. Now right click on the Command Prompt icon on the taskbar and right click again on the COMMAND PROMPT text and select PROPERTIES.

View the SHORTCUT tab within the Command Prompt Properties window. In the text box LABELED START IN: type the file pathway that corresponds to the original data set. In this example the pathway is deleted from the notepad (C:\Users\magstads\Desktop\3shootertxtfiles\Baca9.22.17\).

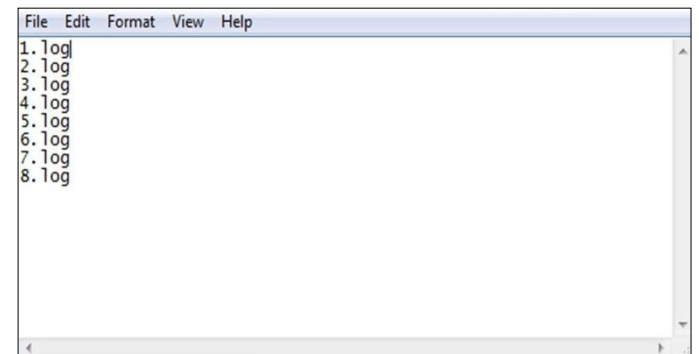


Figure 69. Notepad file of final numbered log files.

If the pathway is unknown, it can be found by navigating to the original data folder and right clicking any one of the several files in that folder and selecting **PROPERTIES**. In the file **PROPERTIES** window, make sure to select the **GENERAL** tab. From this page, observe the pathway for the files being used. Copy the text next to the **LOCATION:** field from the original dataset. This will be used as the pathway in the Command Prompt. Paste that pathway into the **START IN:** text field on the Command Prompt Properties window and hit **APPLY**.

Now the Command Prompt window will be directed to the folder with the copied data removed from the 3-shooter. Open a new Command Prompt window and make sure the directory is set to the desired folder location. It should look similar to this:

```
C:\Users\magstadts\Desktop\3shootertxtfiles\Baca9.22.17> _
```

The underscore at the end should be blinking.

Next combine each individual .log file into one easy to work with .txt file. To do so, type copy in the command prompt followed by a space. Copy, right click and paste the string of files made earlier similar to this:

```
1.log + 2.log + 3.log + 4.log + 5.log + 6.log + 7.log + 8.log
```

After the last log file hit space again and type the desired name for the newly created file followed by .log. The entire command prompt text should be as follows:

```
C:\Users\magstadts\Desktop\3shootertxtfiles\Baca9.22.17>copy 1.log + 2.log + 3.log + 4.log + 5.log + 6.log + 7.log + 8.log anygivenname.log
```

Remember to leave a space after copy, before and after each + and before the anygivenname.log. Hit enter and make sure the command prompt says 1 file(s) copied. The newly created combined text file can now be viewed where the command prompt was directed to (ie.C:\Users\magstadts\Desktop\3shootertxtfiles\Baca9.22.17).

Once in that folder, the file that was created from the Command Prompt can be opened. It is recommended to check the size of the file. Make sure the file is not too large or too small. It should be the sum of all the individual .log files used.

Now, navigate to the Google Drive > 2017 folder. It is here that a template excel file named 3shooter_CAM_GPS_TEMPLATE can be found.

Copy the template excel file from the Google Drive and paste it into the working folder. Do not use or change the excel file in the Google Drive because it is a template with formulas.

Open the template excel file. This excel file should have two tabs labeled 3Shooter_CAM_GPS_Template and 3Shooter_CAM_GPS_Template as well as a tab labeled Sheet 1. Select the tab labeled Sheet 1 and click the **MICROSOFT OFFICE BUTTON** in the top left corner of the excel window. Click **OPEN** and navigate to the combined text file created earlier (remember to change the dropdown menu in the lower left corner from All Excel Files to All Files).

Once the desired file is found, click **OPEN**. A text import wizard prompt will pop up. Make sure **DELIMITED** is checked, not **FIXED WIDTH**, and click **NEXT**. Now make sure the **COMMA** box is checked and the **TAB** box is unchecked and click **NEXT AND FINISH**.

Select the entire data set by clicking the box to the left of column A and above row 1. When all of the data is highlighted blue, right click that box and navigate to **SORT** then select **CUSTOM SORT**. Sort by Column A—Sort on Values—Order A to Z and click **OK**.

Starting at Row 2 in column A, a series of points labeled CAM should be visible. These are point locations where baits were distributed due to camera triggers (for every on trigger point there were three baits distributed). Select all of the rows labeled CAM in column A and copy them. It is important to copy all rows labeled with CAM but only copy Column A through Column J.

Once the CAM points are copied, navigate to the Tab labeled 3Shooter_CAM_GPS_Template and paste that information into cell A2.

Make sure Columns K-P are filled in to the last row of the CAM points. This template only goes to Row 121, so it is necessary to drag Columns K-P down to the last row of data to fill in missing time and date information.

It is important to correct the timestamp (Column Q) so ArcGIS can read the data as a text attribute. Select the time in cell P2 and scroll down until the last timestamp in Column P. Copy those cells and paste them into a new Notepad. Copy all of the times in the notepad and paste them into cell Q2. Column R is a unique identifier and will be used when looking at the data in ArcGIS. Make sure to drag that column to the last row of the CAM dataset.

It is necessary to copy the GPS point locations. The GPS point locations are locations of the 3-shooter taken every tenth of a second. There will be many more GPS locations than there are CAM points.

Starting at a random row in column A, a series of points labeled GPS can be seen. This essentially acts as a record of tracks where the 3-shooter was used. Select all the rows labeled GPS in column A and copy them. It is important to copy all rows labeled with GPS but only copy Column A through Column N.

Once the GPS points are copied navigate to the tab labeled 3Shooter_CAM_GPS_Template and paste the copied information into cell A2.

Make sure Columns O through U are filled in to the last row of the GPS points. Our template only goes to Row 3914, so Columns O-U needs to be dragged down to the last row of data to fill in missing time and date information.

The timestamp needs to be corrected (Column U) so ArcGIS will be able to read it as a timed attribute. Select the time in Cell T2 and scroll down until the last timestamp in Column T is reached. Copy those cells and paste them into a new notepad. Copy all of the times in the notepad and paste them into cell U2.

Column V is a unique identifier and will be used when looking at the data in ArcGIS. Make sure to drag that column to the last row of the GPS data. Save the excel file to the current working folder and close it.

Now, open ArcMap and click BLANK MAP template and click OK. Before the data can be viewed, ArcMap needs to connect to the folder that contains the desired dataset. Click on the CATALOG tab which is usually located along the right side of the ArcMap window. At the top of the CATALOG pane there is a folder symbol with a + symbol. Select that icon and navigate to the folder containing the 3-shooter data and click OK.

This folder can now be seen in the CATALOG pane. Click the + box to the left of the folder and view the contents of the folder. The excel file should be visible amongst the other files. Click the + and the individual tabs that the excel file contains will appear.

Right click on the file labeled 3Shooter_CAM_GPS_Template and navigate to Create Feature Class and then click From XY Table. For the X Field, select LNG from the dropdown list. For the Y Field, select LAT from the dropdown list. Click the Coordinate System of Input Coordinates and click the Geographic Coordinate Systems, then World, then select WGS 1984 and click OK. Click the folder icon next to the output text box. Navigate to the desired file location to save the shapefile. In the Name: text box, label it something descriptive. For the Save as type: click the dropdown menu, select the Shapefile option and click Save and click OK.

