
*Sylvilagus transitionalis*

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Introduction

Natural History
The New England cottontail (Sylvilagus transitionalis) (NEC) is the only endemic cottontail in New England (Probert and Litvaitis 1996, p. 289). The NEC is a medium-large cottontail rabbit that may reach 1,000 grams (2.2 pounds) in weight. Like the conspecific eastern cottontail (EC, Sylvilagus floridanus), the NEC can be distinguished from the snowshoe hare by its lack of seasonal variation in pelage coloration and distinctly smaller hind foot. New England and eastern cottontails can be difficult to distinguish in the field by external characteristics (Chapman and Ceballos 1990, p. 106). However, cranial differences, specifically the length of the supraorbital process and the pattern of the nasal frontal suture, are a reliable means of distinguishing the two cottontail species (Johnston 1972, pp. 6-11).

The NEC, like all cottontails, is short-lived and reproduces at an early age with some juveniles probably breeding in their first season. Litter size is typically five young (range 3-8) and females, which provide little parental care, may have 2-3 litters per year. The breeding season lasts from mid-March to mid-September in Connecticut (Dalke 1942 in Chapman, Hockman and Edwards 1982, p. 93). Initiation of nesting is closely associated with the spring green-up (Chapman, Hockman and Edwards 1982, p. 94). Several attempts have been made to document NEC nesting habitat, however locating nests has proven to be very difficult because nests are concealed in extremely dense vegetation, prohibiting researcher access and discovery (T. Goodie, pers. comm.). Female NECs have a high incidence of postpartum breeding, demonstrate density-independent breeding response, and have a rapid rate of maturity (approximately 40 days from conception to parental freedom) (Chapman and Ceballos 1990, p. 108). These characteristics allow a species to thrive in spite of a high predation rate, provided ample resources are available (Chapman, Hockman and Edwards 1982, p. 105). In the case of cottontail rabbits, these principal resources include ample food and habitat that is free from interspecific competition and provides security from excessive predation (Chapman, Hockman and Edwards 1982, p. 106).

The historic range of the species likely spanned southeastern New York (east of the Hudson River, including Long Island) north through the Champlain Valley, southern Vermont, the southern half of New Hampshire, southern Maine, and statewide in Massachusetts, Connecticut and Rhode Island (Nelson 1909; Litvaitis and Litvaitis 1996, p. 725). The historical range encompassed an estimated 90,000 square kilometers (km$^2$) (34,750 square miles (mi$^2$) (Litvaitis et al. 2006, p. 1191).

NECs are considered habitat specialists, insofar as they are dependent upon early-successional habitats, frequently described as thickets (Litvaitis 2001, p. 466). Barbour and Litvaitis (1993, p. 324) demonstrated a relationship with microhabitats containing >50,000 stem-cover units/ha (20,234 stem cover units/acre). Historically, thicket-dependent species like the NEC may have persisted in core habitats associated with frost pockets, barrens, and the shrubby interface between wetlands and upland forests (Litvaitis 2003, p. 120). Soil conditions, fire or other disturbances limited forest canopy closure in many shrublands (Lorimer and White 2003, p. 41; Latham 2003, p. 34; Brooks 2003, p. 65). From these more persistent core habitats, thicket-dependent species such as the NEC could have dispersed
opportunistically to occupy smaller, disturbance-generated patches of suitable habitat (Litvaitis 2003, p. 120). Stable coastal shrub communities are often overlooked for their importance to thicket-dependent wildlife, yet these habitats may have provided a substantial amount of this habitat type.

Although the amount of shrubland and early successional habitat in the pre-Columbian landscape of the Northeast is not well known, it is generally accepted that these habitats were probably never naturally abundant prior to European settlement (Brooks 2003, p. 65). Fires set by Native Americans set back forest succession and maintained areas of suitable habitat (Bromley 1935, p. 64; Cronon 1983, p. 49). In addition, periodic wildfires and coastal storms such as hurricanes resulted in an estimated 10 to 31 percent of coastal, pine-oak forests in the seedling-sapling stage (age 1-15 years), a condition providing favorable habitat for the cottontail (Lorimer and White 2003, p. 45 and 46). In inland forests, where fires were less frequent, beaver activity and cyclical insect outbreaks set back forest succession. Of the inland forests, about six percent of the landscape is estimated to have been in an early successional stage capable of providing suitable habitat for the NEC (Litvaitis 2003, p. 117). Another model for inland forests suggests that stand regenerating disturbances were very rare and most early successional forest patches were the result of tree-falls (gap phase replacement) in an otherwise broadly-distributed climax forest (Lorimer 1977 in Brooks 2003, p. 70).

The distribution of the NEC has declined substantially and occurrences have become increasingly disjunct. Overall, in comparison to the 90,000 km² (34,750 mi²) encompassed in the estimated historical range, the current estimated range covers 12,180 km² (4,700 mi²) (Litvaitis et al. 2006, p. 1192).

The presence of otherwise suitable habitat, i.e., habitat containing appropriate vegetation structure, does not necessarily mean that it is suitable for sustained occupancy by the NEC. Instead, occupancy of individual habitat patches is dictated by patch-specific parameters relating to habitat quantity and quality, as well as the spatial distribution of patches at a landscape scale. This was illustrated by a multi-state, regional inventory to determine the distribution of NECs (Litvaitis et al. 2006, pp. 1190-1197). Litvaitis et al. (2006, p. 1193) reported that NEC were absent from 93 percent of 2,333 habitat patches within the recent historical range (1990 to present) that were searched for the presence of the species. Many of the unoccupied patches were considered of inadequate size or lower habitat quality due to succession or were occupied by eastern cottontails (J. Litvaitis, pers. comm.).

REFERENCES


Bringing New Animals into the Breeding Program

**Introduction**

There will be times during this project where it will be necessary to capture and remove New England Cottontail rabbits (NECT) from the wild to:

- Provide initial breeder stock
- Replace rabbits which have died due to disease or advancing age
- Provide necessary genetic diversity to the breeding program
- To preserve populations which are at risk due to their location

When to remove rabbits, where to remove rabbits from and how many rabbits to remove from wild populations will be determined by all wildlife agencies involved in this project in coordination with zoo staff who will determine if the breeding facility has the space and equipment necessary to care for additional rabbits. Additional recommendations from geneticists and other specialists may be important in making some of these decisions. The number of rabbits needed for the next breeding season will be provided to the Population Management Work Group by August 31st each year to determine how to meet the demand.

Once a recommendation is made to remove rabbits from the wild this will be communicated to all members of the project via email.

**Capture/Transport of Animals**

The representatives of the wildlife agencies for this project will be responsible for trapping and transportation of NEC rabbits from the wild. Traps will be set in locations determined by participating wildlife agencies and capture of the animals will be made using methods appropriate for this project and in compliance with all state and federal regulations.

Once a rabbit is in a trap, a biologist familiar with the appearance of NECT rabbits will need to visually examine the rabbit to make an initial determination if the rabbit in the trap is a NECT rabbit. If the rabbit does not appear consistent with NECT rabbits, then the trapped rabbit will be released or relocated at the discretion of the wildlife agency.

If the rabbit does appear to be a NEC rabbit based on external appearance, then the wildlife agency will:

1) Contact Lou Perrotti or Scott Silver to inform him we have a suspected NECT rabbit for transportation and determine who they should contact on arrival. If Lou Perrotti or Scot Silver
cannot be reached, the following people should be contacted in order to arrange for transport (See Appendix #1 for contact information):

a. Mike McBride, DVM (RWPZ)
b. Kim Wojick (RWPZ)
c. Donna Graffam, DVM (Queens Zoo)

2) After making contact with the zoo, the agency representative will complete a NECT capture record (See Appendix #4). A copy of this information will be brought with the rabbit when delivered.

3) Transport the animal. Upon arrival, the rabbit will be taken (in the trap) to the quarantine space on zoo grounds. The rabbit will be provisioned with food and water in the trap and left for several hours to acclimate to the new environment.

Note: At this point, the rabbit is in quarantine and standard quarantine procedures should be followed.

The zoo contact will assign an animal number to the rabbit. The animal number will be assigned as follows:

- The first two letters will be the two letter abbreviation for the state the animal was trapped in (RI for Rhode Island rabbits, NH for New Hampshire rabbits, MA for rabbits from Massachusetts, CT for rabbits from Connecticut, etc)
- The second two digits will be the last two digits of the year of capture (“10” for 2010, “11” for 2011, etc)
- The final 3 digits will be the rabbit number for that state for that year.

As an example, rabbit # “MA-11-003” will be the third rabbit trapped in Massachusetts in 2011.

A zoo employee will monitor the rabbit throughout the day and watch for fresh fecal material. Once a fresh fecal is found, the employee will:

1) Get a clean examination glove from the box in the rabbit area. Note you MUST use a new glove for each rabbit to prevent cross contamination of DNA between samples.
2) Using the gloved hand, open a new fecal tube which has been prefilled with alcohol. Prefilled tubes will be stored in the rabbit work space. Set the lid upside down on a flat surface.
3) Using the gloved hand place 5 fresh pellets in the fecal tube.
4) Using the gloved hand, screw the lid back on the tube.
5) Discard the glove in the trash.
6) On the tube, using a permanent marker, write the following information on the label:

```
NECT
<Animal Capture Number>
<Zoo Name>
<Today's Date>
```

7) When you are finished, bring the fecal tube and the Animal Capture Record to the Veterinary Hospital and give it to a member of the Veterinary Department.