Monitoring Bait Removal

Purpose

To determine the rate of bait removal a number of baits (usually 50) will be physically marked with a flag and the location recorded via GPS. Marked baits will be monitored daily after initial distribution to determine rate of bait removal. This procedure may be used if bait removal data is needed.

Materials

- GPS with bait points loaded
- Pencil/pen and notebook/bait check sheet
- Radios (optional)
- Pre-labeled flags with extras

Methods

To mark bait, divide the number of baits to be marked by the number of transects used for bait distribution then select the baits along each transect for marking at random. Example: 50 marked baits divided by 25 transects = 2 marked baits per transect. Select 2 baits in each transect to mark at random. The random selection should be done before starting each transect (ex. the 8th and 42nd bait).

Marked bait needs to be visible to observers, not under shrubs or in thick grass (Figure 70). Mark the bait with a 12-inch surveyor flag placed 1 meter to the north of the bait and record the location with a GPS unit. Label the flag and the GPS waypoint with the same label (Figure 71). Pre-labeling flags is a time saver and helps eliminate missing points. If bait is marked on the GPS but not flagged, it will be impossible to find the precise location of the bait. Occasionally, a technician will flag the bait but forget to mark the GPS location. In this case,



Figure 70. Plague vaccine bait marked with flag for monitoring.

simply select a different bait to mark with the GPS and flag this bait with an extra flag. When finished, check that all pre-labeled flags have been distributed and that there is a corresponding waypoint recorded on the GPS. Correct any errors before leaving the site. Save GPS data to the computer and create a bait check GPX file to guide bait monitoring.

To monitor bait removal, observe the status of marked bait daily for 3 days or until all baits are removed. Divide the bait monitoring locations among technicians. Use a bait check data sheet to help keep track of points to be checked.

Follow the GPS to the first point. Locate the flag and then thoroughly search the area less than 1 meter around the flag. If present, the bait will be within 1 meter of the flag. Flags are numbered the same as the waypoint, so check the flag to see if it is numbered correctly. Mark the data sheet according to bait status. If only partially present, estimate and record the percent remaining. Also record any relevant notes, such as ants or other insects eating the bait and the presence of any blue stained scat near the marked baits. Only remove the flag if you are certain the bait has been removed. Do not remove flags marking baits that are still present. Continue to the next bait until all baits have been checked. When bait monitoring is complete, combine lists and check off missing baits to avoid looking for baits that are not present. Edit the bait check GPX file and delete the waypoints where baits have been removed. This step will help to keep GPS points organized and help to avoid confusion in the field.



Figure 71. Materials for bait monitoring (gloves, bait, GPS and flags).

Burrow Dusting Overview

Purpose

To control fleas, the primary vector of plague, on prairie dog colonies by administering five grams of DeltaDust® into all prairie dog, ground squirrel, small mammal and predator burrows. The number of burrows dusted and the quantity of dust applied are tracked.

Materials

- DeltaDust® **1 jug (5lb) will cover ~17 acres -or- 1 case (4 jugs) will cover about 68 acres**
- Nitrile gloves, N95 mask, other PPE
- Duster backpacks
- Wet wipes and/or hand wash
- GPS
- Extra AA batteries
- Portable kitchen gram scale
- Radios (optional)
- Field books
- Datasheets
- Extra 1 gal zip top bags for personal items

Materials (Air Dusters)

- Duster canister and regulator
- Backpack
- Backpack rack
- Duster wands
- Full paintball tanks
- Bottle wrenches
- Allen wrenches
- Air Duster maintenance kit

Materials (Battery Dusters)

- Dusters
- 2-3 charged batteries
- Wands, wand tips and triggers
- Phillips head screwdriver

Methods

If the site is scheduled for flea collection via burrow swab, **this must be done before dust is applied**. The dust contains the insecticide deltamethrin. Read the Material Safety Data Sheet (MSDS) for deltamethrin prior to use. Take care to limit exposure to skin, eyes and lungs. Wear gloves and mask when handling dust. Wear long pants, sleeves and boots and do not wear shorts or sandals. Wash hands before eating or drinking. Always have contact information of local land managers and biologists. Know where the colony is located and who to call locally in case of an emergency.

Both the Air and Battery style dusters are fragile and need daily cleaning and maintenance. Treat the duster with care to prevent breakdown time and the need for repeat application.

Do not apply DeltaDust® within 50 meters of any natural water source; it is toxic to aquatic life. Do not spray DeltaDust® in burrowing owl burrows, which will likely have bird scat and feathers around the entrance to the burrow.

Figure 72 and Figure 73 visually explain two different scenarios that may be encountered in the field when dusting. In these cases, each of the 5 technicians is represented as separate colors. The blue and yellow lines represent the technicians on the ends of the dusting line. Note how each technician's tracks are slightly overlapped to insure that all burrows are dusted.

Set Up

Each technician should use the same duster every day to familiarize themselves with the duster and make necessary adjustments to consistently dispense 5g/burrow on average. Each duster unit requires trigger pull calibration prior to use. Fill and weigh dust canisters and record that information on the data sheet before dusting. Assemble all dusting equipment and pack any extra supplies needed. Take care to park vehicles near the beginning and ending locations of dusting swaths. Look at the shape of the colony on the GPS and decide how to cover the colony in as few passes as possible.

Line Up

Form a line at one end of the colony, leaving adequate space between each technician (20-30m). Many narrow swaths are better than fewer wide ones. Prior to dusting, assess the colony shape and size to determine beginning and ending locations, as well as how to efficiently cover the colony, Figure 72. Turn on "Tracks" on the GPS.

Begin Dusting

Begin dusting burrows with each technician weaving through their 20-30m transects. Visually track the technician working on adjacent transects and try to move across the colony as a group and in an even line so that each technician's coverage area meets the next, on either side (Figure 72, Figure 73). Technicians should communicate frequently and make spacing adjustments to account for uneven burrow density. Technicians should stay in an even line and technicians should not walk ahead of the group. As technicians move through

their coverage area they should frequently encounter burrows on the edges that have just been dusted by the adjacent technicians. This narrow “overlap” helps to minimize gaps in coverage. The objective is to dust >95% of the burrows. Mark every burrow dusted on the GPS; refer to “GPS Burrow Marking” protocol. When out of air or dust, record the current burrow waypoint and switch paintball tanks or refill dust canister.

Turning Around

When the crew reaches the opposite side of the colony, pivot the line over to the next pass area and repeat the process (Figure 73). The crew should hinge around the technician working on the inner edge of the colony. The technician on the inner edge should dust the area near the swath they just completed. This will allow the technician to visually check their GPS tracks to make sure they are at the edge of the previous pass (Figure 73, yellow line). Placing the most experienced technicians on the ends often helps with orientation and pacing.

Finish, calibration and Pack Up

When dusting is complete, weigh the dust canister at the end of dusting and fill out the required information on the data sheet. Fill out a data sheet for each individual site/colony. Do not condense data from multiple sites onto a single data sheet. After filling out the data sheet, an average amount of dust dispensed into each burrow will be calculated. The target average is 5g of dust per burrow. Make necessary adjustments to equipment and trigger pull to ensure adequate dust is dispensed. When technicians first begin dusting a calibration period of ~50 burrows is required. Data collected during this calibration is used to adjust trigger pull timing and familiarize technicians with the equipment. Transfer data from the field book to the appropriate data sheet and give to crew leader. Clean and store the duster (refer to the Battery Powered or Air Powered Duster Protocol). A site visit data sheet should be completed after dusting each colony. Download all GPS data, waypoints and tracks.

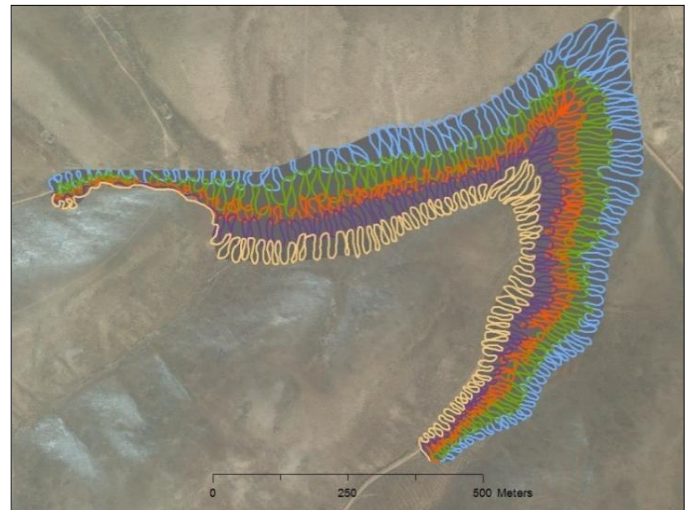


Figure 72. Visual representation of five technicians dusting a colony in one pass.

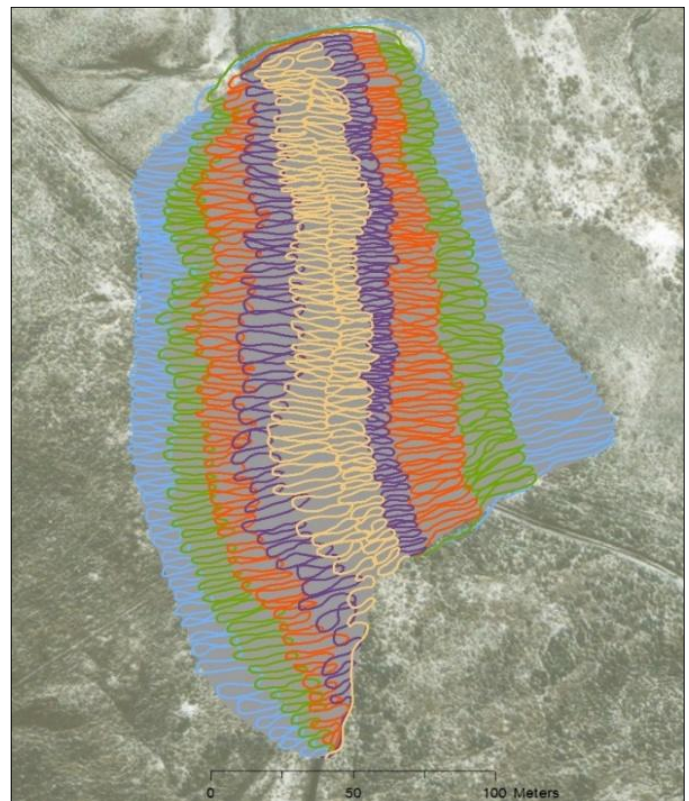


Figure 73. Visual representation of five technicians dusting the entire length of the colony in two passes, crew will rotate and the middle (yellow) person will follow the first path.

High Pressure Air Compressor Operating Instructions

WARNING

The engine becomes hot during operation. If the hose/line is accidentally laid on the engine, it will melt and become inoperable. A heat damaged hose may fail and cause injury if used again. Read the owner's manual (MCH6 User Guide) before using and for maintenance information. These instructions are just a general outline of the procedure. Do NOT perform maintenance directly after operation; wait for the compressor to cool.

Purpose

Scuba tanks need to be filled with compressed air using a scuba tank air compressor. Starting, stopping and maintaining the compressor correctly will help the life and function of the MCH-6 air compressor.

Materials

- Air compressor
- Scuba tanks
- Gas
- Safety glasses
- Ear protection

Methods

Place the compressor outside on level ground, (Figure 74, Figure 75). Make sure the air compressor is in good, working condition. Perform a visual check on the compressor and fill hose, make sure there are no worn or melted sections. Check that the maintenance log is up to date and all maintenance checks have been completed. Check the gas and oil levels and fill if needed. If oil is accidentally overfilled, consult the owner's manual. Fill out the time and starting operating hours in the compressor logbook. Open the condensation valves and shift the fuel lever to the right (ON position). Shift the choke to the right (Open) if the engine is cold or to the left (Closed) if the engine is hot. Shift the accelerator about $\frac{1}{3}$ towards the left from its minimum position.

Flip the emergency shut-off to "ON" and pull the starter handle. If the compressor is hard to start, adjust the choke and accelerator until the engine turns over when the starter handle is pulled. If the choke is to the right (Open) gradually shift it left (Closed) as the engine warms up.

Close the condensation valves and wait until the pressure reaches 3000 psi. At that point, the safety valve should loudly "pop" and a hissing noise will be heard. If the safety valve does not release at 3000 psi, immediately shut off the compressor and do not operate it again until maintenance has been performed.

Turn the compressor off by turning the emergency on/off switch to "OFF". Move the accelerator to the minimum position (right) and then move the fuel lever to "OFF" (right).

Carefully and slowly, open the condensation valves; be prepared for the condensation tube to flap around. The lines are highly pressurized. Place a heavy item like a brick, cinderblock, or rock on the condensation tube and be aware that the pressure can release powerfully. Leave the condensation valves open after draining.

Scuba Tank Fill Procedure

WARNING

The engine becomes hot during operation. If the hose/line is accidentally laid on the engine, it will melt and become inoperable. A heat damaged hose may fail and cause serious injury if used again.

Purpose

Fill the scuba tanks using the compressor. These scuba tanks will be used to fill the paintball tanks in a cascade system.

Materials

- MCH-6 compressor
- Three empty scuba tanks
- Gasoline
- Safety glasses
- Ear protection
- O-rings

Methods

Securely attach the yoke to the scuba tank, hand tight. Open the line valve and make sure the bleed valve is closed. Open the top valve of the scuba tank. Start the compressor and close the condensation valves. The compressor is now filling the scuba tank; an empty tank will take approximately 20 minutes to fill. Once the scuba tank is at 3000 psi, the safety valve will "pop" and a vent will open that makes a hissing sound. Shift the accelerator to minimum and flip the emergency on/off switch to "Off" and close the valve on the scuba tank. Open the bleed valve. WARNING: Do not place hands

or face in front of the release hole. Close the line valve and detach the yoke from the scuba tank. Scuba tanks must be carried with protective caps at all times, (Figure 77).

Condensation builds up in the compressor and has to be released every 20 minutes. Release the condensation valve after each tank is filled. **WARNING:** the system is pressurized! The tank and line valve need to be closed and the compressor needs to be shut off before opening the condensation valves. Carefully open the condensation valves on the compressor, being sure to lay a weighted object on the condensation hose.