The Veterinary Department will arrange to send the sample to Dr. McGreevy for the initial species determination.

*It is important that you do not touch the pellets with contaminated gloves or your bare hands as DNA testing is very sensitive to contamination*

After collecting the fecal sample, the animal will be cared for with other quarantined rabbits. Until an accession number has been assigned, this rabbit will be known by its Animal Capture Number.
**Initial Species Verification**

The Veterinary Department will receive the fecal samples and the animal capture record from the animal care staff.

The Veterinary Department will complete a NECT Animal Collection Log, seal the top of the tube with paraffin and the sample will be placed in the freezer for until mailing. The Veterinary Department will also call Dr. McGreevy (See Contact Sheet, Appendix #1) to arrange delivery. The samples will be delivered in person to URI or sent overnight mail with freezer packs to:

Dr. T.J. McGreevy  
University of Rhode Island  
Coastal Institute Kingston Campus  
Dept of Natural Resources Science, Rm 102  
1 Greenhouse Road  
Kingston, Rhode Island 02881

Dr. McGreevy will process the fecal samples and provide the results of the initial DNA testing back to the zoo.

If results of the genetic testing indicate the rabbit is a NECT rabbit, then the animal will be assigned an accession number that is communicated with the animal care staff and the Veterinary Department.

**Animal Identification**

Animals will not be permanently identified until the initial genetic testing indicates they are NECT rabbits. Once this testing is completed, the Veterinary Department will provide permanent identification using both a passive integrated transponder tag (PIT tag) and an ear tag. Both will be placed on the rabbit during its initial animal health profile while it is under anesthesia and replaced as needed by the Veterinary Department.

The animal will now be referred to exclusively by its accession number and all records related to this rabbit will have the animals accession number noted on them.

**Initial Animal Quarantine**

Rabbits will be quarantined for a total of 30 days from date of trapping until they can be moved in with the breeding colony.

If any animals in quarantine appear sick during the quarantine period, all rabbits currently under quarantine will stay under quarantine until the Veterinary Department releases the rabbits.

If new rabbits are introduced into the same quarantine space, the quarantine period for all rabbits starts over and must continue for 30 additional days.

**When rabbits are in quarantine animal care staff should:**

1. Take care of quarantined rabbits after caring for the breeding colony
2. Wear examination gloves when handling quarantine rabbits. Change gloves between rabbits.
3. Use equipment specifically indicated for quarantine rabbits. Do not use equipment from the breeding colony to care for rabbits under quarantine.
4. Use trash receptacles specifically identified for quarantine animals
5. Wear lab coats specifically for quarantine animals
6. Limit direct contact with rabbits under quarantine.
Animal care staff should not:
1) Go from quarantine back to the rabbit breeding colony
2) Feed, medicate or provide water to the breeding colony after servicing quarantine rabbits
3) Move equipment, dishes, trash, etc from the quarantine area to the breeding colony
4) Interact with any animals prepared for release

Note: While NECT rabbits are either in the breeding colony or in quarantine, all NECT rabbits are in permanent quarantine in regards to the rest of the zoo's animal collection.

Daily Care of Rabbits in Breeding Program

Caging
NECT rabbits will be individually housed in laboratory style cages (Figure 1) this style of caging will allow for easy cleaning and disinfection. Substrate will consist of Timothy hay and all rabbits will have access to a wooden hide box with a closable door that not only will provide shelter and security for the rabbit but will allow the keepers to remove the rabbits from their cages with little or no stress when cleaning, taking weights, or transporting.

Figure 1: Examples of caging appropriate for this project

Security
The NECT captive population at Roger Williams Park Zoo and Queen's Zoo are housed in buildings that have no public access and limited staff access. Only authorized zoo staff and keepers responsible for daily rabbit husbandry are permitted to enter this facility.

Biosecurity
To avoid any possible spread of disease to zoo staff and/or the rest of the zoo collection persons servicing the rabbits in any way are required to wear lab coats or similar protective clothing, rubber boots, surgical masks, and disposable surgical gloves. Boots and clothing will be used exclusively for
rabbit husbandry and will remain in a designated area in the captive breeding facility when not in use. For additional protection, keepers are required to service the rabbit group at the end of the day when all other animal care duties have been completed. To eliminate the spread of disease from area to area within the facility foot baths are to be placed and used at the entrance and exit of all areas that house NECT rabbits.

Disinfectant Policy

The following disinfectant schedule will be implemented in each area that houses the NECT rabbits. Every effort should be made to follow it as closely as possible. Rotation and standardization of disinfectants will discourage the spread of disease and the development of resistant infectious agents.

General Rules for Disinfection

1. Avoid contact with skin. Wear eye protection when mixing disinfectants and if there is a potential for disinfectant to splash in eyes. Wear gloves when using disinfectants.
2. Remove all organic material (feces, food, blood and soil) from area or item prior to disinfecting.
3. Clean items or area thoroughly with detergent. Rinse.
4. Reconstitute disinfectant mixture in appropriate dilution. Failure to do so may harm you, the animal or render the disinfectants less effective. A more concentrated solution does not always mean stronger or more effective.
5. Soak surfaces with disinfectant for a minimum of 10 minutes.
6. Disinfectants can be harmful to animals. Rinse thoroughly!!
7. Three different disinfectants will be used, Bleach, Roccal & Virkon S. Each Disinfectant will be used for one month. These are the only disinfectants approved for use without Veterinary or Curator approval.
8. All disinfectants must be stored in properly labeled containers.
9. Amphibians are not included in this disinfectant schedule as selected disinfectants may be harmful to them.
10. Disinfectants loose their efficacy if not reconstituted at the appropriate frequency.

Concentrate containers with appropriate disinfectant will be distributed to the areas the first of the week. Concentrate containers from the previous disinfectant will be returned by the end of the day.

<table>
<thead>
<tr>
<th>Floors, cages, walls, windows, rakes and shovels are to be disinfected a minimum of once a week, alternating the disinfectant as per schedule.</th>
<th>Footbaths are to be change daily, alternating disinfectant as per schedule.</th>
<th>Dishes and Enrichment items are to be washed and disinfected after each use, alternating disinfectants as per schedule.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Month</strong></td>
<td><strong>Disinfectant</strong></td>
<td><strong>Dilution</strong></td>
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<tr>
<td>January</td>
<td>Bleach</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>February</td>
<td>Roccal</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>March</td>
<td>Bleach</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>April</td>
<td>Vircon S</td>
<td>1 1/3 oz/Gal</td>
</tr>
<tr>
<td>May</td>
<td>Bleach</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>June</td>
<td>Roccal</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>July</td>
<td>Bleach</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>August</td>
<td>Vircon S</td>
<td>1 1/3 oz/Gal</td>
</tr>
<tr>
<td>September</td>
<td>Bleach</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>October</td>
<td>Roccal</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>November</td>
<td>Bleach</td>
<td>½ oz/Gal</td>
</tr>
<tr>
<td>December</td>
<td>Vircon S</td>
<td>1 1/3 oz/Gal</td>
</tr>
</tbody>
</table>
Cleaning

Daily
All rabbit enclosures are to be checked and serviced on a daily basis. All Catch trays are to be dumped and washed with the proper disinfectant and thoroughly rinsed clean (See Disinfectant Policy). Refill all food hoppers as needed, rinse all water bottles, and refill with fresh water. Once all the cages have been serviced, floors are to be swept and hosed down. Be sure all floor drains are rinsed and cleaned of any debris that may have accumulated while cleaning. All footbaths should be rinsed and replaced with clean disinfectant. Be sure to log all husbandry activities on to the daily husbandry log.

Weekly
Once a week all cages are to be completely stripped of all the old Timothy hay substrate and disinfected (See Disinfectant Policy). Wash each cage with disinfectant, rinse thoroughly and replaced with fresh Timothy Hay. Catch trays are to be dumped and washed with disinfectant and thoroughly rinsed clean. Check each wooded hide box to be sure all soiled hay and feces are removed. Fill all food hoppers as needed, rinse water bottles, and refill with fresh water. Once all the cages have been serviced floors are to be swept and washed down with disinfectant and rinsed. Be sure all floor drains are rinsed and cleaned of any debris that may have accumulated while cleaning. All footbaths should be rinsed and replaced with clean disinfectant. Be sure to log all husbandry activities on to the daily husbandry log.

Diet
Rabbits will be maintained on a primary diet of commercial rabbit pellet. Timothy hay will be used as supplement. When available, appropriate grasses and woody cuttings from the NECT rabbits known natural diet will also be provided. Additional records are kept on all natural food items offered and the level of acceptance of that particular food item to help better understand food preferences of the NECT rabbit. Natural food items are selected from a list compiled in a food preference study conducted by Pringle in 1958 (Pringle 1960).

Water is offered and readily accepted in lick type water bottles manufactured specifically for rabbits and other small mammals.