Fill out the end operating hours and other information in the compressor log book. The compressor will be hot. Do not set the yoke line on the engine because it will melt. Be careful when moving or touching the compressor.

Paintball Tank Fill Procedure

Purpose

After three scuba tanks have been filled, they need to be connected with the cascade system. The cascade system will be used to fill paintball tanks for use with the air duster regulators.

Materials

- <3000 psi paintball tanks
- 3 filled scuba tanks
- Cascade line (keep a spare on hand)
- Paintball tank thread protectors
- Paintball tank replacement O-rings

Methods

Perform a visual inspection of the scuba tanks, cascade system and the paintball tanks that are to be filled. Make sure the fittings in the cascade system are hand tight and the hose does not have any cracks, holes, or melted portions (Figure 76). Inspect and clean the threads and O-rings on each paintball tank, (Figure 78). Replace any damaged or worn O-rings and make sure to always keep the thread protector covers in place while the tanks are in transport. Preventing damage to the threads on the paintball tanks will extend the life of the tank. Tanks with damaged threads may be unsafe to use.

Attach three filled scuba tanks to the cascade yokes; make sure all the valves and the bleed valve in the cascade are closed. It does not matter what order the scuba tanks are placed within the cascade. Open all three scuba tank valves and listen for any leaks. If there are leaks, immediately stop filling and close all the valves (if the leak is small and very difficult to hear, the leak can usually be ignored).

Attach the paintball tank to the quick disconnect valve at the end of the cascade. Open the fill valve, which is the knob next to the pressure gauge. Listen for the paintball tank pressure to equalize to the cascade pressure. Close the fill valve which is the knob next to the pressure gauge. Open the bleed valve at the end of the cascade to depressurize the line. Close the bleed valve once all the pressure is released. Disconnect the paintball tank.

Repeat these steps for each paintball tank. The maximum pressure on the cascade gauge is the maximum pressure a paintball tank can be filled. Any paintball tank with more pressure than the cascade system will not be filled. Fill out the log sheet after the last paintball tank has been filled. Close all three scuba tank valves. Open the fill valve and the bleed valve to make sure there is no pressure in the line. Detach the scuba tanks from the cascade line and store all equipment appropriately.

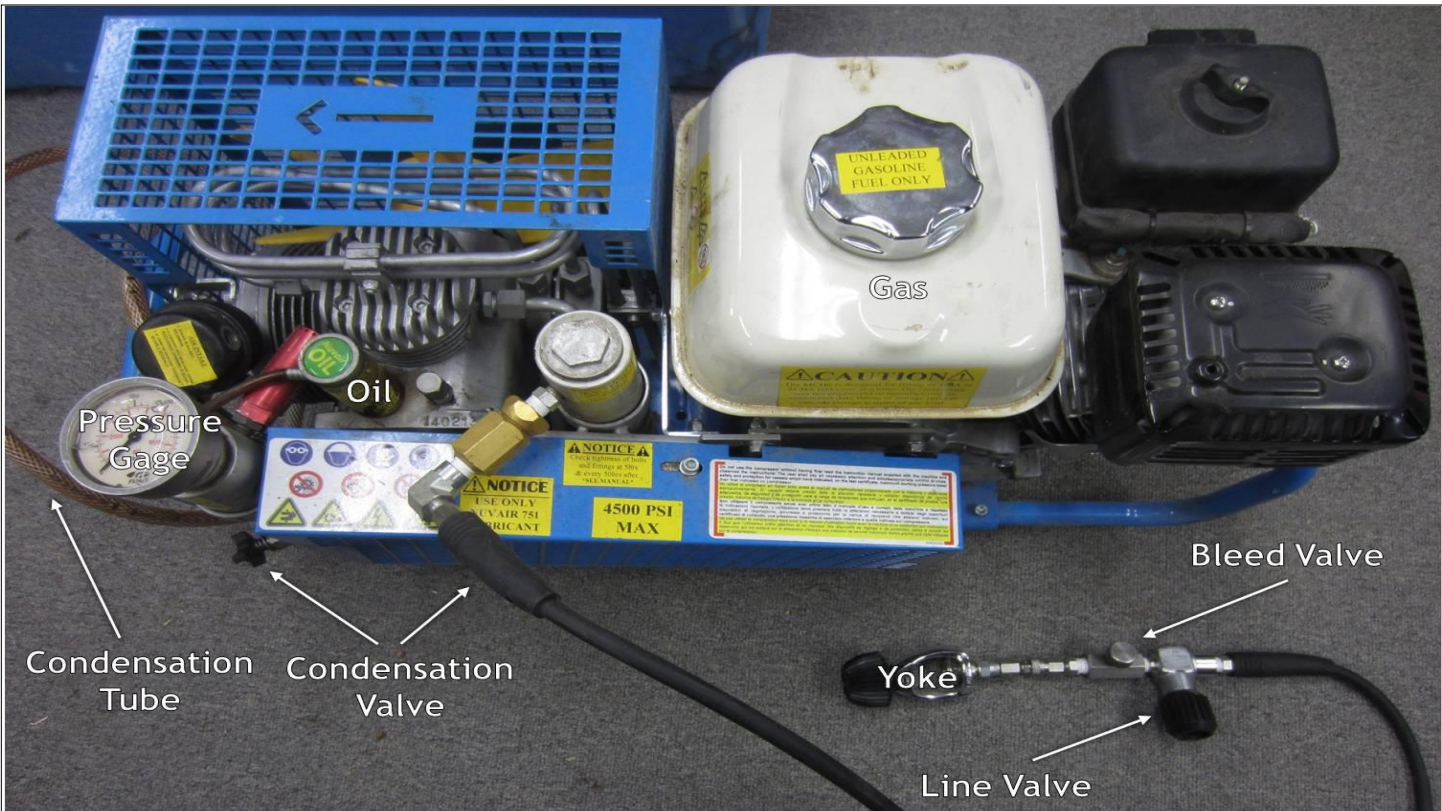


Figure 74. Air Compressor with parts labeled.