Natural Food Items Offered and Accepted by the Captive NECT's:

Apple, (Populus tremuloides)    Black Birch, (Betula lenta)
Oaks, (Quercus sp.)            Beech, (Fagus grandifolia)
Red Maple, (Acer rubrum)       Black Cherry, (Prunus serotina)
Sugar maple, (Acer saccharum)  Quaking Aspen, (Populus tremuloides)
Black Maple, (Acer nigrum)     Mulberry, (Morus sp.)
Sweet Briar, (sp. ?)           Bramble (asst. sp.)

Moving Animals between enclosures
Once the NECT is assigned a permanent cage it should not have to be moved into another cage for any reason unless it is going to be placed in a breeding situation or for medical reasons. Due to the animals high stress level, to avoid escape, injury, or death care must be taken anytime a NECT must be handled and/or moved for any reason. A wooden hide box with a closable door that is used to provide shelter and security for the rabbit is also designed to allow the keepers to remove the rabbits from their cages with little or no stress when cleaning, taking weights, or transporting. The rabbits readily accept these boxes as safe havens and often run into them as soon as the cage door is opened. Be sure to wear disposable surgical gloves when handling NECT’s. Surgical gloves are to be changed between rabbits.
**Captive Reproduction**

**Gender Determination**
Mature male NECT rabbits have paired external scrotum but immature animal gender determination must be done by evertng the penis or clitoris and examining the structure.

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.jpg" alt="Male Image" /></td>
<td><img src="image2.jpg" alt="Female Image" /></td>
</tr>
</tbody>
</table>

The penis usually tapers slowly, does not have a ventral groove and can arch caudally. The clitoris is wider, rounded rather than tapered. There is sometimes a visible ventral groove.

**Breeding**

Adult NECT rabbits that are chosen to be bred will be removed from the lab cages and placed in the breeding facility. The facility is a 15 by 10 foot room consisting of a concrete floor with a thick layer of Timothy hay for substrate. Brush piles and artificial hiding spots such as PVC tubes and wooded hide boxes are provided for shelter. Rabbits will be offered the primary diet of commercial rabbit pellets and supplemented as much as possible with natural food items. Windows high on the outside wall will provide natural photoperiod in the facility.

One female will be placed in the breeding facility first and allowed to acclimate for a few days before introducing the male. Once the male has been introduced the pair will remain together anywhere from a few days to a week to be sure a successful breeding has occurred. Once it has been determined that reproduction may have taken place both male and female rabbits will be returned to his or hers permanent cage location. Females that have been bred will be allowed to give birth in the lab style caging. This process will be repeated depending on the number of pairs that are to be bred. No more than on pair should be placed in the breeding facility at one time.

Cameras will be set up in the breeding facility to monitor and record male and female interaction and breeding behaviors.

**Gestation**

After completion of any breeding opportunity, please note the date the male rabbit is removed from the female rabbit in both animal records.
The female rabbit may be maintained either in a “breeding run” (as a single rabbit or several females) or in a single animal enclosure after breeding.

Twenty-seven days after the first day of access between a male and a female, the female rabbit should be put on “birthing protocol”. Begin by placing the appropriate cage card on the cage door (See Appendix 7).

During birthing watch, efforts will be made to disturb the female rabbit as little as possible while still checking in on her to see if she has given birth to one or more baby rabbits (kits).

**Birthing Protocol**

1. Every day you need to look throughout the cage to try to find a nest or any kits. Be sure to check inside nest boxes, and in piles of hay.
2. If you see kits in the enclosure you should:
   a. Count the number of visible kits
   b. Inform zoo coordinator immediately via radio (Lou Perotti or Scott Silver)
   c. From this point on, you will start using the protocol for mothers with kits (see next section)
3. If you do not see kits, visually examine the mother to make sure she appears bright and alert.
4. Do not disturb the mother by removing large quantities of hay or intact browse.
5. Do not disturb the mother with loud sounds or lots of talking.
6. Do not disturb animals that are on the birthing protocol unless instructed.
7. Do not pick up rabbits unless absolutely necessary.
8. Do not shift the mother to another cage or remove her (unless instructed).
9. Add pelleted food to the food dish
10. Clean and replace water bottle
11. If you have browse, you can add it to the cage, but do so in small amounts and only put the browse in the front of the cage where it will be easy to remove later.

The birthing protocol automatically ends 40 days after the last day that the female had access to a male rabbit.

**Mother with kits**

Once kits are visible in the nest, the following protocol will be used to care for the rabbits. Remember that mother rabbits may only feed their kits once a day and they may spend a great deal of time away from the kits. This is a natural behavior designed to keep predators away from the nest.

**Protocol for Mothers with Kits**

1. Begin by making sure that the appropriate cage card is displayed prominently on the cage door (See Appendix 7)
2. Each day, start by exploring the cage to see if any kits have wandered away from the nest or if you see any blood or diarrhea. Do this trying not to disturb the mother or her kits. If you need to move hay or browse around in the enclosure, do so slowly and quietly.
3. Finish your examination of the cage by counting the kits in the nest. Do this without touching or disturbing the kits or the mother.
   a. Look for kits that appear weak or unthrifty.
4. Visually examine the mother to make sure she is bright and alert.
5. Do not disturb the females and kits for the first three days after birth by removing large quantities of hay or intact browse, cleaning the trays, shaking the hay, or hosing floors. Only food and water should be given during this period. After the three day period it will be find to clean the trays and remove soiled hay from around the young. If the female appears stressed, discontinue servicing the cage.
6. Do not disturb the animals with loud sounds or lots of talking
7. Weight the animals only when asked.
8. Keep the mother and the kits in the same cage until the kits are old enough to move to a cage by themselves.
9. Make sure you are providing plenty of food for the mother.
10. Make sure the water bottle is cleaned and filled daily.
11. Inform the veterinary department if you have any concerns about either the mother or any of the kits.
12. Cages containing mother and young may be completely cleaned and disinfected after 5 days from birth.

In addition to the protocol above, keepers are responsible for providing information on date of birth, parent ISIS numbers, and number of kits for each litter to zoo coordinator. They will provide you with accession numbers for each of the kits in the litter. These numbers must follow the kits as long as they are at the zoo.

**Record Keeping**

**Husbandry Records**
All rabbits that enter the captive breeding group are logged into the Zoologic Information Management System (ZIMS) upon arrival. These records are maintained and updated accordingly by the Director of Conservation Programs. In addition, rabbit keepers are required to maintain a daily log of all husbandry activities and record the time spent servicing the group. A log of all breeding and births will be maintained in a Google Drive for access by members of the NEC Population Management Work Group.

**Medical Records**
The Veterinary Department will maintain all medical records in MedARKS. If at some point in the future, the Veterinary Department transitions to ZIMS for Medical records, all medical records will be moved over to ZIMS. Items which cannot be entered into MedARKS or ZIMS will be maintained either with paper copies or electronic files clearly labeled with the patient information.

**Handling Sick/Dead Rabbits**
When a rabbit dies in the colony or quarantine space the following procedures should be followed:
1) The veterinary department and zoo coordinator should be notified via radio or phone.
2) Wear a surgical mask or face shield and exam gloves for steps #3-6.
3) All other rabbits in the same enclosure should be removed to a new, empty cage. Do not move a rabbit or babies that were with a dead rabbit into another cage occupied by other rabbits.
4) Place the carcass of the dead rabbit in a necropsy bag. Tie the top of the bag tightly.
5) Place this bag into another necropsy bag. Remove your gloves and put them in this second bag. Tie the second bag tightly.
6) Apply tape to the top of the outside bag with the rabbit’s accession number on it.
7) Take the body to the veterinary department for necropsy.
8) Using a new, clean pair of gloves and a mask or face shield, clean the cage out thoroughly and disinfect all dishes, enrichment items, etc.

**Dead Rabbit Disposition Protocol**
Rabbits which die or are euthanized will be necropsied by the RWPZ Veterinary Department to determine the cause of death or illness. The veterinary department will inform the working group of any deaths after completion of the gross necropsy examination. A more complete report will be submitted to the working group after the results of all tests are back and the histology has been evaluated by a qualified pathologist.
After completing the necropsy, the Veterinary Department will secure any additional tissues requested by any member of the working group and freeze the remainder of the carcass until the end of the breeding season. Anyone who wants specific tissue or laboratory samples collected should contact the Veterinary Departments. The Veterinary Department will also collect and bank blood samples in an ultralow freezer for future research projects.

**Potential Zoonoses of Rabbits (Sylvilagus spp.)**

Whenever humans and animals come in contact with each other, the potential for transmission of diseases from the animals to humans exists. While zoonotic disease is a relatively rare problem, the consequences can be serious. Persons involved in this project should be aware of the following potential zoonotic diseases so that they can protect themselves and inform their health care provider if they believe they may have signs consistent with contact with one of the following diseases:

**Tularemia**

The disease is caused by the Gram negative bacterium *Francisella tularensis* and is probably the most significant potential zoonosis of cottontail rabbits. Although human cases are rare, cases have been identified in New England (2000-2008 data; primarily in Massachusetts), where the bacterium is endemic to several islands. Disease is primarily spread via biting arthropods but also through skin contact with infected rabbits, ingestion of contaminated water, or inhalation of contaminated dusts or aerosols. Symptoms are variable depending on how the bacteria enters to body and can include, fever, skin ulcers, swollen lymph nodes, tonsilitis, cough, chest pain, or difficulty breathing. *Francisella tularensis* is also a class A agent of bioterrorism and any confirmed cases should be reported to public health officials.