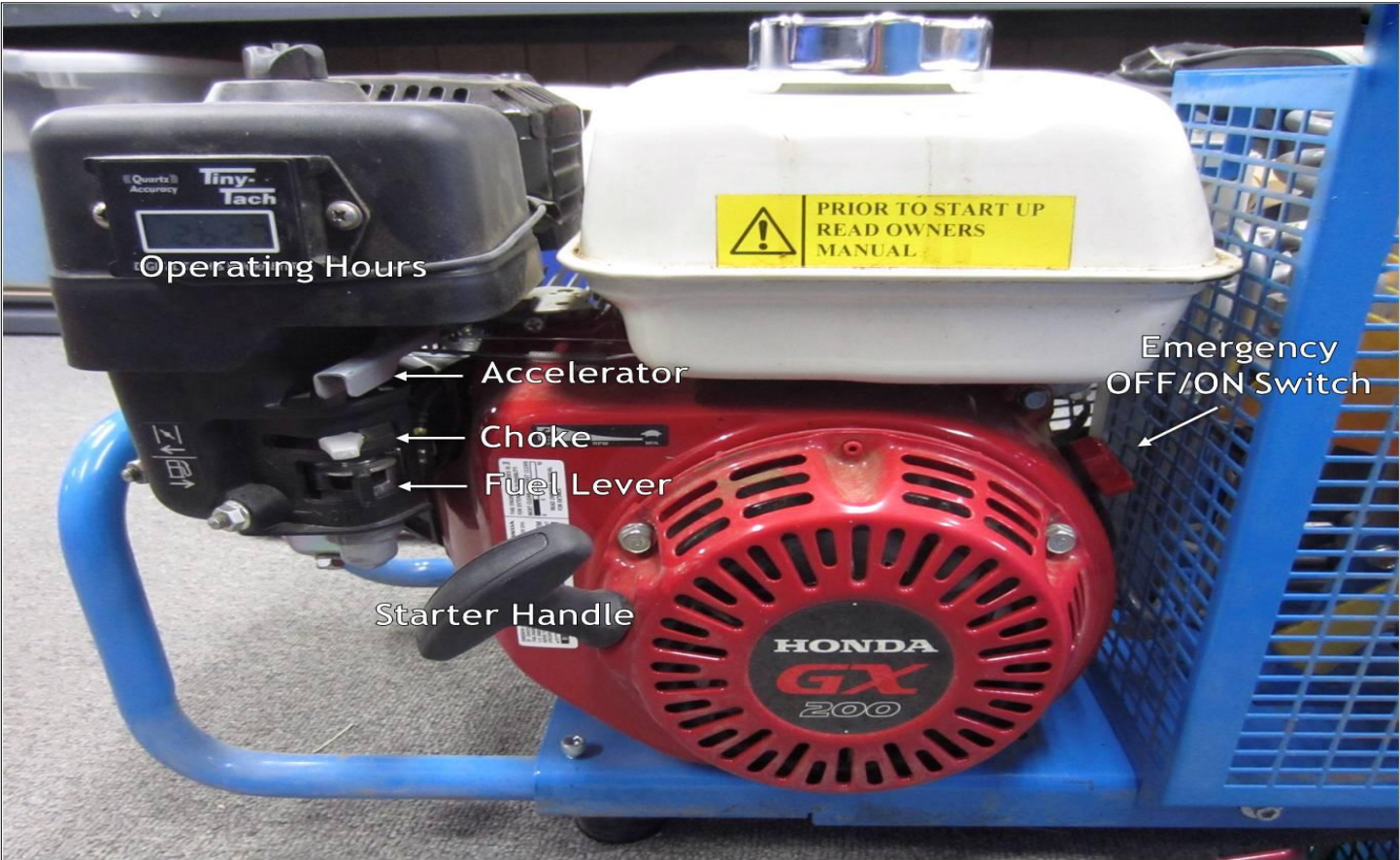


Figure 75. Air Compressor engine with parts labeled.

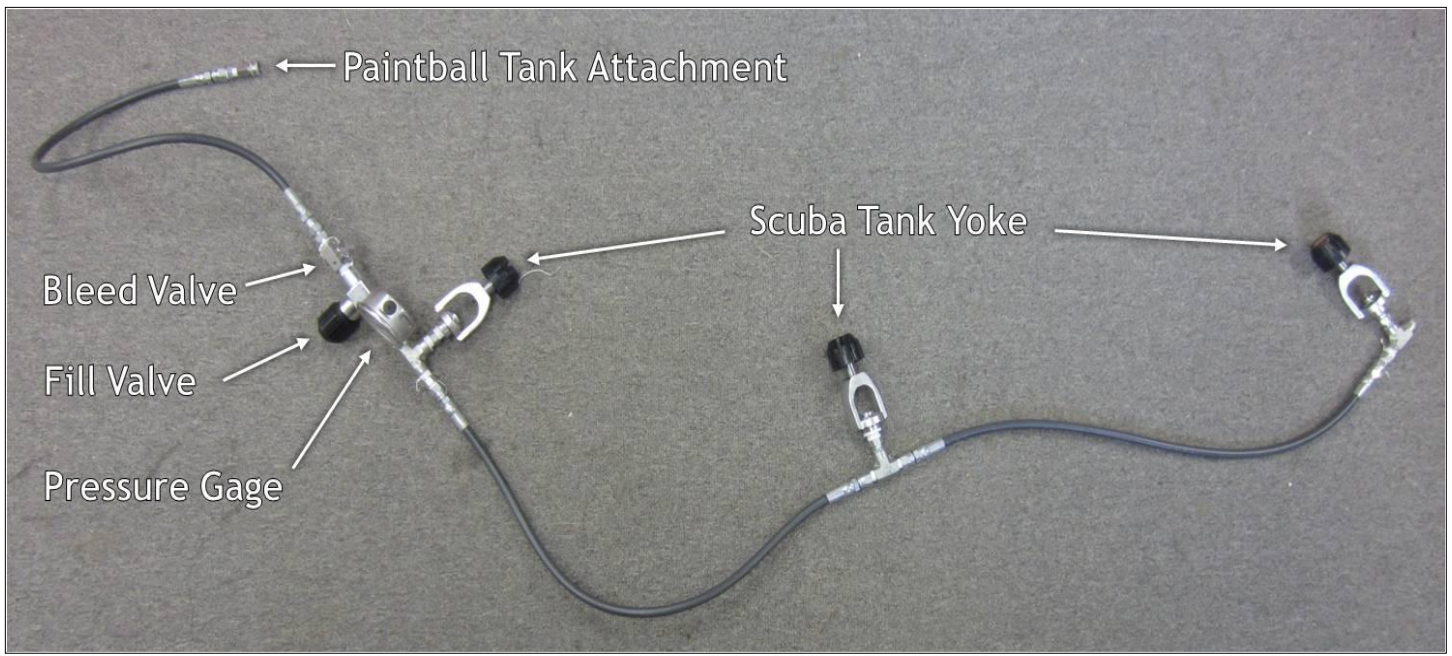


Figure 76. Cascade system with parts labeled.



Figure 77. Scuba tank with protective cap.



Figure 78. Paintball tank gage (left), threads (top) and quick coupler (right).

Basic Air Duster Instructions

Purpose

To control fleas, the primary vector of plague, on prairie dog colonies by administering five grams of DeltaDust® into all prairie dog, ground squirrel, small mammal and predator burrows. As well as track the number of burrows dusted and quantities of dust applied.

Materials

- Air duster bottle and regulator
- Filled paintball tanks
- DeltaDust®
- Allen wrench
- Bottle wrench
- Backpack
- Duster frame

Methods

Set Up

Fill and weigh the duster bottle and record the weight on the data sheet. Tighten the air tank (hand tight) to the regulator and record the starting air tank psi and size on the data sheet. Do not over tighten threads, fittings can get stuck and become difficult to unscrew. Dust canisters, regulators and O-rings must be kept clean and free of dirt, sand and debris. Open the air tank by gently turning the top knob clockwise until the air tank opens (a sound similar to opening a soda can will be heard).

On the datasheet, record the output psi which is the smaller gauge on the regulator, toward the bottom. Close all valves on the bottle and attach the air hose to the bottle. Open the air valve to be sure there are no leaks. Put the dust bottle and air tank in a plastic rack in a dusting backpack. Attach a dusting wand and open the dust valve on the bottle.