**Babesia divergens**

This is a tick-borne disease and the main source of human babesiosis in Europe. The organism is endemic in some rabbit populations in New England and is transmitted by bites from *Ixodes spp.* ticks. Cases are extremely rare, however, the course of disease can be severe. Infection is considered a medical emergency, and a 38% case fatality rate has been observed. Symptoms are similar to malaria with fever (up to 105°F), chills, and severe anemia observed.

**Human Granulocytic Anaplasmosis**

This is a tick borne disease previously known as Human granulocytic ehrlichiosis caused by the organism *Anaplasma phagocytophilum*. Cottontail rabbits have been documented carriers of *A. phagocytophilum* and appropriate tick vectors. Individuals should use particular caution when handled tick-infested rabbits. Clinical signs of disease include fever, myalgia (muscle aches), headache – symptoms similar to influenza. Labwork abnormalities include leukopenia and thrombocytopenia.

**Lyme Disease**

This is a tick borne disease caused by the spirochete agent *Borrelia burgdorferi sensu lato*. The white-footed mouse (*Peromyscus leucopus*) is considered the principle reservoir but tick vectors are also found on cottontail rabbits and cottontails appear to have great reservoir capacity for *B. burgdorferi*. Individuals should use particular caution when handled tick-infested rabbits. Clinical signs of disease in humans include fever, skin rash, myalgia (muscle aches), headache, and malaise. As disease progresses individuals can develop joint, neurologic, and other systemic disease.

**Rabies**

Rabies is a significant viral disease capable of causing neurologic disease in all species of mammals. Rabbits are not considered wildlife reservoirs for rabies, however, they are susceptible like other mammals to disease following bite or exposure from other rabid animals. Diseases is typically transmitted by bite but can also occur via body fluid transmission or other contact with carcasses. Caution is advised when handling rabbits exhibiting neurologic signs or wounds of unknown origin especially in human individuals unvaccinated for rabies. Rabies is almost invariably fatal without post-exposure treatment. Exposed persons should seek immediate medical attention and hold suspect rabbit carcasses to submit the head for rabies testing.
Cryptosporidiosis
The protozoan organism *Cryptosporidium parvum* can cause diarrhea in young rabbits, peaking at 30 to 40 days of age and is also transmissible to people. *Cryptosporidium parvum* causes mainly self-limiting diarrhea in immune-competent individuals but may cause more severe disease in individuals with immunosuppressive disorders or receiving immunosuppressant medications. Disease risk can be significantly reduced with wearing gloves and appropriate disinfection protocols.

Prevention
Individuals can help protect themselves from zoonotic disease by:
1. Using handling protocols which reduce the likelihood of bites or scratches
2. Using personal protective equipment such as gloves, masks and long sleeved clothing to reduce exposure to blood and feces of rabbits
3. Show particular care in the handling of animals which appear ill or are acting abnormally
4. Washing hands with soap and water after any contact with rabbits or their body fluids.

Additional information on rabbit diseases can be found in Appendix 5.

Sources:


Health Care

**Initial Animal Health Profile**
The initial animal profile will not be performed by the Veterinary Department until the initial genetic testing indicates the animal is a NECT rabbit unless the rabbit in question is showing signs of a medical problem and/or the veterinary team determines the rabbit needs evaluation sooner.

The Veterinary Department will perform the following on all rabbits entering the breeding program:
- Complete Physical Examination including:
  - Fundic examination
  - Complete dental evaluation
- Complete Blood Count with Differential
- Complete Chemistry Panel
- Blood will be collected for blood banking for future testing or research purposes
- Blood will be collected for DNA testing (See Appendix #2 for details)
- Tissue will be collected for DNA testing (See Appendix #2 for details)
- Urinalysis (if urine can be collected during anesthesia)
- Two nasal swabs will be taken and frozen (for PCR testing at a later date if needed)
- Radiographs (Whole body)
- Abdominal and cardiac ultrasound
- Morphological measurements including:
  a. Body weight
  b. Length of femur
  c. Ear Length (Base to Tip)
  d. Ear Width (Widest Part)
- Breeding Soundness Exam (Including checking females to see if they are currently pregnant)
- Placement of PIT tag between scapula
- Placement of ear tag (Males in right ear, females in left ear)

All procedures will be performed under anesthesia.

If any serious abnormalities are noted during the Initial Health Exam, the Veterinary Department will not place the PIT tag or the ear tags until it is determined the rabbit will become part of the breeding colony.

After recovery from anesthesia, the rabbit will be returned to quarantine to complete the quarantine period.

**Reintroduction**

The ultimate goal of this program is to successfully transfer young NEC born in captivity to suitable habitat within their former range. Translocation and re-introduction programs for *Sylvilagus* sp. have often resulted in high mortality rates, most often attributed to predation, especially when rabbits are released in sub-optimal habitat or occupied sites. Adults in general are less tolerant of new environments when trans-located than juveniles and suffer higher mortality rates whether raised in a captive pen or wild caught (Letty et al, 2008). Additionally, there is some evidence with other species studied that individuals raised in a captive situation have lower survival rates when released than wild caught individuals (Griffith et al, 1989).

In translocation experiments with European rabbit, predation and physiological condition were the most important factors in survival during the first 18 days post release, whereas food availability and disease played a more important role between day 19-180 post release (Cabezas et al, 2011). Disease in young individuals has also resulted in significant mortality rates in captive situations. Another recent translocation in Maine resulted in high mortality rates (73%), although habitat quality with regard to cover and shrub species composition seemed to be excellent. Mortality rates may have been a result of insufficient availability of high protein foods, or the difference in habitat and food availability in the release site as compared to the initial capture site (Jakubus, 2011). Ultimately, any variables that reduce the overall fitness of individual rabbits or lead to more risky behavior can result in NEC being more susceptible to predation (Litvaitis et al. 2008). In general, rabbit species also are expected to adapt better to a new release site if the plants that they are exposed to as a juvenile are also present in the habitat they are released into, and the habitat is similar in general. In a re-introduction program with juvenile riparian brush rabbits (*Sylvilagus bachmani riparius*), individuals were especially vulnerable in the first four weeks post release and survival increased as a function of the amount of time spent in soft release pens (Hamilton et al, 2009).

Overall, to maximize chances of success in this program it will be critical to 1) select release sites with high quality forage and cover available in sufficiently large habitat patches, 2) minimize the time that juvenile NEC spend in artificial conditions 3) provide juvenile NEC with opportunities to feed on plants that will be present at the release site 4) ensure optimal physiological condition of juvenile NEC prior to release, and 5) provide incentives to minimize dispersal during the first week post release. If soft release
pens are deemed unnecessary or in-feasible based on site specific variables, providing additional cover or a familiar ‘safe area’ may be warranted.

As this program progresses and we are able to learn from the process, we expect that any protocols developed will be adapted to improve upon program successes and minimize failures. In the meantime, the recommended actions include:

**Release Protocol**

**Transport of captive born NEC to an acclimation pen**

Transport of NEC away from the captive situation is recommended at an age when they would normally be dispersing from their natal sites and prior to achieving adulthood (i.e. reproductively active). This should serve to reduce the amount of stress and potential for rabbits to lose any innate predator avoidance behavior, and also prevent them from becoming too accustomed to the captive situation. This needs to be balanced however with allowing rabbits a chance to acclimate and mature to a stage at which they have the best chance of avoiding predators and efficiently identifying food and shelter. The current strategy is to transport individuals from the caged situation to a more natural acclimation pen as soon as possible, with the following general guidelines:

1. Captive born NEC will be separated from the female at weaning or no later than 6 weeks of age, unless a larger pen is available where aggression is unlikely to occur.
2. A health assessment will be completed for each individual prior to 6 weeks to identify baseline levels of parasites and confirm that they are free of disease (this may include sampling for blood, fecal and nasal swab). Handling will otherwise be kept to an absolute minimum.
3. Once results from the health screening are complete, young NEC will be transported to a natural setting with appropriate food and shelter indicative of their ultimate release site.
4. Transfers will be timed to occur between May 1 and October 31 of each year to maximize survival and acclimation.
5. Prior to release each individual will be weighed and marked for later identification. This will potentially include an ear tag, a PIT tag, or a radio transmitter of some kind for monitoring post release (i.e. collar or implant).

**Acclimation pen configuration**

1. Outdoor pens will ultimately need to be established in different portions of the historic range where we anticipate wanting to release captive born rabbits. This will be critical since habitat structure and woody forage varies dramatically in different portions of New England. Ideally, captive born NEC would be able to identify and develop preferences for food that will also be present in their final release site.
2. Initial holding pens should provide adequate food such that NEC would be required to find suitable food and do not become dependent on supplemental food sources that will not be available in their final release site.
3. The holding pen should be completely free of other rabbits to prevent the transmission of disease, and any rabbits removed from the site should go through a health assessment to determine what pathogens may be present in the area. If warranted a vaccination program should be considered to prevent disease outbreaks among newly released individuals.
4. The holding pen should be constructed to restrict all predator access. This will include an apron underground to prevent digging as well as a fine mesh to restrict weasels, flashing at the top to prevent climbing and a cover to prevent avian predation (see Appendix 8 for complete design).
5. Monitoring cameras or track plates will be installed within the holding pen to detect any predators that may have escaped prior detection. Ideally monitoring will occur for a few weeks before the introduction of NEC.
6. Acclimation pens may be sectioned off to separate cohorts of various ages, gender, and/or intended release site as needed. Section should contain a maximum of five individuals per ¼ acre.

**Acclimation pen monitoring**

1. Monitoring cameras will be set in areas where images of rabbits are most likely to be taken. At least one camera should be placed in front of each feeding station. Cameras will be checked twice per week, and observations on the camera photos will be recorded on the appropriate data sheet. All photos containing rabbits or other observations of interest should be saved.
2. Supplemental food and water stations should be set up so that all individuals have access. Feeding stations will be filled with commercial alfalfa rabbit chow twice per week, or as needed to offset ingestion of rabbit chow by small mammals (e.g. voles). Food stations should be placed under brush or in some kind of cover, as to not acclimate individuals to spending time in open areas to obtain food.
3. Water bottles are to be set up so that all individuals have access and filled with fresh water as needed.
4. At the beginning of each breeding season prior to setting up stations, all equipment (bins, bowls, and water bottles) should be cleaned with soap and water.
5. While rabbits are present in the acclimation pen, it should be visited at least twice per week. At each monitoring visit, food and water will be checked and filled as needed, trail cameras will be checked for photos and battery life, and the perimeter and roof (when applicable) will be checked for breaches and repaired as needed. Pen condition and all observations will be recorded on appropriate data sheets.
6. If a dead or injured rabbit is found during a monitoring visit, appropriate USFWS and/or zoo staff should be notified immediately. An injured rabbit should be transported to the Roger Williams Park Zoo veterinarians immediately for care. Dead rabbits should be placed in a plastic bag and inside a cooler until it can be refrigerated until transfer to the zoo for necropsy. If possible, take photos and record all observations at the time of discovery of the rabbit including its location, ear tag number, the temperature/other weather conditions, presence of other animals or animal scat, and evidence of breaching of the pen by a predator.

**Potential issues for ongoing evaluation as the program progresses**

A maximum density should be determined for each holding pen such that rabbits do not become aggressive with other rabbits or degrade the vegetation to the extent that sufficient food is no longer available. This will vary from site to site and seasonally so it may be useful to develop standard methods for monitoring browse and rabbit numbers so that we can adapt the release strategy as needed.

If the pen were to be used as a breeding facility it would be important to determine the number of individuals that could be sustained without altering their natural behaviors. Supplemental food may be provided to enhance the body condition of females and increase production of young.

**Transport from holding pen to release sites**

1. After approximately 2-4 weeks NEC will be re-captured using standard rabbit traps placed in well utilized trails and baited with apple. Each individual will be weighed, sexed, and checked for overall health (physiological condition). If standard traps are not efficient at trapping rabbits, alternative methods will be considered.
2. NEC that have maintained or gained weight during the prior three week period and appear healthy will be collected for transport to their pre-determined release site.
3. Prior to release, any NEC could be chosen to be fitted with a radio-collar for post – release monitoring, ideally this has been done upon release into the pen to reduce stress upon final release.
4. NEC that have lost weight and are not faring well in the outdoor setting could be moved to a smaller holding pen and provided supplemental feed, or returned to a zoo to serve as a breeding stock depending on the goals of the program and the genetic disposition / sex of the individual.

**Release site recommendations**

Ranking and selection of release sites will be determined through a process described previously (Ranking System used to Determine Suitable Release Sites). Decisions will be made at the regional scale as well as on a site by site basis depending on the goals of the overall program and the strategy developed within the Captive Breeding Working Group. Although soft release pens have been recommended to improve short term survival of rabbits in low cover habitats (Rouco et al, 2010), recent research has confirmed that soft release pens may not provide significantly higher survival rates post release in areas with high quality habitat and dense cover (Letty et al., 2008). Identification of high quality habitats with adequate cover and those that are not already occupied with rabbits will be the most critical component to the survival of the young that are released. The physiological condition of the rabbits also plays an important role in post release survival, mitigating the potential impacts of being in an unfamiliar habitat and needing to find food and avoid predators. Therefore, it is critical that we monitor the health of individuals prior to release to maximize survival rates. Depending on the quality of the habitat it may also be advantageous to consider construction of brush piles or warrens to provide cover and also potentially leaving the artificial den sites that the rabbits have become accustomed to in the captive breeding and acclimation pens on site following transport.

There is no clear guidance on the exact number of individuals that should be released at any given time to an area identified for re-population. However, since mortality rates are very high during the first year even in wild populations of NEC (up to 85%), the recommendation is to release large numbers of individuals at each site to improve the success rate. Increasing juvenile recruitment into the breeding population was identified as the most critical component for success in the Columbia basin pygmy rabbit recovery program (Zeoli et al. 2008), and low success rates have been recorded when small populations are released even when habitat conditions are considered good (Griffith et al. 1989). The number of NEC that would be released at a site should be determined based on patch size, habitat suitability and site specific attributes, with the assumption that a minimum of 10-40 NEC would be released during any given season.

Throughout the initial two week period post release, a pre-determined subset of the released NEC will be monitored daily to determine survival and movement. Additional monitoring will continue beyond that timeframe as described in the following monitoring protocol. Any releases should occur between May and October 31 of any year to allow for acclimation to the site prior to winter. Studies with European rabbits have found that age of the released animals may play a larger role than time of year in survival, but in general survival is expected to be lower following fall or winter releases that for those conducted in spring or summer (Letty et. al., 2008). There is also some evidence that predators respond to an influx of prey in a relatively confined area resulting in an increase in predation rates during the short term. Hence, the decisions with regard to the number of individuals to release at a site and over what time period may need to be made on a case by case basis as we begin to implement releases and monitor the results. Additionally, these protocols will be adapted to improve survival if we find that mortality is high and there is the chance that excluding predators or providing additional cover or protein rich food would make a difference in survival rates.

**Literature cited:**
Monitoring Protocol

Monitoring Released Rabbits

A subset of rabbits will be outfitted with radio collars prior to release to track survival and dispersal. The information gained from this work will provide information to assist managers with adapting the release strategy. Rabbits will be monitored 5-7 times for the first two weeks, 3-4 times per week for the next month, and then twice a week for the following 2 months. At that time the rabbit will be checked 1 time per week through the life of the collar’s battery or the rabbit perishes.

Patch Population Monitoring – see Appendix 9 for protocol
Research Associated with Project

Roger Williams Park Zoo
The zoo is currently collecting blood from adult and juvenile rabbits. Rabbits without evidence of disease will be used to create normal physiologic values for the NECT.
Appendices

Appendix 1 – Contact Sheet
Appendix 2 – Protocol for collecting DNA samples from Cottontail Rabbits
Appendix 3 – Sample Collection Log
Appendix 4 – New England Cottontail Capture Record
Appendix 5 – Overview of Rabbit Diseases
Appendix 6 – Husbandry Log
Appendix 7 – Cage Cards
Appendix 8 – Acclimation Pen Construction Details
Appendix 9 – Patch Population Monitoring Protocol
## Appendix 1 – Contact Sheet

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<td><a href="mailto:Amy.gottfried@gmail.com">Amy.gottfried@gmail.com</a></td>
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<td>Thomas Husband, PhD</td>
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<td><a href="mailto:thomas.husband@gmail.com">thomas.husband@gmail.com</a></td>
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<tr>
<td>T.J. McGreevy, PhD</td>
<td>401-743-4675</td>
<td></td>
<td><a href="mailto:tjmcg@my.uri.edu">tjmcg@my.uri.edu</a></td>
<td>Cell Phone: 401-481-6151</td>
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<td>Mary Sullivan</td>
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<td></td>
<td><a href="mailto:mniebels@gmail.com">mniebels@gmail.com</a></td>
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### US Fish and Wildlife Service

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<td>Cynthia Corsair, Biologist</td>
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<td><a href="mailto:cynthia_corsair@fws.gov">cynthia_corsair@fws.gov</a></td>
<td>Rachel Carson National Wildlife Refuge 321 Port Road, Wells, ME 04090</td>
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<tr>
<td>Kate O'Brien, Refuge Biologist</td>
<td>207-646-9226 x 27</td>
<td>207-646-6554</td>
<td><a href="mailto:Kate_OBrien@fws.gov">Kate_OBrien@fws.gov</a></td>
<td>Coastal Program Southern New England - NY Bight 50 Bend Road, Charlestown, RI 02813</td>
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<td>Suzanne Hoover Paton, Senior Biologist</td>
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<td>401-364-0170</td>
<td><a href="mailto:Suzanne_Paton@fws.gov">Suzanne_Paton@fws.gov</a></td>
<td>New England Field Office 70 Commercial Street, Suite 300 Concord, New Hampshire 03301</td>
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<tr>
<td>Anthony Tur, Endangered Species Biologist</td>
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<td>Steve Fuller, Ph.D</td>
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<td>Steve Weber</td>
<td></td>
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<td><a href="mailto:sweber56@roadrunner.com">sweber56@roadrunner.com</a></td>
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Appendix 2 – Protocol For Collecting DNA Samples From Cottontail Rabbits

**SKIN SAMPLE:**
Anesthetize the rabbit using standard veterinary procedures. Use a standard biopsy punch (~6-mm) to collect sample from the outer edge of the rabbit’s ear. Use a sterile biopsy punch for each individual. If a sterile biopsy punch is not available, then use a sterile razor blade. **Please be extremely careful to avoid cross-contamination between samples, as well as contamination from humans or other animals.** Place the tissue sample into a sterile 15ml tube and add enough 100% ethanol to submerge the sample. Label tube on the white writing surface with a permanent marker. Please include the species of cottontail (if known), animal’s studbook number, name of the institution where animal is housed, and the date that the sample was collected. Seal the cap with parafilm and place the sample in the freezer until shipping. Please record information requested in the data collection sheet and ship the sample FedEx next business day with ice packs.