The lower, output, gauge may need to be adjusted. Adjust gauges by slowly turning the screw on the bottom of the regulator a quarter turn at a time. Quickly squeeze the dust wand handle and check the output pressure and adjust until the pressure reads approximately 22 psi. Air pressure in the dust canister should not exceed 25 psi.

Tear Down

Close the air tank knob by gently turning the knob counter-clockwise. Then close all valves on the bottle. Compress the dusting wand to release hose pressure. Detach the dust hose and the air hose from the bottle.

Compress the regulator air hose tip using an Allen wrench to release any remaining pressure in the regulator.

Unscrew the air tank; if it is difficult to unscrew there is likely still pressure in the system. Record the ending psi of the air tank on the data sheet. Open the dust valve on the bottle slowly to release any remaining bottle pressure. Reclose the dust valve and weigh the bottle and record the final weight on the data sheet. Turn off the tracks and record the number of burrows dusted on the data sheet. Transfer GPS and log book data to permanent locations.

Detailed Air Duster Instructions

WARNING

Contents are under pressure. Visually inspect air tanks, O-rings, hoses and duster bottles for cracks and leaks. Always be aware of valve positioning (Horizontal=Closed). The dust contains the insecticide deltamethrin. Wear appropriate PPE when handling. Avoid contact with skin and eyes and avoid inhalation. Wash hands after contact. The dusters and the regulators are fragile. Treat dusting regulators with care. Dust canisters, regulators and O-rings must be kept clean and free of dirt, sand and debris.

Purpose

Control fleas, the primary vector of plague, on prairie dog colonies by administering five grams of DeltaDust® into all prairie dog, ground squirrel, small mammal and predator burrows. As well as track the number of burrows dusted and quantities of dust applied.

Materials

- Regulator and Bottle
- Bottle Wrench and Allen Wrench
- DeltaDust®
- Air tanks
- Plastic duster frame
- Dusting Backpack
- Dusting wand
- Dusting hose for wand
- GPS unit
- Data sheet
- Scale
- Nitrile gloves

Before set up, record in the logbook or data sheet: The specific symbol or unit # of the duster being used (ex. “Square”), as well as the site being dusted (ex. BLM-9) and the date (Figure 79 and Figure 80).

The Dust Bottle

Put gloves on and set out the clear plastic dust bottle (Figure 81). Check that both valves on the lid are closed (Horizontal=Closed). Unscrew the lid of the duster bottle with a bottle wrench. Keep dust bottle clean and free of dirt, sand and debris. Inspect the empty bottle and draw tubes for damage and remove any obstructions or debris. Do not store dust in the dust bottles long-term.

The bottle should be filled to the full line which is either blue or red. Position the bottle downwind and away from other technicians and add dust to the full line. Screw the lid back on and tighten the lid with a bottle wrench. Weigh the entire unit (lid, dust and bottle) and record the weight on the data sheet. Set the unit aside and set up the air regulator.

The Air Regulator

Select an air tank and record the starting psi and air tank size on the data sheet. Keep the regulator clean and free of dirt, sand and debris. Inspect the regulator for damage and remove any obstructions or debris (Figure 82). Screw the air tank into the regulator arm until it is hand tight. Rotate the knob on the top of the regulator arm clockwise to depress the pin that opens the air tank. The gauges on the regulator should be pressurized and can be read and recorded at this point.

Test the gauge response by compressing the regulator air hose tip with the Allen wrench or attaching the air hose to the duster bottle. The top gauge should read between 200-250 psi and the bottom gauge should read between 22-25 psi. If the gauges need to be adjusted, use the Allen wrench to rotate the pins a quarter turn at a time on the top and bottom of the gauge post. Turning the pin clockwise will increase the pressure. Compress the regulator air hose tip after each adjustment to check new readings on the gauges. Depending on the duster, the gauge readings may change after the air hose has been attached to the dust bottle. If this is the case, adjust the gauges appropriately and note that the gauges will read high when the hose is not attached. It is recommended that technicians use the same regulator and dusting system throughout a season, this allows technicians to become familiar with specific models and will reduce the need for minor adjustments during setup.

Complete the Duster Unit

Attach the regulator air hose to the bottle. Open the air valve on the bottle lid to pressurize the bottle. The bottle will fill with air and an audible pressure rising in the bottle can be heard. If the sound does not taper off and continues to make noise, reclose the air valve. Dust being released from the lid or hissing indicates a leak. Unscrew the lid and clean any dust from the O ring. Tighten the lid with a bottle wrench and open the air valve.

Put the completed duster unit into a plastic duster frame (Figure 83). Position the frame in the backpack so that the flat edge is against the users back and the gauges are facing away from the users back.

Extra air tanks can be packed around the dusting unit but be careful not to damage the regulator. Connect a dusting wand to a dust hose. Attach the dust hose to the dust valve on the top of the bottle lid. Double check the connection is secure by gently tugging on the hose. Open the dust valve and check that the wand is releasing dust.

Dusting

Put the dusting-wand tip into a burrow as far as it easily extends (6 inches or more). Compress the dusting-wand handle fully and hold it down for 1 full second.

Draw the wand tip towards the burrow entrance while dust is being extruded. This will coat a larger section of the burrow and leave a visible trace of dust near the entrance of the burrow to show it has been dusted.

Mark the burrow and burrow activity on a GPS unit or count the burrow with a clicker depending on equipment available. Move in a systematic pattern in line with other technicians, making sure to dust all possible burrows. Dust all burrows including non-prairie dog burrows. **Do not** dust burrows occupied by burrowing owls, which are characterized by feathers and white scat around entrance of burrows, or within 50 meters of any water source.

Running Out of Air

If a duster is weak or does not appear to spray DeltaDust® as expected, it may be low on air. Close both valves on the dusting bottle (Horizontal=Closed). Check the psi gauge on the air tank. If there is still sufficient air, reopen the valves and keep dusting.

If the tank is close to 0 psi, record the ending psi on the data sheet and detach the regulator air hose from the dusting bottle. Rotate the knob on the regulator arm counter-clockwise until resistance is felt (do not over tighten). This will close the air tank. Use an Allen wrench to release residual pressure in the regulator and air hose. Both gauges should read 0. Unscrew the air tank from the regulator. Record the number of burrows dusted with the empty air tank.

Record the starting psi of a new air tank. Screw the new air tank into the regulator arm, rotate the knob clockwise until the tank is open (do not over tighten). Check the gauges, re-attach the regulator air hose to the lid, reopen the valves and continue dusting.

Running Out of Dust

When the dust level is near the bottom of the lower tapeline on the bottle, dusting will be noticeably less efficient. To improve dusting efficiency, the dust bottle will need to be refilled. Close both valves on the lid (horizontal=closed). Depress the dusting wand for several seconds into a burrow, away from people. This will release residual pressure and dust in the wand. Detach the dust hose from the lid. Close the air tanks by rotating the knob on the regulator counter-clockwise until resistance is felt, then detach the air hose from the lid.

Stay downwind and away from other technicians then slowly open the dust valve to release any remaining pressure in the bottle. If the valve is opened too quickly, dust may shoot out of the opening of the valve. Reclose the dust valve when the bottle is depressurized. Weigh the depressurized bottle with the lid on. Record the weight in a log book. Unscrew the lid with the bottle wrench. Fill the bottle to the top tapeline, screw on the lid and make sure all valves are closed. Weigh the bottle with the lid on, reattach the dust and air hoses and reopen the air tank. Open the air valve to pressurize the bottle, check for leaks, open the dust valve, put everything back in the backpack and continue dusting.