**BLOOD SAMPLE:**
Anesthetize the rabbit using standard veterinary procedures. Collect at least 2 cc of blood in an EDTA tube, invert tube several times to prevent clotting, and refrigerate the sample until shipping. Label the tube on the white writing surface with a permanent marker. Please include the species of cottontail (if known), animal’s studbook number, name of the institution where animal is housed, and the date that the sample was collected. Please record information requested in the data collection sheet and ship the sample FedEx next business day with ice packs.

**FECAL SAMPLE:**
Collect a fresh fecal sample (<3 hours is ideal, but up to 8 hours is acceptable) using clean gloves and place 5 pellets into a sterile 15ml tube. Add 100% ethanol to the tube at a volume of ten times the volume of the fecal sample. At a minimum please make sure the pellets are submerged. Label the tube on the white writing surface with a permanent marker. Please include species of cottontail (if known), animal’s studbook number, name of the institution where animal is housed, and the date that the sample was collected. Seal the tube with parafilm and store in a freezer. Please record information requested in the data collection sheet and ship the sample FedEx next business day with ice packs.

Please contact Dr. T.J. McGreevy to co-ordinate the shipping of all samples.
tjmcg@my.uri.edu
401-481-6151 (cell phone)

Send samples overnight to:
Dr. T.J. McGreevy
University of Rhode Island
Coastal Institute Kingston Campus
Dept of Natural Resources Science, Rm 102
1 Greenhouse Road
Kingston, Rhode Island 02881
Appendix 3 – Sample Collection Log

Institution Name: Roger Williams Park Zoo

Contact Person: Lou Perrotti

Phone #: 401-785-3510 x 335

E-mail Address: lperrotti@rwpzoo.org

Fax #: 401-941-3988

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Any questions? Please Contact Dr. T.J. McGreevy at 401-481-6151, tjmcg@my.uri.edu; Dr. Thomas Husband at 401-487-2912, thomas.husband@gmail.com; or Mary Niebel at 401-743-4675, mniebels@gmail.com
Appendix 4 – New England Cottontail Capture Record

Date trap set: ___/___/______

Location: ___________________________ GPS Coordinates: __________________________

State of Capture: _______ Type of Trap Used: __________________________

Animal Identified By: __________________ Animal Transported By: __________________________

Contact Phone Number: __________________

Wounds or Evidence of Illness: Yes No Unique Markings/Features __________________________

(If Yes, please describe under “Additional Notes”)

Additional Notes ____________________________________________________________________

To be filled out by RWPZ Contact:

Capture # _______—_______—_______ Received by: __________________________

Animal Number (3 digits) Last Two Digits of Year (2 digits) State Abbreviation (2 letters)

Directions to Security Gate at the Zoo:

From Points North of the Zoo:
1) Take I-95 South to Exit 17
2) At the bottom of the off ramp, turn left (at the light)
3) Move over to the right lane and turn right at the first light (This will take you back across the road towards the Zoo)
4) As soon as you have crossed the major road (Elmwood Ave), make an immediate left.
5) This is Gate #3. Give them your information and the name of your contact.

From Points South of the Zoo:
1) Take I-95 North to Exit 16
2) On the off-ramp, stay to the right
3) At the light at the bottom of the off-ramp, take a left onto Elmwood Avenue
4) At the next light make a right into the park
5) Take an immediate left turn.
6) This is Gate #3. Give them your information and the name of your contact.
Appendix 5 – Overview of Rabbit Diseases

Diseases of Potential Concern for the Reintroduction Program

Bacterial Diseases

Tularemia – ZOONOTIC. The disease is caused by the Gram negative bacterium *Francisella tularensis*. This bacteria has a broad host range but principally causes disease in rabbits and rodents. Two major subspecies of F. tularensis are recognized: *Francisella tularensis* biovar *tularensis* (type A), which is associated primarily with rabbits in terrestrial environments, and *Francisella tularensis* biovar *holarctica* (type B), associated commonly with rodents in aquatic environments. Within Lagomorpha, *Sylvilagus* spp. and *Lepus* spp. are the most important hosts. Although human cases are rare, cases have been identified in New England (2000-2008 data; primarily in Massachusetts), where the bacterium is endemic to several islands. Disease is primarily spread via biting arthropods but also through skin contact with infected animals, ingestion of contaminated water, or inhalation of contaminated dusts or aerosols. The disease has several forms in humans: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal with the ulceroglandular form as the most common presentation. Direct inhalation of organisms leads to the pneumonic form of disease. Clinical signs in wild animals are poorly documented, largely because of the acute nature of disease. In rabbits, clinical disease is often acute with a disease course of 2-10 days with individuals exhibiting severe lethargy and rapid septicemia resulting in death. Post-mortem diagnosis is based on gross lesions and histology and culture identification of the organism. Ante mortem diagnostic tests available include blood culture or serology (tube agglutination test or ELISA). In most ecosystems, tularemia cannot be controlled, and as such, should be recognized as a risk for animals and human personnel working with rabbits for the reintroduction program. *Francisella tularensis* is also a class A agent of bioterrorism and any confirmed cases should be reported to public health officials.

Bacterial typhlitis/colitis – Colibacillosis or Clostridial Enterotoxemia. Enteritis complex with signs ranging from soft stool and diarrhea to enterotoxemia, septicemia, and death are a frequent disease presentation in captive domestic rabbits. Bacterial typhlitis and colitis have also been reported as a cause of morbidity and mortality in the pygmy rabbit (*Brachylagus idahoensis*) captive rearing program. Enterotoxemia is caused by the toxin produced from the Gram positive, anaerobic bacterium *Clostridium spiroforme*. Other Clostridium species, especially *C. difficile* and *C. perfringens* have been reported but are now not considered to be the primary cause of disease. Factors such as diet, stress, antimicrobial use, and genetic predisposition to GI dysfunction may contribute to the proliferation of these pathogenic bacteria and toxin production. Disease is most significant in young rabbits likely due to an underdeveloped population of GI bacterial flora and a high gastric pH that favor growth of *C. spiroforme*. Older rabbits tend to be more resistant and generally require some dietary, environmental, or other stress to induce dysbiosis and pathogenic bacterial growth. In acute disease, rabbits become anorexic and markedly depressed. Diarrhea is observed which may contain blood or mucus. Affected rabbits become hypothermic and moribund and die after 24 to 48 hours. Post mortem findings include petechiae and ecchymoses on the serosal surface of the cecum (appendix and colon may also be involved). Diagnosis may be made on isolation of toxin via PCR. Colibacillosis is caused by pathogenic strains of *E. coli*. Disease is less common in captive domestic rabbits than enterotoxemia. *E. coli*-related diarrhea in postweanling rabbits may be caused by variety of different serotypes and pathogenic bacteria may proliferate due to similar factors as those listed for enterotoxemia.

Pasteurellosis – Disease is caused by the bacterium *Pasteurella multocida*. The bacterium is a normal inhabitant of the upper respiratory tract and pharynx of rabbits but can cause upper respiratory infections, otitis, endocarditis, bacteremia, localized or generalized abscesses, and/or orchitis. Pasteurellosis has not been documented as a significant cause of morbidity and mortality in wild U.S. lagomorphs but can be a significant health risk in domestic (European) rabbits and is a common cause of mortality in wild and farmed European brown hares (*Lepus europaeus*) as well as hares elsewhere in Europe. The
significance of pasteurellosis in captive-reared *Sylvilagus* spp. is not known but the potential for clinical disease should be considered in any rabbit subject to immunosuppression or stress (such as newly captured wild or captive housed rabbits for the reintroduction program). Available ante mortem diagnostics include ELISA for screening colonies and culture of nasal swabs for culture. Since the organism is a commensal, positive nasal culture swabs are not necessarily indicative of disease so screening *Sylvilagus transitionalis* entering into the captive breeding program is likely of limited value.

**Bordetella bronchiseptica** – This is another common inhabitant of the respiratory tract of captive rabbits that can also cause upper respiratory tract infections. Infection can predispose rabbits to pasteurellosis, or complicate *Pasteurella multocida* infections.

**Staphylococcus spp.** – These are common pyogenic bacteria that cause local abscesses and less commonly, generalized infections. Among wild mammals, most disease is attributed to *S. aureus* and infection is of greatest significance in rabbits *Sylvilagus* spp., *Oryctolagus* sp., and hares *Lepus* spp., where infection may result in severe morbidity and sometimes mortality. The organisms are opportunistic pathogens that require damage to the skin or mucous membrane to establish infection. This most commonly occurs secondary to arthropod bites or injuries from bites from or fighting with conspecifics. Infected lagomorphs may be listless, emaciated, and lame if tendons or joints are involved. Subcutaneous abscesses may be present. Treatment is with antimicrobials.

**Viral Diseases**

**Rabbit fibroma virus** – Genus *Leporipoxvirus*. (Synonym – Shope fibroma virus). This virus occurs in *Sylvilagus* spp. in eastern and midwestern North America and typically causes mild self-limiting cutaneous tumors (fibromas) at the site of infection. Lesions observed are firm, white cutaneous tumors typically on the legs of affected animals that generally persist for 150 days after which time the lesions regress and disappear. Rabbit fibroma virus can cause fatal disease in young cottontails, though, with death occurring 4 weeks after inoculation. Natural disease is common (typically in summer and fall seasons) and presumed spread via arthropod bites. Control measures for captive rabbits include quarantine and vector exclusion.

**Myxomatosis** – Genus *Leporipoxvirus*. This is a potentially significant disease in European rabbits (*Oryctolagus* spp.) in which variable to high mortality rates can be seen depending on the strain type. In the U.S., the virus is endemic in the brush rabbit (*Sylvilagus bachmani*) in California. Infection of other species of *Sylvilagus* and *Brachylagus idahoensis* (pygmy rabbit) with the virus has not been reported in free-ranging populations and the status of the virus in *Sylvilagus transitionalis* is unknown. Myxoma virus is transmitted by blood-feeding arthropods or shed via discharges and clinical signs observed include swollen eyelids, and mucopurulent conjunctival and nasal discharges. Cephalic and aural edema are often present with disease. Control measures for captive rabbits include quarantine and vector exclusion.

**Rabbit coronavirus** – This virus was discovered in 1980 as a cause of diarrhea in laboratory rabbits. The virus has been associated with high rates of morbidity and mortality but has also been isolated from clinically normal adult rabbits. Coronavirus affects 3 to 10 week old rabbits with clinical signs observed during outbreaks including lethargy, diarrhea, abdominal swelling, and death. Coronaviral enteritis has also been observed as a cause of morbidity in the pygmy rabbit captive rearing program. Diagnosis is confirmed by viral identification in feces or cecal contents.

**Rabbit rotavirus** – This is another cause of diarrhea in captive rabbits, primarily in commercial rabbitries but also in pet rabbits. Infections in animals caused by rotavirus alone can be mildly pathogenic, but in rabbits, rotaviral infection has been associated with very high morbidity (and variable mortality rates).

**Herpesvirus sylvilaguses** – This is a lymphotropic gamma herpesvirus confined to its natural host, cottontail rabbits (*Sylvilagus* spp.), that is characterized by chronic infection. Transmission is thought to occur via direct contact and not transplacentally. This virus produces clinical and histopathologic changes in adult cottontail rabbits similar to those observed in humans with acute Epstein-Barr virus infection - lymphoproliferative disease (lymphoid hyperplasia, lymphoma) in adults.
Parasitic Diseases

Intestinal coccidiosis – Disease is due to the Apicomplexan protozoal parasite, *Eimeria* spp. Coccidia are the most common GI parasites of captive rabbits (coccidiosis, especially in juvenile rabbits up to 3 months of age, has been observed as a cause of morbidity in captive reared pygmy rabbits). Many rabbits are subclinically infected and finding of oocysts on fecal floatation does not necessarily indicate this as the cause of disease. Clinical signs vary widely depending on age, organism involved, and degree of infection but are most often seen in young rabbits and include weight loss, intermittent mild to severe diarrhea, and dehydration. Death most often occurs due to dehydration and secondary bacterial infections. Post mortem lesions may be seen in the small or large intestine and can include intestinal epithelial ulceration. A hepatic form of coccidiosis caused by *E. stiedae* also occurs in domestic rabbits and may result in anorexia, weight loss, and diarrhea. Treatment with amprolium or sulfa drugs is most common, although other newer drugs may be more effective.

Cryptosporidiosis – Infection due to *Cryptosporidium parvum* has ZOONOTIC potential. *Cryptosporidium parvum* can cause diarrhea in young rabbits, peaking at 30 to 40 days of age. Clinical signs include diarrhea lasting 3 to 5 days, depression, lethargy, and dehydration. Currently, no effective treatment exists and treatment is largely supportive. Cryptosporidiosis has been observed to cause morbidity and mortality in young captive pygmy rabbits. Frequency of occurrence in free-ranging *Sylvilagus transitionalis* is unknown, but all mammals are considered potentially susceptible.

Trematodiasis – Significant disease in rabbits is specifically due to the liver fluke, *Fasciola hepatica*. *Fasciola hepatica* is typically associated with liver infections in domestic and non-domestic ruminants but there is also an extensive history of infections in various lagomorph species including jack rabbits (*Lepus californicus*), cottontail rabbits (*Sylvilagus* sp.), European hare (*Lepus europaeus*), and mountain hare (*Lepus timidus*). Infected hares and rabbits are common in enzootic areas and may contribute to maintaining or spreading the fluke population. Flukes can infect rabbits that graze in wet pasture areas or other areas of heavy moisture (banks of streams, rivers, etc.) in endemic regions where rabbits acquire infections via ingestion of the snail intermediate host. Adult flukes live in the gallbladder and bile ducts, and can cause severe lethargy, weight loss, poor hair coat, and death. Diagnosis is based on identification of eggs on fecal sedimentation or adult flukes at necropsy. Treatment is with praziquantel.

Diseases of Less Concern for the Reintroduction Program

Bacterial Diseases

Rabbit syphilis – Disease is caused by the spirochete bacterium, *Treponema paraluiscuniculi*. Transmission can occur venerally or by direct contact with infected rabbits. Disease usually presents as papules, ulcers or vesicles on external genitalia and occasionally lesions are seen on the face. The disease is reported in captive rabbits only so this is not currently considered a disease of significance in *Sylvilagus* spp. Asymptomatic carriers are possible. Diagnosis is based on clinical signs, skin biopsy for silver staining, and skin scrapes for darkfield microscopy. A fluorescent antibody test and ELISA are also available to screen for antibodies. Treatment is with antimicrobials (parenteral penicillin) and response is to treatment is typically rapid.

Leptospirosis – *Leptospira interrogans* serovar Grippotyphosa has been isolated from wild cottontail rabbits in Florida and southwestern Georgia. Rabbits do not appear to serve as important reservoirs for leptospirosis.

Viral Diseases

Rabbit viral hemorrhagic disease – Disease is caused by a calicivirus. (Synonym – viral hemorrhagic disease, rabbit caliciviral disease). The virus first emerged in farmed rabbits in China in 1984, then in farmed *Oryctolagus cuniculus* in Europe starting in the mid-1980s killing millions of rabbits, particularly in
Italy and Spain. The disease was first recognized in the U.S. in 2001 in a captive colony of rabbits in New York. Accidental introduction of the virus to mainland Australia, has caused significant mortality in *O. cuniculus* populations (mortality rates ranging from 65-95). The disease affects wild and domesticated *Oryctolagus cuniculus* but other rabbit species including cottontails (*Sylvilagus floridanus*) do not seem to be susceptible. The virus causes three forms of disease – 1) peracute, fulminant fatal disease (dead rabbit found in cage), 2) acute disease with individuals exhibiting lethargy, depression and possibly bloody nasal discharge prior to death 1-2 days post-presentation, and 3) subacute disease leading to asymptomatic carriers. Adult rabbits are affected with rabbits < 8 weeks old typically spared from disease. A similar calicivirus causing European Brown Hare Syndrome linked with the distribution of *Lepus europaeus* is not known outside Europe. Serological tests are available (ELISA). Diagnosis is usually achieved via histopathology, PCR, etc. Careful sanitation and disinfection are imperative to halting or limiting the spread of VHD.

**Aflatoxicosis** – Rabbits are considered very sensitive to the aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*, which can grow on improperly stored feeds. These organisms are also ubiquitous in the environment. Rabbits are especially sensitive to the B1 aflatoxin fraction. Feed items should be screened for the presence of aflatoxins prior to use for feeding captive *Sylvilagus transitionalis*.

**Parasitic Diseases**

**Babesia divergens** – Potentially ZOONOTIC. The disease is caused by a piroplasm infecting red blood cells of rabbits. Disease is transmitted through tick vectors (primarily *Ixodes* spp.) and the organism is considered enzootic in populations of eastern cottontails (*Sylvilagus floridanus*). Recent study showed a 16% prevalence of infection in *Sylvilagus floridanus* surveyed in Nantucket Island, MA. Severe disease in humans is rare occurring only in splenectomized persons.

**Sources:**


# Appendix 6 – Husbandry Log

Roger Williams Park Zoo

___________________________________Weekly Report

INITIALS: Veterinarian _________ Curator __________

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Appendix 8 – Acclimation Pen Construction Details

Description of Ninigret National Wildlife Refuge New England Cottontail Pen

Location-
A NEC soft-release and hardening enclosure is being constructed within the “Triangle Field” of the Ninigret NWR (Diagram 1). The enclosure is approximately 1.2 acres. The chosen location is prime maritime shrubland with a diversity of shrubs and forbs, which provide cover with herbaceous microhabitat. The site is not so dense that it places significant limits on monitoring or rabbit collection. Species within the site include wild raspberry (Rubus sp.), goldenrod (Solidago sp.), wild grape (Vitis labrusca), switchgrass (Panicum virgatum), asters (Asteraceae sp.), northern arrowwood (Viburnum dentatum), sumacs (Rhus sp.), bayberry (Myrica pensylvanica), highbush blueberry (Vaccinium corymbosum), and shadbush (Amelanchier canadensis). Invasive species, such as oriental bittersweet and multiflora rose, cover less than 5% of the total area.

Access to the Pen will be from the West. There are maintenance roads and trails that lead to the pen. During monitoring and maintenance activities, workers will be expected to park on the trail and walk 30 meters to the entrance to the pen.

Construction of Pen-
The pen will be approximately 100 ft x 500 ft (att.2). The outside perimeter will be 6 ft above the ground, and covered by #2 hardware cloth (1/2 inch), supported by 3” diameter galvanized steel posts. The hardware cloth will extend 20 inches below ground, angling outward, to create an apron to prevent canids from digging into the enclosure (att. 1). Nylon netting (2 inch diamond mesh) will cover the entire enclosure. The center (lengthwise) of the pen will have 9 foot posts every 10 feet (att.3). Each post will have an eye-bolt at the top. There will be a ¼ inch diameter cable that runs along the top of the perimeter posts through these eye-bolts. From the perimeter posts, there will also be cables that will run to the center posts. The netting will attach to the cables with hog-rings and karabiners, providing a structured ceiling to the enclosure.
A 20 ft x 20 ft vestibule will be constructed on the northwest end of the pen. This Vestibule will be covered in hardware cloth and have 2 sets of doors for entrance and exit, and will also hold the solar electric fence unit and other supplies.

A solar electric fence will surround the pen at 1ft high, 5 ft high and 5.5 ft high (att 1). At 1ft high, the electric fence will be 10 inches above the ground and 10 inches away from the hardware cloth in order to discourage large mammalian predators. At 5ft and 5.5ft heights, the electric fence will be installed at 1 inch from the hardware cloth to discourage even the smallest weasels and cats. The solar fence charger with a battery will keep the fence charged up to a week during cloudy/stormy weather.

Water-
Watering holes will be created at 2 locations within the pen(diagram 1). Water troughs will be excavated, both 2 feet deep and 4 foot wide. They will be constructed with a clay base so as to hold water under natural conditions, but will be maintained by Refuge staff during dry periods.

Costs(including shipping)-

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Inch Metal Posts</td>
<td>$7,100.00</td>
</tr>
<tr>
<td>Hardware Cloth</td>
<td>$7,200.00</td>
</tr>
<tr>
<td>2 Inch Diamond Netting</td>
<td>$7,000.00</td>
</tr>
<tr>
<td>Misc Cables/Brackets/Bolts, etc.</td>
<td>$3,000.00</td>
</tr>
<tr>
<td>Electric Fence materials</td>
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</tr>
<tr>
<td>Cultural Resource Inventory</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$40,000.00</strong></td>
</tr>
</tbody>
</table>
New England Cottontail
Spatially Explicit Mark-Recapture Protocol
University of New Hampshire
Adrienne Kovach and Thea Kristensen

What sites to survey: patches of ~20+ ha in size
- If smaller patches are more typical of your focus area, discuss patch selection with Adrienne/Thea.

How many surveys: 2 independent surveys at each site
- Pellets should be collected after two separate snowfall events.
- Ideally, these snowfall events will be close in time. However, it is more important to do the 2 sampling sessions rather than be concerned about how close they are in time.

When to survey: 3-5 days after a snowfall event
- This allows for accumulation of pellets and tracks.
- Ideally 4 days after snowfall event, however weather is a factor, hence the 3-5 days after an event.
  - It will be important to monitor the upcoming weather conditions to be able to make optimal decisions for collection.
    - If it warms above freezing and causes snow to melt this can degrade DNA. Hence if temperatures are expected to warm in the 4th or 5th day, it would be advisable to sample on the 3rd day after snowfall.
    - Cold weather <-10° C and high winds, may limit rabbit activity. If these are the conditions, sampling 4-5 days after snowfall is advised.
  - Note that there is a limit to the benefit derived from increasing pellet deposit days after snowfall, because of the negative effects of environmental exposure on pellet DNA quality (freeze-thaw, rain, warm temperatures, and dampness in particular are known to degrade DNA). For this reason, 4 or 5 days after snowfall is considered a maximum wait period for surveys.
- Preferable to have <12 inches of snow.
  - While snow cover increases the detectability of fecal pellets and tracks, deep snow may inhibit cottontail activity or even facilitate subnivean behavior (rabbits may travel through air pockets beneath the snow, especially around dense vegetation). For this reason, surveys conducted in deep snow may be less effective than surveys conducted with <1 foot snowpack. However, it may not be possible to survey three times during these ideal conditions. Thus, it may be best to survey whenever there is snowfall to do so.
How much of patch to survey: as much as possible, representative of landscape

- If it is possible, it would be ideal to survey the entire patch or a large representative portion (not just what is assumed to be suitable habitat), allowing for inclusion of variation in the habitat. This will let us identify features that correlate with occupancy/abundance and may help us develop future predictive models.

Spacing between samples: every 30 m along transect

- Aim to sample intensively, collecting pellets approximately every 30 m along the sampling transect (20-40 m is acceptable).

Spacing between transects: 30 meter spacing between transects

- Search the patch systematically using a continuous transect that winds its way back and forth across the patch keeping approximately 30 meters between passes.
- Due to the difficulty of traversing thicket habitat, the search transects need only serve as a rough guide from which one may need to deviate to facilitate movement around denser thickets. If cottontail tracks are discovered they should be followed until either pellets are found or the tracks are lost (due to crusted snow or dense vegetation). The survey should then continue from the point where the tracks were first discovered.
- While 30 m transect spacing is ideal, slightly greater spacing between transects may be allowed if necessary. Limit the greatest distance between transects to 60 m.
Tracking: Use a handheld GPS unit to track movements of surveyors during the duration of the survey and mark 4 corners of polygon sampled.

Pellet Collection: collect pellets from single cluster into a unique vial labeled with location coordinates

- Pellets may be found within a distinct or slightly diffuse clump, or alternately, several pellets may be scattered individually along a short trail. The objective is to collect several pellets from the same individual rabbit (2-10); these pellets will be placed together in a unique vial. Restrict collection for each vial to an area approximately 5 x 5ft or smaller to minimize the number of individuals represented in each vial (ideally, one rabbit per vial). Use disposable sterile gloves and place the pellets in a unique vial; try to keep snow out of the vial but avoid rubbing the pellet as it may remove DNA. Do not touch the pellets with bare hands, as this may cause contamination, and use a fresh glove for each vial.
- Use a GPS to record the exact location of pellets collected. Record this in a data sheet and on the pellet vial. It may be useful to record other identifying information on the vial, such as:

Figure shows the survey design focused on intensive sampling of pellets every 30m, along loose transects winding back and forth across the sampling area at approximately 30 m intervals. Note that surveys include the core, suitable habitat (in grey) as well as less suitable habitat areas surrounding the core.
initials or name of person collecting samples, site name, date, survey number (1, 2 or 3) and a
unique sample identification number.

Sample storage: *keep samples cold/frozen at all times to the extent possible*
- Because DNA can degrade quickly if not frozen, return the vial(s) to a cooler upon returning to
your vehicle and deposit in a freezer as soon as possible. A good trick is to temporarily store
your pellets in a ziplock bag filled with snow while you are in the field (e.g., to prevent them
from heating up in your pocket), and transfer them to a cooler once you get to your vehicle.

Sample shipment: *on ice overnight*
Ship samples, preferably by FedEx, overnight on ice/cold packs to:
Adrienne Kovach
University of New Hampshire
46 College Road
Durham, NH 03824
603-862-1603

Data to record:
- GPS location and patch name for each set of pellets collected, and an identification number
  for each separate sample.
- Number of days since snowfall and weather conditions since snowfall
- GPS tracks of surveyors
- 4 points surrounding sampling area (the 4 corners of a polygon surrounding the patch)
If a technician is able to record sample IDs, patch location information, survey dates, and geographic
coordinates into a spreadsheet, this is v