At the End of the Day

When dusting is completed for the day, turn both valves on the lid to the closed position (Horizontal=Closed). Depress the dusting wand into a burrow to release residual pressure in the hose. Detach the dust hose from the lid. Detach the dusting wand from the dust hose and put both away. Rotate the knob on the regulator arm counter-clockwise to close the air tank. Detach the air hose from the lid and then re-attach it or compress the

tip of the air hose with the Allen wrench to release residual pressure until the gauges read 0. Record the ending psi and the number of burrows dusted with that air tank in the log book.

Unscrew the air tank from the regulator and put both away. Downwind and away from other technicians, slowly open the dust valve on the bottle lid to release any remaining pressure in the bottle. Reclose the dust valve when the bottle is depressurized. Weigh the depressurized bottle with the lid on and record the weight in the log book. Put the bottle with the lid on into the tub with its assigned regulator. Put all equipment back in their storage containers. Transfer all the data in the log book onto a datasheet. Download GPS points and enter data into the computer.

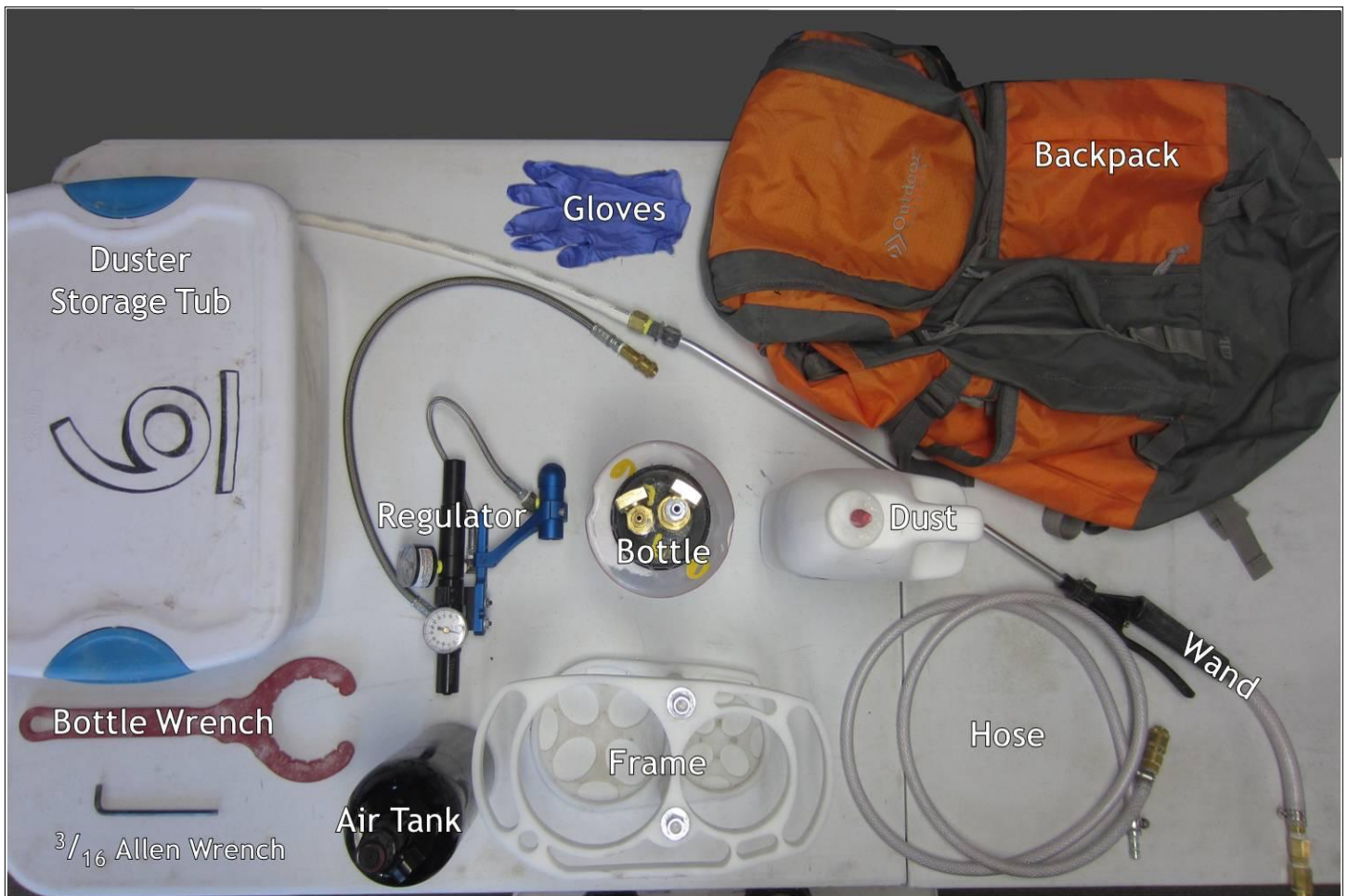


Figure 79. Air duster supplies needed for each technician in the field.



Figure 80. Materials needed to record dusting data in the field.



Figure 81. Air duster dust bottle with parts labeled.

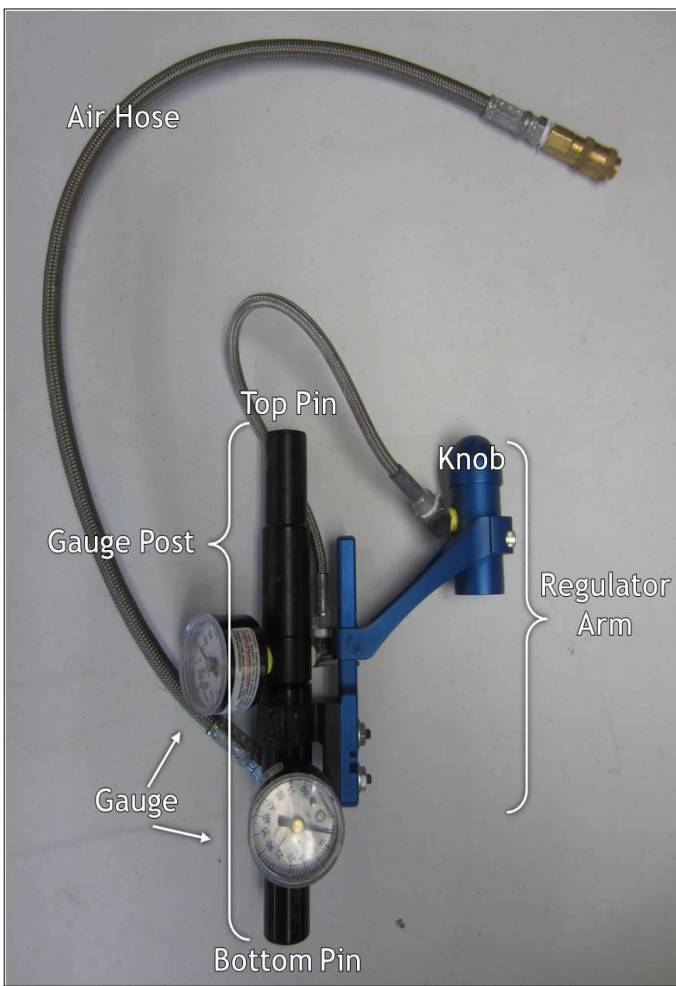


Figure 82. Air duster regulator with parts labeled.



Figure 83. Fully assembled air duster unit.

Battery-Powered Duster

Purpose

To control fleas, the primary vector of plague, on prairie dog colonies by administering five grams of DeltaDust® into all prairie dog, ground squirrel, small mammal and predator burrows. As well as track the number of burrows dusted and quantities of dust applied.

Materials

- GPS
- Dusters
- Batteries for dusters
- Wands, wand tips and triggers
- DeltaDust®
- Dust canisters
- Backpack carabineers
- Phillips head screwdrivers
- Wire
- Duct tape
- Wet wipes
- Needle nose pliers

Methods

Fill a dust canister with dust and weigh the canister. Record the duster unit and the starting and ending canister weights on the data sheet. Weigh an extra full canister of dust and record weight on the data sheet. Screw the canister tightly onto the duster. Use the carabineers and wire to attach the duster to a backpack. The duster should be mostly level with the canister end tilted slightly downward. Put the extra dust and fresh batteries for the duster in the backpack. Change the battery at least twice each day. Attach a wand and trigger to the duster. If the trigger attachment fitting is too loose to securely hold in place a small section of duct tape may be used to hold the connection point firm to the duster frame. Turn on the motor and GPS tracks and start dusting. Mark dusted burrows and record track logs on the GPS.

At each burrow, insert the duster nozzle at least 6 inches into the burrow with the nozzle facing up. Be careful not to plug the nozzle with mud or dirt. Spray dust for a minimum of 10 - 30 seconds per burrow. Each duster is different and will require 10 - 30 seconds to dispense 5g of DeltaDust®. The dust must be weighed regularly to calculate the average grams of dust dispensed in each burrow. Each dusters trigger pull time must be calibrated individually to dispense 5g/burrow.

If the nozzle is not spraying dust while pressing the trigger and the motor is running, the nozzle might be clogged. It is possible to hear the motor straining against the blockage. Immediately stop the motor and unclog the nozzle with a flag wire. Dust may build-up in the hose causing dust to stop flowing. It is possible to disconnect the hose from the duster and shake the hose to clear the line. Make sure to do this downwind of other technicians. The dust canister needs to be agitated often to ensure that air pockets do not build up in the canister. If the trigger is engaged but the motor will not run consistently or sounds irregular, adjust the connections at the trigger and battery. The trigger connection may need to be re-taped down. Additionally, a thin layer of foil wrapped around the connector post may help to tighten the connection.

Weigh dust at the end and divide by the number of burrows dusted to get the amount of dust per burrow and record this data on the data sheet. At the end of the day, clean off the duster and impellor wheels with an air compressor (about 50 psi) and run the duster without dust for 15 seconds. Store dusters in the appropriate bin and charge all batteries.



Figure 84. Battery powered duster.

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Appendix 1: Data Sheets and Logs

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Prairie Dog Colony Site Visit

Date: _____ Arrival Time: _____ Leave Time: _____

Observer: _____ Contact Info: _____

Colony/ Site: _____

UTM Coordinates: _____

Species: GUPD BTPD WTPD

Reason for Visit: _____ Binoculars Used? Y N

Weather Conditions --

Estimated? Y N Temp: _____ Precip(past 24hrs): _____

Wind: _____ Cloud Cover: _____

Weather conditions and time of day during visit were... *circle one*

Poor Suitable Excellent

Prairie Dogs Observed --

Seen? Y N Heard? Y N

#Adults: _____ #Juveniles: _____

Burrow Appearance --

- Active Inactive
- Fresh Scat Fresh Digging

Burrow Density-- _____

What percentage of the area, as defined by the colony perimeter, contains active or inactive burrows?

0= none, 1= sparse, very low (1-25%), 2= low to moderate (26-50%), 3= moderate to high (51-75%), 4= very dense (+75%)

Burrow Activity-- _____

Of the burrows observed; what percentage are active?

0= dead, no activity, 1= 1-25%, 2= 26-50%, 3= 51-75%, 4= +

Brief Survey Summary--

Examples worth noting: colony expanding, patchy distribution, inactive zones, cattle presence, predators (mammals or birds), ground squirrels, vegetation description, drought conditions, flash flooding, shooting, hikers, vehicles passing by.

Prairie Dog Burrow Survey

Date: _____

Site: _____

Recorder: _____

GPS Unit: _____

CHECK ALL THAT APPLY

Site ID	WPT	A	I	A?	I?	S	M	FD	FS	AUD	VIS	W	V	C

NOTES:

NOTES:

NOTES:

Site ID	WPT	A	I	A?	I?	S	M	FD	FS	AUD	VIS	W	V	C

NOTES:

NOTES:

SITE COMMENTS:

Key: WPT=GPS waypoint, A=Active, I=Inactive, A?= likely 'Active', I?= likely 'Inactive' , S=Burrow Type Single, M= Burrow Type Multiple, FS=Fresh Scat, FD=Fresh Dig, VIS=Visual, AUD= Audio, C=Collapsing, V=Veg, W=Webs

Dusting Site Summary

Date: _____ Colony/site: _____ Region: _____

Start Time: _____ Temp: _____ Wind: _____ Cloud Cover: _____

Initials	GPS Unit	Duster	Start Weight	End Weight	Dust Used	Total Burrows	Grams/Burrow	# Air Tanks	Notes

End Time: _____ Temp: _____ Wind: _____ Cloud Cover: _____

Observations --

Seen? Y N Heard? Y N

Burrow Density-- _____ 0= None, 10= Very Dense

Colony Activity-- _____ 0= Dead, 10= Thriving

Additional Comments/ Notes-- _____

also complete a "Prairie Dog Colony Site Visit" form

Individual Dusting Output

Date: _____ Colony: _____ Duster: _____ Initials: _____

Start Weight (g): _____ Added Weight (g): _____

Tank 1			
Tank Size (circle)	Start Pressure:	End Pressure:	Notes:
62 cui 48 cui	_____psi	_____psi	
	Output Pressure:	Total Burrows:	
	_____psi	_____	
Tank 2			
Tank Size (circle)	Start Pressure:	End Pressure:	Notes:
62 cui 48 cui	_____psi	_____psi	
	Output Pressure:	Total Burrows:	
	_____psi	_____	
Tank 3			
Tank Size (circle)	Start Pressure:	End Pressure:	Notes:
62 cui 48 cui	_____psi	_____psi	
	Output Pressure:	Total Burrows:	
	_____psi	_____	
Tank 4			
Tank Size (circle)	Start Pressure:	End Pressure:	Notes:
62 cui 48 cui	_____psi	_____psi	
	Output Pressure:	Total Burrows:	
	_____psi	_____	
Tank 5			
Tank Size (circle)	Start Pressure:	End Pressure:	Notes:
62 cui 48 cui	_____psi	_____psi	
	Output Pressure:	Total Burrows:	
	_____psi	_____	

End Weight (g): _____ Dust Used (g): _____

Total Burrows: _____ grams/burrow: