

# White-nose Syndrome

## Summer Bat Survey Protocols



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# Table of Contents

<b>White-nose Syndrome</b>	<b>4</b>
<b>Preparing for Fieldwork</b>	<b>6</b>
<b>Checklist of Personal Items</b>	<b>8</b>
<b>Disinfectant Methodology</b>	<b>9</b>
<b>In-field WNS Considerations</b>	<b>12</b>
<b>Checklist: Mist Net Site Gear</b>	<b>16</b>
<b>Critical WNS Gear</b>	<b>17</b>
<b>Protocol: Mist Net Sites</b>	<b>18</b>
<b>Latex Glove Removal</b>	<b>24</b>
<b>Checklist: Processing Gear</b>	<b>25</b>
<b>Protocol: Processing Station</b>	<b>26</b>
<b>Checklist: Roost Visit Gear</b>	<b>32</b>
<b>Protocol: Roost Visits</b>	<b>33</b>
<b>Checklist: Decon Station</b>	<b>36</b>
<b>Protocol: Decon Station</b>	<b>37</b>
<b>Checklist: Harp Trap Decon</b>	<b>41</b>
<b>Bat Survey Kit Bag: Contents</b>	<b>42</b>
<b>More Summer Survey Gear</b>	<b>43</b>
<b>WNS Gear Checklist</b>	<b>44</b>
<b>Vendor Resources</b>	<b>45</b>

<b>Definitions</b>	<b>46</b>
<b>References</b>	<b>47</b>

# White-nose Syndrome



**White Nose Syndrome (WNS)** has killed well over one million bats in the U.S. since the winter of 2006/2007. First noted near Albany, New York, it has spread at an astonishingly rapid rate. If WNS continues to spread, it will greatly affect both subterranean and surface ecosystems and may cause species extinctions. There are indications that people might spread WNS when they visit caves and/or handle bats and bat research equipment. Therefore, we must take every reasonable precaution to minimize the risk of BCI workshop participants and anyone involved in

summer bat surveys from spreading WNS at faster than normal rates, and to keep the syndrome from reaching new areas.

The following WNS decontamination procedures, prepared by Bat Conservation International (BCI) in June 2009, follow published U.S. Fish and Wildlife Service, Pennsylvania Game Commission (PGC), and U.S. Geological Survey Wildlife Health Service decontamination protocols. These protocols have been reviewed, revised, modified, and described in detail after initiating WNS protocols in the field during the remainder of the 2009 summer field season. Additional insights gleaned from work in Pennsylvania, Arizona, New Mexico, and California during the summer of 2010 and after consultation with colleagues about their similar experiences trying to implement WNS protocols in the field, we have arrived at these suggested procedures and in-field decontamination/disinfection practices.

The procedures outlined in this document are not an exhaustive representation of all possible implementation instructions for the accepted WNS decontamination and disinfection protocols known at this time. They merely represent the “best practices” as determined by BCI staff and colleagues who have worked in field situations, while faced with the evolving nature of what is known about WNS in bats, transmission vector(s) and persistence on the landscape. It is hoped that users can learn from the experiences documented herein and expand on the procedures as needed. Feel free to forward any comments or suggestions about this document to:

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# Preparing for Fieldwork

## **Personal Gear and Research Equipment**

NO equipment (clothing, helmets, boots, lights, cameras, ropes, packs, etc.) used in a WNS-confirmed bat roost should be used at any other roost or net site. For BCI workshops and summer field surveys, we ask all participants, staff and colleagues to bring new personal items and equipment if possible, or items and equipment that has been thoroughly decontaminated, and bring *absolutely no gear from affected or possibly affected bat roosts*.

## **Bat-handling Gear**

Any persons expecting to handle bats during a workshop or summer survey must bring: (1) their own personal supply of latex exam gloves (or equivalent) *and be familiar with glove removal method to keep bare hands free of contaminants and trap potentially infectious agents inside gloves* (see: “Latex Glove Removal” on page 24 of this document), (2) a supply of air-tight bags or totes suitable for stowing possibly contaminated gear for later decontamination or disinfection, and (3) suitable quantities of outerwear that can be considered “single use” clothing for each night of netting or each planned roost visit.

Bat handlers should be urged to bring at least *50 pairs* of gloves *per night* of netting/trapping. Gloves should be test-fitted prior to use in the field. A tight-fitting yet easy-to-don-and-remove glove is best. Loose gloves make bat handling more difficult. If a leather glove will be worn over the non-dominant hand for additional protection against bat bites, different sizes of latex gloves may be required to facilitate ease of use. Most U.S. and Canadian bat species are unable to pierce through both latex and human skin. Bat handlers with enough expertise to hold and manipulate bats to minimize chances of getting bitten can wear tight-fitting disposable



gloves over bare hands, thereby being more effective and efficient in swapping out gloves, while providing a safer, more comfortable experience for the bats.

Finally, depending upon the exact nature of the netting/trapping location or issues that arise during the event, boots, outerwear, and other personal items may need to be removed at the site and bagged for later decontamination. Gallon-size “Ziploc” style bags can be used for most items of clothing. Tall kitchen trash bags, preferably of the “drawstring” variety can be used to “double-bag” coveralls and boots.

### **Roost-visit Gear**

Roosts are considered to have the highest-potential for containing soils contaminated with WNS spores or are most likely to be cross-contaminated by un-clean practices. Equipment for working in and around roosts should include disposable or “single use” outerwear (e.g., Tyvek coveralls), hair and head covers, extra-strength rubber gloves, and rubber boots (e.g., Wellington-style) or other non-porous footwear that can be soaked during decontamination and disinfection procedures. A personal supply of bags and/or plastic tubs with air-tight seals for storing and/or disposing of single-use outerwear will be required. Sufficient bags to allow double-bagging of possibly contaminated items is required. Additional optional gear for roost visits may include respirators, helmet and headlamp covers, dry bags, ropes, harnesses, or other personal protection equipment.

### **Decontamination and Disinfection Requirements**

Every effort will be made to provide ample decontamination and disinfection solutions at sites to treat survey gear during the workshop or inventory event. Extra solution may be available for use on personal gear and equipment but is not guaranteed to be available. Persons handling bats and/or entering roosts must be prepared either to decontaminate and disinfect their personal items on site in accordance with accepted protocols or must properly secure their items in air-tight containers for transport to their home areas prior to decontaminating and disinfecting them in accordance with accepted protocols as outlined in this document.

# Checklist of Personal Items

The following Personal Items, beyond those items regularly brought into the field for summer bat surveys and/or roost inventories, are required for anyone desiring to handle bats and/or enter roosts in accordance with current WNS decontamination and disinfection procedures.

## Required Items

- 50-pairs (100ct.) box of disposable latex exam gloves (or equivalent) *per night of bat handling and/or per roost inventory/entry event*
- 1 pair rubber (Wellington-style) boots or other non-porous, submersible footwear that can be disinfected/decontaminated after each use at each site
- 1 set of outer-wear or over-wear, single use clothing *per night of bat handling and/or per roost inventory/entry event*
- Sufficient non-porous, air tight, plastic bags, bins, or stuff sacks to store any footwear, outerwear, or over-wear, allowing items to be “double-bagged,” at the site of use for later disposal or disinfection/decontamination.

## Optional Items

- Tyvek coveralls
- Hair, head, and/or helmet covers
- Face masks or respirators
- Decontamination/Disinfection solution (e.g, Promicidal™ or Professional Lysol®)
- Dilution means (i.e., measuring device and water)
- Soaking, drying, and storage containers (e.g., 5-gal. buckets, clothesline, Ziploc bags, air tight totes)
- Plastic casings or custom designed plastic bags for any non-submersible gear (e.g., flashlights, cameras, video recorders, bat detectors) used at or inside bat roosts



# Disinfectant Methodology

In a March 2009 study conducted by the New York State Department of Environmental Conservation, Wildlife Pathology Unit, two compounds were found to be especially effective in killing the WNS fungus under laboratory conditions: a germicidal detergent (Promicidal™) and an anti-bacterial all purpose cleaner (Professional Lysol®). Due to their commercial availability it is recommended that all personal items, equipment, and materials brought to and used during roost visits and bat handling activities be disinfected with one of these compounds prior to and immediately following exposure. If items cannot be disinfected with these substances, or according to the procedures outlined below, they cannot not be brought to the field site.

Specific decontamination products and procedures for items are outlined below and further described in the subsequent sections. It is critical that the chosen disinfectant contain the active ingredient(s), at the listed concentrations, and that the disinfectant solution is prepared at the recommended strength, applied in accordance with the specific decontamination protocols, and stored properly to be effective. *Failure to do so will provide inadequate disinfection.*

Please note that many of the chemicals found to be effective in disinfection and decontamination are hazardous compounds and all necessary and appropriate precautionary, use, storage, and disposal information should be apparent on each of the product labels. It is critical that all researchers and biologists read and follow all label instructions provided on the products mentioned in this protocol. It would be a violation of federal law to use, store, or dispose of a regulated product in any manner not prescribed on the approved label and/or MSDS (material safety data sheets) accompanying the product.

It is especially important that the chosen decontamination product be stored and handled properly to remain effective and to protect all users coming into contact with the product and items being soaked in diluted solutions of products. In most cases, full protective gloves and clothing should be used when handling these chemicals. This includes, but is not limited to: long rubber safety gloves, safety goggles, and appropriate outerwear to protect against contact with soaking solutions, and/or splashes or over-spills/sprays from the solutions while decontamination procedures are being conducted.

In 2011, the following chemicals and natural products were tested in the laboratory and found to be effective for killing the conidia of *Geomyces* spp.

Product	Brand	Active Ingredient(s)	% by weight	Dilution ratio	Concentration in water
germicidal detergent	Promicidal™	o-benzlyl-p-chlorophenol	4.75%	1:128	1 oz. / gallon 1/2 cup / 4 gal.
		o-phenylphenol	4.75%		
antibacterial cleaner (e.g., quaternary ammonium)	Lysol® Professional Antibacterial All-purpose Cleaner	alkyl-dimethyl-benzyl ammonium chloride	2.4%	1:128 to 1:64*	1 oz. / gallon 1/2 cup / 4 gal. to 2 oz. / gallon 1 cup / 4 gal.
		didecyl-dimethyl ammonium chloride	0.8%		
antibacterial cleaner (e.g., quaternary ammonium)	Formula 409® Antibacterial All-purpose Cleaner	n-Alkyl (C12-16) dimethylbenzyl ammonium chloride	.3-.6%	1:128	1 oz. / gallon 1/2 cup / 4 gal.
		n-Propoxypropanol	.5-2%		
		Monoethanolamine	.5-1.5%		
disinfectant	Lysol® IC Quarternary Disinfectant Cleaner	didecyl-dimethyl ammonium chloride	10.14%	1:128	1 oz. / gallon 1/2 cup / 4 gal.
		n-Alkyl (C12-16) dimethylbenzyl ammonium chloride	6.76%		
		sodium hydroxide	1-3%		
disinfecting wipes	Lysol® Disinfecting Wipes	Alkyl (50%C14, 40%C12, 10%C16) dimethyl benzyl ammonium chlorides	0.1-1%	n/a	n/a
chlorine bleach	Clorox®	sodium hypochlorite	5.25%	1:10	1 part bleach in 9 parts water
boiling water	n/a	n/a	n/a	n/a	n/a

\* 1:128 is the recommended concentration for cleaning and sterilizing and 1:64 is the concentration for disinfecting. In the NY DEC study, no growth of *Geomyces destructus* occurred on media containing concentrations  $\geq$  1:128. Soaking baths should be diluted at 1:128 and - allow items to soak for 10-minutes and then triple rinse; Spraying or washing solutions should be diluted at 1:64 allow surfaces to remain wet for 10-minutes and air dry.. Wipes: to sanitize, allow surface to remain wet for 30-seconds; to disinfect, allow surface to remain wet for 10-minutes - allow surfaces to air-dry Boiling water: to disinfect/sanitize, boil for 15-minutes

All non-porous items (e.g., rubber, Wellington-style boots; harp trap strings and frames; mist net poles and stakes) and typical porous items (e.g., clothing, backpacks, stuff-sacks, bat holding bags, mist nets, harp trap catch bags, ropes, and guy-lines) should be scrubbed, washed, or rinsed free of coarse debris at the site of use, then double-bagged for later off-site decontamination and disinfection.

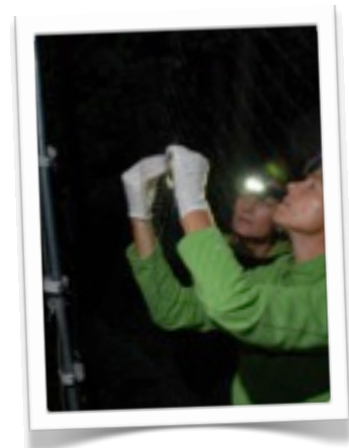
Field-site decontamination and disinfection procedures consist of diluting the selected disinfectant to the approved concentration, then soaking the item(s) in the solution for at least 10-minutes, rinsing at least three times, and allowing to air dry, preferably in the sun. An easy methodology employs 5-gallon lidded buckets: 1 filled with the decontamination solution, and 3 filled with fresh rinse water. Items should then be spun dry in an electric spin dryer, allowing waste water to drain to a rinse bucket, then arranged in the sun to dry before re-packing into clean stuff-sacks or storage containers. *NOTE: Used decontamination solution and rinse water cannot be disposed of on the ground or in septic systems and must be drained into an appropriate sanitary disposal location (i.e., municipal sewer or RV dump station).*

# In-field WNS Considerations

As WNS has spread through America's bat populations, it has become of grave concern to bat-workers and resource managers everywhere. Recent evidence points toward human activities as a significant factor influencing the rapid spread of WNS. This may occur even during seasons when bats and/or obvious signs of the fungus are not present. The fungus has been observed to grow on many different organic materials and appears to persist in bat roosts year-round. Fungal spores can become attached to skin, hair, clothing, and equipment and it is possible that they can remain viable for an undetermined time after leaving a host site. As a result, strict protocols for the entry near to or in bat roosts, and the handling of bats in general, have been initiated. Bat Conservation International requires all employees, contractors, volunteers, assistants, participants, and invited guests conducting or attending programs and activities involving any bat-human contact to adhere to WNS in-field policies and protocols during the netting and trapping of bats, the handling and processing of bats, the visiting of or entry into roosts, and the decontamination or disinfection of personal and field gear. These activities are described below and specific WNS protocols for each are detailed in later sections of this document.

## **Deploying Nets/Traps and Collecting Bats**

Sufficient gear is deployed at the net/trap sites to capture and remove bats, handle bats, transport bats to a nearby "processing station," and stow all used nets, traps, and WNS trash for later decontamination, disinfection, or disposal. All gear and actions are designed to minimize potential for bat-to-bat and/or human-to-bat transmission of possible WNS spores. One of the most important



pieces of WNS summer survey gear is a good quality, mesh-style, mist net storage bag that can be used for keeping nets organized by length and tangle free during setup, teardown, washing, and drying during decontamination. It is essential before going into the field for the first time, post-WNS, that all mist nets destined for use be re-packaged into a suitable bag. A great option that stands up to multiple seasons of use and decontamination activities is the “Handy Bag” (\$6 ea.) by Bat Conservation and Management (Carlisle PA, [www.batmanagement.org](http://www.batmanagement.org)).



### Processing Bats

All bat processing (i.e., data recording, identification, measuring, weighing, and other photographic or physical documentation) will take place a short distance away from the net/trap site and should ideally be conducted by a person designated as a “recorder” who will remain “clean” by not handling any bats or

equipment that has come into contact with a bat during the netting/trapping or processing activities. Persons designated as “handlers” will work the net/trap sites and visit the processing site without acting in any manner that could potentially contaminate the processing site.

### Roost Visits

Roost visits during summer survey efforts will be eliminated or strictly minimized and conducted only when absolutely necessary. Any persons visiting roosts will agree to bring new gear or personal items into the roost that have **never** been used in a confirmed WNS area or from any geographic location currently identified to be reporting WNS-positive bats. Any gear, including outer clothing brought to or worn at a roost, must be new or never used/worn at a WNS site. Additionally, no gear or clothing should be used in multiple roosts in a single day unless they can



be cleaned and disinfected in between visits according to required protocols. Sufficient materials (e.g., non-porous bags and or stuff-sacks or airtight containers) must be brought to the site to allow users to brush off coarse debris and change into and out of appropriate clothing and bag clothing and any other gear used at the roost for transport and later off-site decontamination.



### **Decontaminating Nets, Traps and Gear**

Cleaning, disinfecting, drying and re-packing of all field gear will be done on a daily basis for multi-day events, or as soon as possible after the event for single-day outings. The cleaning station ideally should be located where there is access to: (1) electricity, (2) fresh water, and (3) a municipal sewer system (or RV dump station) for disposing of used decontamination

solutions and rinse water. Access to an area where cleaned items can be allowed to air-dry, in full sunlight, is preferred, although a household or commercial dryer can also be used.

To facilitate decontamination and disinfection, 5-gallon, lidded, plastic buckets (readily available from home improvement super stores) are used to transport and store WNS gear in and out of the field and to store potentially contaminated nets and other gear after survey efforts, keeping transport vehicles “clean.” In this manner, buckets can perform “double duty” as both storage containers and as soaking and rinsing vessels during the decontamination and disinfection efforts. A minimum of four (4) buckets are needed to accomplish the necessary decontamination and rinsing efforts.



**IMPORTANT NOTE:** Open dumping of decontamination solutions (quaternary ammonium compounds or germicidal detergents) is prohibited by law. Neither the decontamination solutions nor the rinse water from these solutions can be disposed of on the ground or in a septic system. They must be disposed of in a municipal sewer (i.e., poured down drains connected to a sewer line) or disposed of at a water treatment plant or at an RV dump station.

In the following sections, a suggested check-list of equipment that can be used for each aspect summer bat surveys is included followed by specific protocols for each aspect of the tasks, including set-up and take-down measures, best practices during activities to minimize contamination and cross-contamination, and important caveats to protocols which were discovered during field implementation of these procedures. What follows is by no means an exhaustive list of all potential equipment and protocols, it is simply what many bat workers during the 2009 and 2010 field seasons have found to be helpful, useful, and appropriate for initiating summer field surveys with bats in the age of WNS.

Prior to going out into the field, all workers participating in netting or trapping, processing bats, or roost visits should become familiar with the checklists of gear, examples of actual gear, and protocols for use and conduct. To facilitate this, laminated copies of check-lists and protocols are included with the equipment and gear destined for each aspect of the summer survey activities. When appropriate, optional or alternate information, techniques, and equipment are listed, including vendors or other resources for obtaining certain items. A compendium of all equipment mentioned in these protocols, including vendor source(s) for some items can be found at the end of this document.

# Checklist: Mist Net Site Gear

The following Items, beyond those items regularly brought into the field for summer netting/trapping bat surveys, are required for anyone desiring to collect and handle bats in accordance with current WNS decontamination and disinfection procedures.

## Suggested Items

- Mist-net “Kit Bag” (e.g., one or more soft-sided duffles) Containing:
  - Sufficient “clean” mist nets to deploy single- , double- , and/or triple-high net sets
  - Sufficient poles and rigging (guy-lines, ropes, stakes, pitons, etc.) to deploy nets
  - Sufficient “clean” single-use bat bags to store bats for processing or later release
  - Electronic equipment to assist documenting activities (e.g., bat detector, GPS)*(See Appendix for complete list of suggested Mist Net Kit Bag Contents)*
  
- 1 5-gallon Bucket\* Labeled “Dirty Nets” Containing:
  - 1 mesh-style “delicate laundry” bag labeled “WNS Mist Net Site Supplies”
  - 1 quart-sized Ziploc bag with 5 white, drawstring-style tall kitchen trash bags
  - 1 quart-sized Ziploc bag with 10-20 extra pairs of latex gloves
  - 1 gallon-sized Ziploc bag with 12-20 cloth bat-holding bags

-or-

  - 1 gallon-sized Ziploc bag with 25-50 paper lunch bags
  - 1 sharpie marker
  - 1-2, 12-15 foot long lengths of rope (for hang lines)
  - 10-15 spring-style wooden or plastic clothes pins
  - 1 bottle of alcohol-based hand sanitizer (e.g., Purell)
  - 1 canister antibacterial/germicidal wipes (e.g., Sani-wipes or Lysol wipe)
  - 1 laminated sheet with “Mist Net Site WNS Protocols”

## Optional Items

- 1-2 folding camp chairs
- 2-5 Two-way radios
- Harp Trap (see: “Checklist: Harp Trap Decon” for more information)

*\*Tip: Use a sharpie marker to label the inside of the bucket lid with the bucket contents to facilitate re-stocking the bucket after each netting/trapping event.*

# Critical WNS Gear



## Paper Bat Holding Bags

Single use paper bags are popular bat holding containers because they are cheap, readily available and disposable.



## Paper Bag Caveat

Bats can chew thru paper, especially paper weakened by urine. Some species (esp. Lasiurines) don't respond well to being held in paper.



## Single Use, Washable Cloth Bags

A better choice for holding bats is a single use cloth bag that can be decontaminated after use.



## 5-gallon Lidded Plastic Buckets

Buckets are the mainstay of this WNS summer survey protocol, serving as totes, airtight containment, & soaking containers.

# Protocol: Mist Net Sites

Mist-net (and trapping) sites are set up using standard field protocols. It is best to have at least 2 people at each net/trap site to facilitate WNS protocols during set-up, manning and handling, and tear-down activities. All Kit Bags, poles, and associated items brought to the site are assumed “clean” either because they are new or never used in a WNS area, or because they have been completely decontaminated, disinfected, stored, and transported in accordance with accepted WNS protocols. A “Dirty Nets” bucket with associated contents is also brought to the site. All nets (and traps) and additional WNS items from the “Dirty Nets” bucket are to be handled and set-up prior to handling bats so they remain in a “clean” condition until the first bat is captured.

Bat workers should come to the site wearing rubber (wellington-style) boots and have a clean pair of footwear to change into after the netting effort. Workers should also have their own supply of latex gloves, a means for carrying the supply on their person, and outerwear that can be removed and bagged at the end of the evening should it come into contact with potentially contaminated bats, nets, gloves used to handle bats or nets, or any other items. In some cases, additional “clean” outerwear may need to be brought to change into after removing soiled items.

## **Additional Set-up Considerations**

- Once nets are set, all handlers should assemble their personal supply of latex gloves in a waist pack, fanny pack, or in a new, draw-string cloth bag (e.g., a bat holding bag) that is worn on the belt or belt loop. This will allow gloves to be readily accessible for use, as needed, to touch nets and/or remove bats. Handlers must don a fresh pair of gloves before handling a new bat and/or touching nets.
- Any non-handlers present can assist with set-up and tear-down without coming into contact with potentially contaminated surfaces or items. Handlers should review the net/trap site protocols with all people present to assure compliance and to answer any questions. Refer to the laminated copy of the “Net/Trap Site WNS Protocols.”
- All handlers should also review (or practice) the procedures for “Latex Glove Removal” (see page 24 of this document) to become familiar with proper ways to

prevent skin and other surfaces from coming into contact with potentially contaminated substances on the exteriors of gloved hands.

- Next, remove all WNS gear from the 5-gallon bucket labeled “Dirty Nets,” hanging the mesh bag labeled “Net Site WNS Gear” from a net pole or other nearby location. Mesh bags are used to store “clean gear” and tall kitchen trash bags are used to store potentially “dirty” gear. This makes it easy to differentiate at a glance what should be handled with gloves and what does not need to be handled with gloves.
- Use the guy lines provided to string two “hang lines,” one for empty cloth bat bags, and one for any back-log of bagged bats should the site become too busy to process bats as they are caught. Empty cloth bags should be hung wide open and “upside down” using clothespins to secure a bottom corner of the bag to the hang line. Leave 5-10 extra clothespins pinned to the line. NOTE: Un-used single-high mist net poles may be useful as hang line anchors in the absence of appropriate vegetation or other items at the site.
- Place empty, lidded “Dirty Nets” bucket near the hang lines and place zip lock bags of paper bags, latex gloves, and tall kitchen trash bags on the bucket lid.
- Place disinfecting wipes and hand sanitizer bottle on the bucket lid.
- Take one tall kitchen trash bag and using the Sharpie marker, label it: “WNS Trash” and hang it near the hang lines so handlers can dispose of used gloves, wipes, or other disposables that come into contact with potentially contaminated bats or surfaces. This also helps prevent handlers from placing used gloves or potentially contaminated trash in pockets or on or near other “clean” items.
- Take a second tall kitchen trash bag, and using the Sharpie marker, label it: “Dirty Nets” and hang it near a net pole, guy tie, or stake.
- Upon completing these tasks, nets can be opened, optional bat detectors can be deployed and the survey effort can commence.

## **Manning the Site and Handling Bats**

- Net checks can be done by handlers or non-handlers. Handlers should not “glove up” prior to determining the presence of bats in nets (or traps). Once bats are discovered, 3-high or 2-high nets should be lowered before “gloving up” to remove bats. This will keep poles, pulley lines, and guy lines in a “clean” condition and prevent them from being cross-contaminated. It also teaches situational awareness that gloves are worn only when touching potentially contaminated items.
- To save on gloves (and trash created) only one handler should “glove up” at a time. Un-gloved workers can hold lights, provide advice, and assist with raising and lowering nets as needed.
- No personal items or body parts should be touched while wearing gloves. This means that opening zippers, turning on or adjusting headlamps, adjusting clothing or glasses, and/or donning additional outerwear should only be done with clean, bare hands.



- Likewise, extreme care should be taken not to tangle headlamps, clothing, or footwear in nets while moving around the site or removing bats.
- Any body parts or other items coming into contact with potentially contaminated nets (i.e., a net which has caught bats) should be wiped down as soon as possible with disinfectant wipes at the “Dirty Nets” bucket.
- Upon removing a bat from a net, the handler should take it directly to the Processing Station, before placing it in a holding bag, and follow the WNS Protocols described for the Processing Station in the next section.
- If it is necessary for a handler to remove additional bats from a net to respond to an unexpectedly rapid capture rate or to prevent bats from becoming too tangled or chewing their ways out of the nets, then the first bat should be bagged and hung from the hang line according to one of the following procedures:

Bagging Bats: Assisted by a non-handler:

- A non-handler will remove a cloth bag from the “empty bags” hang line or a paper bag from the Ziplock bag on the “Dirty Nets” bucket and hold it open with the open end up, while touching only the outside of the bag.
- The handler then places bat and gloved hand all the way into the bag and the non-handler massages the bat out of the handler’s hand by touching the outside of the bag and maneuvering the bat to the bottom of the bag, through the bag and allowing the handler to remove the gloved hand.
- The non-handler then snugs the drawstring on the bag, ties it off, and hangs it upside down from a clothespin on the second hang line. (The bat will usually crawl to the “bottom” of the bag, which should now be facing “up” and will relax and hang out and is therefore less likely to try to escape thru the drawstring while more removing bats from nets and bagging is going on.)
- All site workers should try to hang bats in the order that they came into and therefore were removed from the net so they can be processed in that order once they are transported to the processing site. Because the outsides of the bags remain “clean” using this method, either non-handlers or un-gloved handlers can transport the bats to the processing site as soon as activity levels at the net(s) decrease.

Bagging Bats: Un-assisted bagging:

- The handler will approach the hang line of empty, open bat bags, reach into one without touching the outside with any gloved hands and release the bat in the bag, encouraging it to climb to the top of the upside down bag.
- Then the handler takes an un-used clothespin off the line and pins it within 2-4 inches of the bottom of the bag, closing off as much of the opening as possible.



- The handler then removes and discards the latex gloves in the “WNS Trash” bag and returns to the bat holding bag to cinch it shut and tie it off.
- The handler then unpins the bag from the first hang line and moves it to the second hang line.
- A disinfectant wipe is then used to remove the second clothespin from near the bottom of the bag and deposit it in the “Dirty Nets” trash bag, putting the wipe in the “WNS Trash” bag.
- Bats in bags can be transported to the Processing Site by anyone not wearing gloves as the outsides of the holding bags should remain clean. Clothespins used to hang bags should be transported with the bags so they can be hung on hang lines at the Processing site if needed.

Hand sanitizer can be used to wipe latex powder off hands in between glove changes.

Anytime a net/trap site worker feels as if a surface might have become “dirty” with a possible contaminant, disinfecting wipes can be used to cleanse the area.

***TIP: Two-way radios are helpful tools during numerous activities, especially between multiple net-trap sites and their associated processing stations and any time activities involving holding, processing, or releasing bats are separated by any significant distance. They can also be used during roost visits to help keep in contact with surface personnel to organize on-site processing and/or decontamination/disinfection activities. But, remember only handle radios with “clean” hands!***

If a site is so busy that all the cloth bags are used up, paper bags can be readied on the hang lines by hanging them upside down from the “empty bag” line. Both assisted and unassisted bagging protocols can be followed using paper bags as a substitute.

Whenever possible, reproductive females (pregnant, lactating, or post-lactating individuals) and juveniles of both sexes should be processed as soon as possible and not held in bags at all or at most only for very short periods of time until they can be expedited. Permitting stipulations may require that federal or state, threatened or endangered (T&E) species of either sex be given similar treatments.

Sites that are so busy that they (1) tax supplies of bat holding bags or (2) the availability of personnel to process bats in a timely manner, should consider the temporary or permanent closure of one or more nets.

**IMPORTANT NOTE:** Not all bats respond well to being kept in paper bags. Our colleagues in the Eastern U.S. report that hoary bats (*Lasiurus cinereus*) will often break their finger bones (or rarely arm bones) while thrashing around in paper. It is unknown if other Lasiurines behave similarly. If bats urinate in paper bags, it is easier for them to scratch or chew their ways out. Some bats will chew thru dry paper too. Best practices suggest limiting use of and length of time bats are in paper bags.

### **Tear-down and Transport to Decontamination Site**

- At the end of the survey effort, non-handlers and handlers (without gloves on) collapse all nets at the poles upon conclusion of netting efforts and tie off net main lines at the poles using standard methods.
- Any nets that did not catch bats (i.e., single ground based nets; double-high nets where neither net caught a bat; or triple-high nets where all three nets had no bats) could be removed from poles, wrapped up, and stored in their original net bags and returned to the Mist Net Kit bag(s). NOTE: Consult local WNS permitting stipulations to confirm this practice, and/or to determine if “dirty” nets can be re-used on a second-survey night at the same location without going thru the decontamination/disinfection process.
- Hang-lines and clothespins are removed and returned to their labeled bags and packed up in the mesh laundry bag labeled “Net Site WNS Gear.”
- One person wearing gloves removes any “dirty” nets from poles, assisted by one person not wearing gloves who will wrap up the clean guy ties, poles, and stakes and re-pack them in the Mist Net Kit bag(s) and return all equipment to the vehicles.
- All remaining “Net Site WNS Gear” from the bucket is packed up in the mesh laundry bag labeled “Net Site WNS Gear” and returned to the vehicle for transport back to the Decontamination/Disinfection Station for re-packing in time for the next outing.
- “Dirty” nets are always handled with gloves and returned to their original net bags and deposited in the tall kitchen trash bag labeled “dirty nets.”
- If harp traps are used, they are taken down with gloves as the trap parts are disassembled and re-packed in the stuff sack. At this point, the entire trap and interior of the stuff sack is considered “dirty” and is carried to the vehicle and lashed to the roof. (Care is taken to keep the exterior of the stuff sack “clean” by removing gloves prior to touching the outside after packing the trap and while carrying the trap to the vehicle.) The harp trap is then transported to the Decontamination/Disinfection Station. The exterior of the harp trap stuff sack may be cleaned with wiped.
- The trash bag labeled “Dirty Nets” and the trash bag labeled “WNS Trash” are cinched down, tied off and/or doubled back on themselves and then placed in the

“Dirty Nets” bucket which is then tightly capped. This effectively “double bags” any potentially contaminated gear.

- The “Dirty Nets” bucket is then transported to the Processing Station to receive any “WNS Trash” and “Used Bat Holding Bags” trash bags from the Processing Station before being returned to the vehicle for transport to the Decontamination/Disinfection Station.

If netting in or near a roost entrance, all bat workers will need to visit the Processing Station to scrub their boots, remove outerwear, and double bag items in tall kitchen trash bags for decontamination/disposal at the Decontamination/Disinfection Station.

The easiest way to clean boots is to open a tall kitchen trash bag, stand inside, brush the coarse soil and debris from the boots using the long-handled bristle brush from the WNS Supplies bucket, then spray the boots thoroughly with the spray bottle of disinfecting solution provided in the WNS Supplies bucket. Upon completing this, wearer will step out of the boots, put on clean footwear, cinch and tie the boots in the bag and double-bag the boots for transport to the Decontamination/Disinfection Station.

Any other clothing or personal items that may have come into contact with potentially contaminated bats, nets, or gloved hands also need to be removed, inside out, and double bagged for later decontamination or disposal at the Decontamination/Disinfection Station.

When possible, contaminated items that are bagged can be single-bagged and placed inside the Dirty Nets bucket with the nets, used bat holding bags, and WNS trash, otherwise they are double-bagged, and labeled with a sharpie marker as “dirty” prior to transport to the Decontamination/Disinfection Station.

***TIP: To prevent cross-contamination between potentially “dirty” surfaces and items that need to stay in a clean condition, use the “gloving up” process as a cue to teach situational awareness. Gloved hands may only touch that which may be contaminated (e.g., bats, nets, or roost surfaces). Touch mist net poles, guy lines, pulley ropes, and personal items like headlamps, jacket pockets etc., with bare hands only. Glove up only after turning on headlamps, lowering nets, and before touching a bat.***

# Latex Glove Removal



1. Hold arm of non-dominant hand at side, palm up and flex wrist, creating a gap between wrist and glove.



2. Using dominant hand, grab raised edge of glove without touching skin with gloved hand.



3. Make a fist with non-dominant hand then use dominant hand to pull glove up and off hand.



4. Slowly open non-dominant hand while pulling glove off inside out and hold in dominant hand.



5. Make closed fist with dominant hand, holding removed glove.



6. Hold arm of dominant hand at side, palm up and flex wrist, creating gap as in step 1.



7. Using thumb and finger of bare hand, pinch *inside* edge of remaining glove.



8. Pull glove off, inside out, trapping first glove inside and dispose both gloves in trash.



9. If desired, use sanitizer to wash latex powder off hands.



# Checklist: Processing Gear

The following Items, including those items regularly brought into the field for processing bats during summer netting/trapping bat surveys, are required for anyone desiring to collect and handle bats in accordance with current WNS decontamination and disinfection procedures.

## Suggested Items

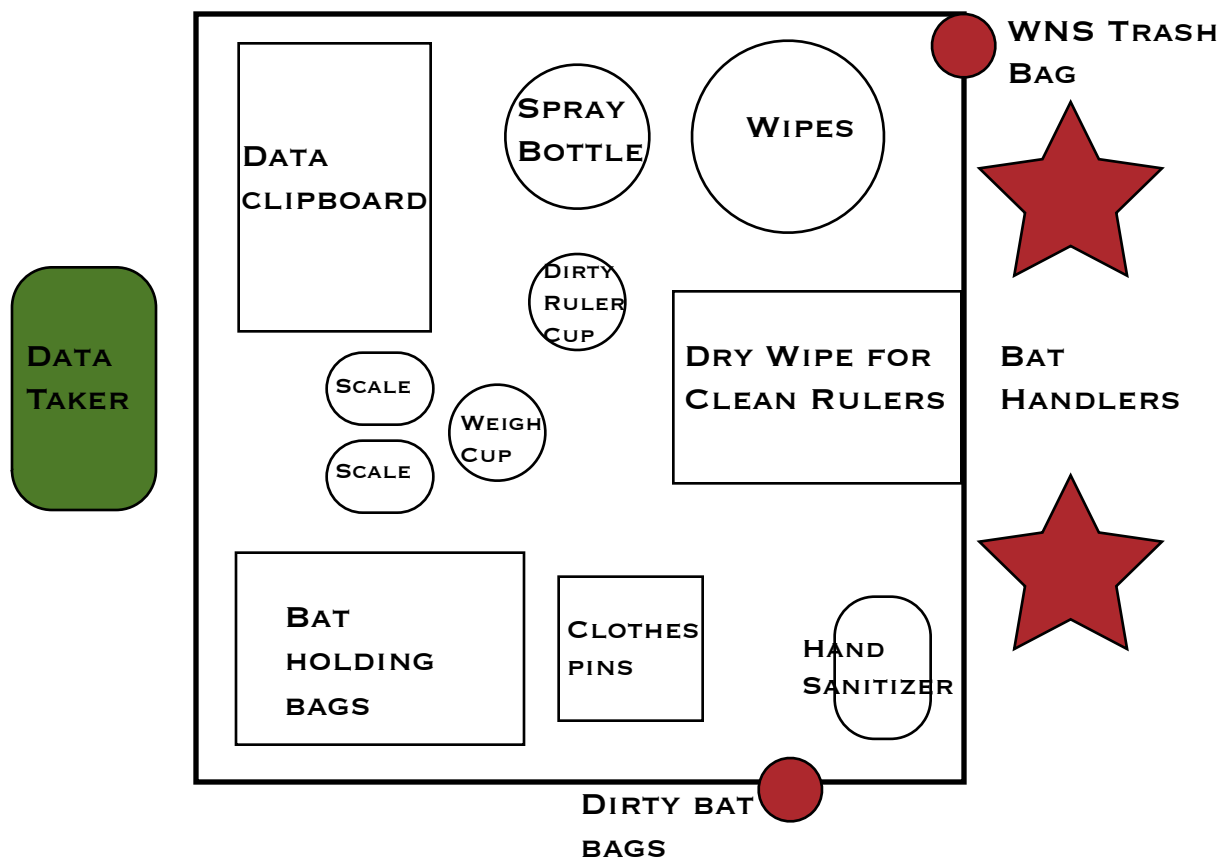
- 1 Folding aluminum camp table
- 1 Folding camp chair
- Tray-style Clipboard for data-taking Containing:
  - Blank data sheets
  - Laminated ID key(s)
  - 3-5 Millimeter rulers
  - 3-5 Pens and/or pencils
  - 2-3 Sharpies
  - bat bag labels (e.g., peel off, blank, address-type labels)
  - 2 Digital scales
  - 1 Digital thermometer
  - GPS unit
- 1 shoe-box sized lidded tote labeled “WNS Wing Damage Photo Kit” Containing:
  - 1 AA battery operated light box with grid ruler and blank labels for identifying photos
  - 1 AA battery operated digital camera + extra AA batteries
  - Tissue sample materials and WHC submission guidelines
  - Laminated WNS Wing Damage Index sheet (see pages 30-31)
- 1 5-gallon Bucket Labeled “WNS Supplies” Containing:
  - 2 12-15 foot long guy ties
  - 10-15 spring style wooden or plastic clothes pins
  - 1 quart-sized Ziploc bag with 5 white, drawstring-style tall kitchen trash bags
  - 1 quart-sized Ziploc bag with 10-20 extra pairs of latex gloves
  - 1 gallon-sized Ziploc bag with 12-20 cloth bat-holding bags
  - 1 gallon-sized Ziploc bag with 25-50 paper lunch bags
  - 2 plastic cups (1 for storing cleaned rulers, 1 for weighing bats)
  - 1 spray bottle of decontamination solution (2-1/4 tsp. product per 24 oz. water)
  - 1 long-handled bristle brush
  - 1 pump-style bottle of alcohol-based hand sanitizer (e.g., Purell)
  - 1 canister antibacterial/germicidal wipes (e.g., Sani-wipes)
  - 1 laminated sheet with “Bat Processing WNS Protocols”

# Protocol: Processing Station

Processing sites are set up using standard field protocols and consist of all items necessary to describe the site, take data on captured bats, and comply with permit requirements for the study proposal. In order to keep the area free from potential WNS contamination, bat handlers are not to touch anything at the site except for millimeter rulers for measuring bats and trash bags for disposing of gloves and bags. The processing site works best when a dedicated “data taker” is selected to man the site and help keep the area clean, work with the bat handlers to identify and process bats, and record all relevant information about the netting/trapping event.

## Additional Set-up Considerations

- The data taker sets up folding aluminum camp table, folding chair, clipboard (and certain clipboard contents), and WNS supplies from the “WNS Supplies” 5-gallon bucket. The camp table has a schematic indicating where certain items should be located to ensure that the area remains clean and isn’t inadvertently contaminated with potentially “dirty” items when bat handlers bring bats to be processed.





- Bat-handlers should never cross the “dirty edge” of the processing table (indicated in the schematic by the red icons).
- Two tall kitchen garbage bags are hung from the table, one labeled “WNS Trash” and one labeled “Dirty Bat Holding Bags.” Gloves, wipes, and paper bags brought in and used by bat handlers are put in the “trash” bag and used cloth bat holding bags are put in the other bag.
- Two guy lines are used to create “hang lines” for storing bats in holding bags, one line for those that are awaiting processing, and one line for those that have been processed but are awaiting release. NOTE: Un-used single-high mist net poles from the net/trap site may be handy for use as hang-line anchors.
- Bat handlers and net workers should be encouraged to leave their clean shoes, fresh outerwear, and other personal items at the processing site to make changing at the end of the evening more efficient.

## **Manning the Site and Handling Bats**

- With assistance from the net/trap workers, the data taker fills out the data sheet with site information including start time, start temperature, GPS location, number and sizes of nets and traps deployed, and a schematic of the setup on the reverse.
- The data-taker then takes a single antimicrobial wipe and lays it on the table in the spot indicated, allowing it to dry out, and lines up millimeter rulers on the wipe so bat handlers can easily select a ruler without contaminating anything else on the table.
- Upon receiving a bat from the net/trap workers, the data taker asks for time of capture, species ID, age, sex, and reproductive condition.
- At this time, the handler should inspect all wing surfaces and membranes for evidence of WNS damage in accordance with the WNS Wing Score Index information provided in the Appendix of this document. If damage consistent with indices of 2 or 3 are noted, then the Wing Damage Photo Kit must be used to document the condition. (Note: Some permitting agencies may require that bats with any damage at all be photographed, tissue samples taken, or other methods of documentation. Review permits and adjust protocols if necessary.)

### Using the Wing-Damage Photo Kit

- The data-taker opens the kit and places the light box on the aluminum table or other appropriate surface (e.g., the top of the “WNS Supplies” bucket), opens the lid, positions the grid ruler, and turns the unit on.
- The data-taker prepares a bat-label with the date, site name, species, sex, and bat number from the data sheet and places it on the grid so it will be in the frame of the photo.
- The data-taker then takes the camera and positions it to photograph the grid.
- The bat handler holds the bat, extending one wing at a time over the grid ruler for pictures. NOTE: most bats prefer to be held dorsal surface up (with the ventral

surface towards the light box and grid; holding them “upside down” often causes them to struggle more and be more difficult to photograph).

- If the tail-membrane also shows evidence of scaring, it can be displayed separately for an additional photograph.
  - Data-takers may want to experiment with the best settings to record usable photos of bat wings. Using the telephoto setting, zooming all the way out and positioning the photographer so the bat on the lightbox fills the frame often renders a well-lit photograph. Alternatively, if the built-in flash can be manually dialed to a lower intensity, an ASA of 400-800 used, and the shutter speed adjusted to 1/800 at F 7.0; then the “macro” setting on the camera may be used. Until competency with the technique is achieved, use two or more methods to ensure a good result.
  - Photograph numbers should then be recorded in the appropriate section of the data sheet for the identified bat. **NOTE:** *Consult local WNS permitting stipulations to determine if tissue samples need to be taken from suspect animals and follow the protocols (provided) for that process.*
  - When photography is complete, the data taker sprays the grid ruler with prepared disinfectant spray from the spray bottle, saturating the surface and letting solution sit for 10-minutes, then wipes grid ruler and any over spray off light box with a disinfectant wipe. The box is then allowed to air-dry and stored with the camera in the tote for the next use.
- 
- After assessing the bat for WNS damage, the bat handler then takes a clean millimeter ruler and provides measurements for: (1) forearm, (2) ear, (3) tragus, and (4) hind foot. (Note: tragus and hind foot measurements may be required for T&E species only)
  - The bat handler then places the dirty ruler into the appropriate storage/soaking cup that contains antimicrobial wipes saturated with decontamination solution from the spray bottle.
  - The data taker then selects a bat storage bag, places it in the weigh cup and tares the cup and bag on one of the digital scales. Then the data taker holds the bag open, over the bat handler’s hand so the bat can be placed in the bag and weighed. It is important that the data taker touches only the outside of the bag and the bat handler touches only the inside of the bag during the transfer of the bat. The bat is then rolled up loosely in the bag and placed in or on the cup to be weighed.
  - If bats are to be kept for any length of time, cloth bags should be used. Otherwise, bats can be bagged in paper, released after weighing, and the paper can be disposed of in the “WNS trash” bag along with the bat handler’s used gloves.
  - Bat bag tags are used to identify kept bats with the location name, capture number, and ID information from the data sheet. The data taker should fill out and affix the tag to the upper corner of the bag and then the bag should be hung on the second hang line, reserved for already processed bats awaiting additional attention.
  - The data-taker is responsible for hanging bats in the holding bags on the appropriate hang line. This keeps the outsides of the holding bags in a “clean” condition.
  - During cold or windy nights a cloth, blanket, towel, or jacket should be placed over occupied bat holding bags on the hang line to help protect bats from the weather.

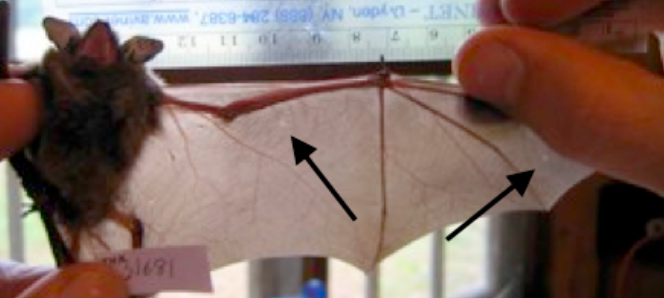

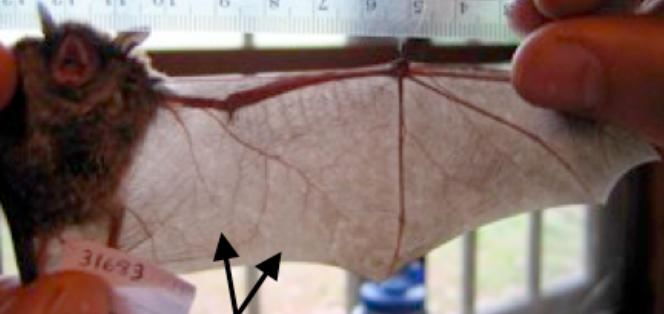

- If there are no additional uses for processed bats (i.e., hand release for acoustic recording or collecting guano samples), then the processed bats can be released immediately after processing is complete.
- To release a bat from a paper bag, the bag can simply be opened and held horizontally so the bat can crawl out and take flight. To release a bat from a cloth bag, the bag can be untied and un-cinched and the bat can be worked towards the opening until it can peek out and take flight.
- Alternatively, the bat handler can take the bag from the data taker (after weighing) and reach in and remove the bat and hold it aloft so it can take flight. Then paper bags can be disposed of in the “WNS trash” bag and cloth bags can be stored in the “Used Bat Holding Bags” bag. **NOTE:** *To aid in the decontamination/disinfection process, used cloth bat-holding bags should be turned inside-out when they are placed in the “used bat holding bag” trash bag, this helps eliminate any accumulated guano in the bags and will keep decontamination solution water cleaner longer.*
- The data taker uses a dry antimicrobial wipe or damp cloth to rinse used millimeter ruler(s) from the “dirty” storage/soaking cup and return ruler(s) to the area reserved for clean millimeter rulers. **NOTE:** *It is important that rulers are rinsed with water or hand-sanitizer and allowed to dry completely before using on bats. Failure to do so can allow live bats to come into contact with dangerous or deadly concentrations of chemicals which could seriously compromise their fitness or kill them.*
- This process is repeated until all bats from the site are processed.
- If bats are kept for additional uses they should be transported to the use area with materials for creating a new hang line (i.e., poles, guy lines, protective cloth, “WNS trash” and “Used Bat Holding Bags” trash bags) so they can be kept safe until eventual release and items can be returned at the end of the night.

## **Tear-down and Transport to Decontamination Site**



- At the end of the sampling effort, any potentially contaminated gear on or around the processing table (e.g., rulers) must be disinfected using the spray bottle solution.
- Items should then be re-packed in either the clipboard or the “WNS supplies” bucket as appropriate. **NOTE:** *The clipboard and bucket will have their respective contents listed on the insides of their respective lids.*
- Use the spray bottle and a disinfecting wipe to clean the aluminum table and re-pack it in its stuff sack.
- Trash bags need to be cinched and tied then placed in the “Dirty Nets” bucket that arrives from the mist net/trap site or double-bagged (and appropriately labeled; either “WNS trash” or “Used Bat Holding Bags”) for transport.
- Data-takers can assist net/trap staff with additional removal, decontamination, or storage of personal items as needed.

# WNS Wing Damage Index

The damage to membranes and the forearms are scored 0 (none) to 3 (high) according to the criteria listed below and digital photographs are taken to document any damage. Each photograph should include a reference scale and the bat ID number, cross-indexed on the data sheet.

<p><b>Score = 0</b></p>	<p><b>No Damage</b></p>
<p>Fewer than 5 small scar spots are present on the membranes. The membranes are fully intact and pigmentation is normal.</p>	
<p><b>Score = 0</b></p>	<p><b>No Damage</b></p>
<p>Fewer than 5 small scar spots are present on the membranes. The membranes are fully intact and pigmentation is normal.</p>	
<p><b>Score = 1</b></p>	<p><b>Light Damage</b></p>
<p>Less than 50% of flight membrane is depigmented (splotching), which is often visible only with transillumination. The membranes are entirely intact. Some discoloration or flaking is visible on forearms. Such flaking on the forearm may exist even if the patagium appears unaffected.</p>	
<p><b>Score = 1</b></p>	<p><b>Light Damage</b></p>
<p>Less than 50% of flight membrane is depigmented (splotching), which is often visible only with transillumination. The membranes are entirely intact. Some discoloration or flaking is visible on forearms. Such flaking on the forearm may exist even if the patagium appears unaffected.</p>	



<p><b>Score = 2</b></p>	<p><b>Moderate Damage</b></p>
<p>Greater than 50% of wing membrane covered with scar tissue (splotching), Scarring is visible without translumination. Forearm skin may be flaking and discolored along the majority of the forearm, but this condition alone does not earn this score level.</p>	
<p><b>Score = 2</b></p>	<p><b>Moderate Damage</b></p>
<p>Membrane exhibits some necrotic tissue and possibly few small holes (&lt; 0.5cm in diameter). Small holes are surrounded by discolored tissue. Necrotic tissue is sometimes associated with less severe splotching.</p>	

<p><b>Score = 3</b></p>	<p><b>Heavy Damage</b></p>
<p>Deteriorated wing membrane and necrotic tissue. Isolated holes <math>\geq 0.5</math>cm are present in membranes. Necrotic or receding plagiopatagium and/or chiropatagium are evident. This score is characterized by notable loss of membrane area and abundant necrosis.</p>	
<p><b>Score = 3</b></p>	<p><b>Heavy Damage</b></p>
<p>Flight membranes show damage similar to Level 2 (score = 2) damage with additional loss of flight membrane are due to holes and/or receding edges of the wings.</p>	
<p><b>Score = 3</b></p>	<p><b>Heavy Damage</b></p>
<p>Plagiopatagium loss may be severe.</p>	

# Checklist: Roost Visit Gear

The following items, including those items regularly brought into the field for processing bats during summer bat roost visits, are required for anyone desiring to enter cave, mine, or building roosts and/or handle bats in these sites in accordance with current WNS decontamination and disinfection procedures. Additional “Personal Items” may also be required. See “Checklist of Personal Items” on page 8 of this document for details.

## Suggested Items

- Folding aluminum camp table\*
- WNS Supplies\* bucket
- Tray-style Clipboard\* for data-taking
- Extra Tyvek suit(s) for anyone needing to enter roost
  
- 1 tote labeled “WNS Wing Damage Photo Kit”\* Containing:
  - 1 AA battery operated light box with grid ruler
  - 1 AA battery operated digital camera + extra AA batteries for camera and light box
  - Tissue-sample supplies and submission guidelines from WCH
  - Laminated WNS Wing Damage Index card (see Appendix)
  
- 2 lidded totes (20x15x5”) Labeled “Disinfectant” and “Rinse” Containing:
  - 1 mesh-style “delicate laundry” bag labeled “Roost Visit WNS Supplies”
  - 2 gallon-size jugs of fresh water
  - 1 quart decontamination solution (e.g., quaternary ammonium compound)
  - 1 1/8-cup measuring cup
  - 1 1-tsp measuring syringe
  - 4 bristle brushes
  - 1 gallon-sized Ziploc bag with 10-20 white, drawstring-style tall kitchen trash bags
  - 1 quart-sized Ziploc bag with 10-20 extra pairs of latex gloves
  - 1 Sharpie marker
  - 1 spray bottle of decontamination solution (2-1/4 tsp. quat. per 24 oz. water)
  - 1 pump-style bottle of alcohol-based hand sanitizer (e.g., Purell)
  - 1 canister antibacterial/germicidal wipes (e.g., Sani-wipes)
  - 1 laminated sheet with “Roost Visit WNS Protocols”

*\*these items are also used at a “Bat Processing Site” and can be shared, see the “Processing Site” section for descriptions of bucket and clipboard contents*



# Protocol: Roost Visits

Sufficient gear accompanies each Roost Visit to disinfect/decontaminate visitor footwear and other submersible items used at or in the roost. Under special circumstances, visitors may be required to obtain and use additional single-use, disposable, protective gear at the discretion of the trip leader and/or instructors on site. **NOTE:** *These protocols only apply to summer survey roost visits. Additional precautions may be required during winter surveys, especially since winter entry into roosts where bats are already compromised by WNS infection are likely to prove additionally deadly. If winter roost entry is required it should be done in close coordination with federal and state agency personnel already well acquainted with the special considerations of winter WNS survey protocols.*

## **Additional Roost Entry Considerations**

- Visitors must wear non-caving clothes/shoes on the way to the site. All clean, underground equipment and clothing should be carried to the site in a sealed container. None of the underground equipment should have ever been used in the past three years at a roost where WNS has been confirmed. Visitors will change into underground clothes and equipment at the site and put clean clothes worn to the site in the sealed container.
- Non-submersible gear brought into the roost for the purposes of non-contact documentation of the bats or the roost (e.g., cameras, video recorders, etc.) should be either placed in plastic casings (e.g., underwater housings) or bagged in plastic, leaving only the lens glass unwrapped. Lenses can then be decontaminated using disinfectant wipes and plastic can be removed and disposed of appropriately.
- Visitors must bring rubber boots (or other suitable, submersible footwear) to wear during the roost visit. This footwear is to be carried to and from the site and not worn off-site unless thoroughly decontaminated.
- Visitors must also bring spare footwear to wear to and from the roost.
- Visitors must have at least three pairs of latex gloves (more, if bat handling is planned) which will be worn inside the roost. Double-gloving will help prevent rips or tears while maneuvering around inside the roost.
- If bats are going to be collected for processing, sufficient quantities of disposable or single-use bat bags must be brought into the roost for storing and removing bats to the processing station outside the roost.

- No equipment should be taken into a roost unless it can be disposed of or thoroughly decontaminated after the visit.
- All bat processing will take place outside the roost, at least 10-meters from the entrance, and be handled under “clean” conditions similar to the processing that occurs in conjunction with mist net/trap sites.
- The folding table, clipboard, WNS Supplies bucket, and Wing Damage Photo Kit tote are left outside the roost, at least 10-meters from the entrance and any bats collected in the roost will be brought outside for processing.
- A data-taker, who will remain outside the roost, sets up the processing equipment as during a Mist Net/Trap effort, preparing hang lines to receive any bats collected from the roost. NOTE: Because exteriors of bat holding bags coming out of the roost will be “dirty” only bat handlers may handle bats during processing. After processing, bat bags, clips, hang lines, etc. must be bagged for transport to decontamination station.
- The data-taker can also set up the foot baths for returning roost entry personnel. One gallon of water is poured into each of two roost visit totes. In the “decontamination” tote, one ounce (1/8 cup) of quaternary ammonium compound is added. Lids are placed on totes until needed.

### **Entry Behavior and Handling Bats**

- While in the roost, care must be taken not to stir up excessive dust or dirt from roost floors or walls, as any WNS spores present will have greater potential to contaminate gear, equipment, personnel and bats.
- Bats should be handled minimally and with latex gloves. Care should be taken to bag and store bats in a safe manner and while they are transported to the processing station.
- Bats should be single-bagged in disposable or single-use bat holding bags and returned to the processing station as soon as possible.
- Other minimally intrusive roost entry protocols should be followed to reduce disturbance to bats.

### **On-site Clean-up and Transport to Decontamination Site**

- Upon exiting the roost, every visitor must decontaminate footwear and outerwear first by brushing coarse dirt and debris from surfaces using long-handled bristle brushes. This is best achieved while standing in an open tall kitchen trash bag to control dirt and debris so it is not tracked around outside the roost entrance.
- Next visitors stand in decontamination solution for 10-minutes, swishing boots around. Then step into rinse solution, then step into a double-bagged, tall kitchen trash bag and remove boots, cinch and knot tops of bags for transport to decontamination station where boots can be properly submersed for complete decontamination.

- Alternatively, visitors can eliminate foot baths by standing in a double-bagged, tall kitchen trash bag and thoroughly spray boots with spray bottles of 1:64 decontamination solution, step out of boots and bag boots for later submersion.
- Visitors then remove roost visit clothing, turning it inside out to trap any potentially contaminated surfaces and double bag clothing for transport and decontamination or disposal. **NOTE:** *Tyvek suits aid immensely in providing a disposable outerwear option.*
- Visitors then put on the clean clothing and boots worn to the site.
- No roost visit gear is loaded into vehicles until it is double-bagged or sealed in air-tight containers so vehicles stay clean.
- No WNS trash (e.g., gloves, used bat bags, etc.) is loaded into vehicles until it is double-bagged or sealed in air-tight containers so vehicles stay clean.
- Decontamination solution and rinse water from totes is then disposed of appropriately in a municipal sewer or water treatment plant or RV dump station.

**TIP:** Decontamination solutions (anti-bacterial, germicidal, or bleach solutions) should be prepared fresh daily or more often if the solution becomes visibly dirty or diluted. (A “visibly dirty” solution is one in which items at the bottom of the bucket cannot be easily seen.) Failure to do so will provide inadequate disinfection. Care should be taken to thoroughly rinse all items (using a triple rinse) to remove all residues of solution from items that will come into contact with bats or bat workers. Failure to do so could become a health hazard. Review product label and warnings for additional directions about use, storage, and disposal.

# Checklist: Decon Station

The WNS Decontamination/Disinfection station is generally located at a lodge, hotel, office, house, or other facility away from the field site where WNS-contaminated and potentially contaminated gear is collected during summer survey efforts. Ideally this station will have access to electricity, fresh water, and a sewer system. If not, options for running off batteries or generators, bringing sufficient water, and/or disposing of used decontamination solution and rinse water must be exercised. Alternatively, nets, bat holding bags, and other gear may be boiled for 15 minutes to successfully decontaminate items. See “optional equipment” for materials.

## Suggested Equipment

- 1 electric spin dryer
- 1 12-foot extension cord
- 1 5-gallon bucket labeled “Clean Nets” containing:
  - 1 1-cup measuring cup
  - 1 pair scissors
  - 1 gallon quaternary ammonium “decontamination solution” and/or bleach
  - 2 12-15 foot guy lines for a laundry line, preferably located in direct sunlight
  - Clothespins (use supplies from the “net/trap site” or “processing station”)
  - 1 laminated sheet with “WNS Decontamination Station Protocols”
  - 1 laminated sheet with “Harp Trap WNS Decontamination Protocols”
  - 1 spray bottle with prepared decontamination solution (2-1/4 tsp/24 oz. water)
  - 1-3 pair elbow length rubber gloves (optional: include rubber bands to secure)
- 2 folding camp tables (can be the same as those used at the “Processing Station”)
- fresh water spigot, preferably with hose attached
- extra WNS supplies for re-stocking net-site and processing-station buckets and roost-visit totes

## Optional Equipment

- AC/DC converter (to run the spin dryer off a vehicle battery)
- 4 5-gallon jugs of fresh water (in case fresh water is not available at the site)
- 2 12-quart covered pots (for “boiling” decontamination option)
- 1 double-burner camp stove (for “boiling” decontamination option)
- 3-6 fuel canisters (for “boiling” decontamination option)
- Matches (for “boiling” decontamination option)

# Protocol: Decon Station

Decontamination, disinfection, disposal of potentially WNS-contaminated items, and re-packing gear and supplies for subsequent outings should all be conducted away from the netting/trapping or roost entry site, preferably at a location with access to: (1) electricity, (2) water, (3) trash removal service, and (4) proper means for disposing of decontamination solutions and rinse water. The decontamination steps will add 1-2 hours to each survey effort and should be done on a daily basis. But, because many items require drying before re-packing the decontamination process can begin as early as the night of the survey effort upon returning in from the field, or no later than first-thing the following morning. The decontamination process itself should take about 90-minutes. All equipment should be dry and ready for re-packing by mid-afternoon, a process that can be accomplished in about 30-minutes. If off-site disposal of solutions and rinse water is required, the time to locate and transport waste to an appropriate site and return should be built into the process.

## Set-up Considerations

- All lidded buckets and/or double-bagged trash bags from mist net/trap sites and processing stations and totes from roost visit activities should be deposited at the decontamination/disinfection station at the end of the survey effort.
- Mesh bags with WNS Supplies are also deposited at the site near the main WNS supplies.
- Set-up spin dryer on its carry box or other elevated surface. This helps the dryer to drain neatly and completely.
- Assemble folding aluminum camp tables in full, all-day sunlight, stringing guy lines between them to use as hang-lines if necessary.
- Separate the WNS Supplies bucket(s) and empty contents so quantities can be inventoried, re-stocked, and re-packaged for later re-packing.
- Empty “Clean Nets” bucket, and arrange contents nearby.
- Empty “Dirty Nets” bucket(s), combining the “WNS Trash” bag with any other “WNS Trash” bags from the evening and dispose of in dumpster or trash can on site.
- If harp trap(s) require decontamination, follow specific trap instructions included.

- Considerations for Boiling Gear: If the “decontamination by boiling” option is exercised, fuel, camp stove, and pots filled with water will need to be set up. The largest reasonable boiling pot which can be used on a camp stove is a 12 quart (3 gallon) stock pot. This is considerably smaller than the 5-gallon buckets used in chemical decontamination so the boiling process will take a bit longer due to the limited amount of gear which can be decontaminated at a single time. Care must also be taken to have enough fuel to keep pot(s) boiling for 30-90 minutes. A kitchen facility may be used, but care must be exercised in transporting potentially contaminated “dirty” gear indoors and during the cleaning and disposal of containers used for storage and transport.

## **Manning the Site and Handling Potentially Contaminated Items**

- Remember, approved disinfection/decontamination solutions are caustic, potentially dangerous products. Personal safety should always be exercised while handling these solutions. Wear gloves, goggles, appropriate over-wear, and footwear to prevent contact between chemicals and exposed skin, especially eyes, nose and mouth. Decontamination solution ***should not*** be handled with bare hands. Gloves are provided in the “Clean Nets” bucket. Workers are required to bring their own glasses/goggles and other protective outerwear to ensure a proper fit.
- In “Clean Nets” bucket(s), prepare decontamination solution according to dilution ratios for the chosen compound. (i.e., 1 oz. quaternary ammonium per gallon of water or 1/2 cup per 4-gallons of water, added to “fill line” on outside of bucket.)
- A Note on Bucket Management: It is up to the site coordinator to determine if multiple decontamination solution buckets will be used or if just a single bucket will suffice for the quantity of “dirty” nets and other gear that is returned to the site. Because only “Dirty Nets” buckets will have potentially contaminated gear in them, if they are used as rinse buckets, at least one cup of decontamination solution must be added to each empty “Dirty Nets” bucket and swished around for 10-minutes, completely saturating the entire inside and lid, then drained back into the bucket with the remainder of the decontamination solution. “Dirty Nets” buckets may then be used as rinse water buckets, leaving the “WNS Supplies” buckets un-used, which can then be re-packed for the next outing. ***NOTE: For each “decontamination solution” bucket, three (3) rinse-water buckets are required. Or, a single bucket can be used ONLY IF rinse-water can be disposed of properly and refreshed at the site, during the rinsing process. More likely, if multiple decontamination solution buckets are used, additional rinse water buckets may also be required.***
- Cut top(s) off any bag(s) of “Used Bat Holding Bags” and ensure that bags are “inside out” prior to placing into the decontamination solution. If bags are not turned inside out, don latex gloves and turn bags inside out, shaking any accumulated guano into the bottom of the trash bag before placing bags in the solution. This will keep the solution cleaner longer. Then soak bags for up to 15-minutes, agitating often during the process. Solution must completely cover all bags in bucket, otherwise bags must be soaked in batches. ***NOTE: It is important to do bat holding bags first as they are likely to come into contact with the most potential spores***



*should bats be shedding WNS fungus, and so bat bags can be thoroughly rinsed in the freshest possible rinse water prior to drying and re-use to protect bats from any residual chemicals that could potentially survive the triple rinse.*

- Station workers should wear elbow-length rubber gloves to move bat bags from decontamination solution to the first rinse bucket. Items should remain in the rinse buckets for at least 10-minutes and should be agitated periodically throughout the process. It is important to drip dry and thoroughly wring out items soaked in decontamination solution back into the decon-solution bucket before transferring wet items to the first rinse bucket.
- Drip dry and thoroughly wring out rinse water back into the rinse bucket before transferring wet bags to the second rinse bucket. Repeat as above.
- Repeat again as above for the third rinse bucket, placing wrung out bags, up to 10-12 at a time, into the spin dryer, set timer for 5-minutes, allow dryer to drain into the 3rd rinse bucket.
- Hang bags on clothesline, using clothespins to keep them in place until they are thoroughly dried.
- During this entire process, care should be taken not to spill any decontamination solution or rinse water on the ground or splash solution on workers.
- Cut off top of “dirty nets” trash bag and use latex gloves to place nets into decontamination solution and soak for up to 15-minutes. Solution must completely cover all nets in bucket, otherwise nets must be soaked in batches.
- Using the same wringing and draining, rinsing and agitating process as that used for the bat holding bags, transfer nets from decontamination solution to rinse buckets, repeating the rinsing process three times before transferring nets, up to 4-6 at a time, to spin dryer.
- Spin nets dry for 5-minutes, then transfer nets to aluminum tables to air dry in the sun for the rest of the day, flipping them over half-way thru the day if needed.
- Soak any personal gear, clothing, or other porous or non-porous items, including the lid(s) from the “Dirty Nets” bucket and rinse thoroughly, spin dry, and place in sun.

### **Tear-down, Re-packing, and Re-stocking**

- If on-site municipal sewer systems exist, buckets of decontamination solution and rinse water can be drained and rinsed in showers or sinks, buckets are then returned outdoors, up-ended, and allow them to dry in the sun with the rest of the decontaminated/disinfected gear.
- If on-site municipal sewer systems do not exist, buckets must be capped and transported to a sanitary disposal site or RV dump station, rinsed at the site, and returned to the processing station to dry.
- After gear is completely dry, bat holding bags should be re-packed in gallon-size Ziplock bags and re-distributed to now dry “WNS Supplies” and “Net Site Supplies” buckets.

- Nets should be re-packed in mist net kit bags, taking care to distribute adequate quantities of each net length in each kit as needed.
- From the WNS Supplies stash, make up appropriate quantities of gloves, paper bags, guy lines, disinfectant wipes, and other items as indicated in the check-lists for each bucket.
- Cap buckets and return them to vehicles for the next survey effort.
- Note any supplies that are running low and need replenishment before returning to the field for future survey efforts.

***TIP: The “electric spin dryer” is an extremely worthwhile addition to the WNS decontamination process. It is highly efficient in extracting the vast majority of moisture from washed items and is essential for preventing moldy, dank nets, bat bags, and field gear; prolonging the lives of these items in the process. This is especially true during cool, humid seasons or portions of the country, but is also quite helpful even in our hot, lowland desert locations. Properly dried and stored gear, especially expensive mist-nets, will last longer.***

# Checklist: Harp Trap Decon

Harp traps must be cleaned after use at each site to remove any dirt or debris from lines and bags. “Dirty” harp traps should not be transported inside vehicles, instead, repack in carry bag and tie to roof rack. Rinse all parts of the trap, especially the catch bag, and dry thoroughly before re-packing for the next use.

## Suggested Equipment

- |  |   |
|--|---|
| <input type="checkbox"/> 4 5-gallon buckets with lids                              | <input type="checkbox"/> plastic tarp   |
| <input type="checkbox"/> 1 1-cup measuring cup                                     | <input type="checkbox"/> elbow length rubber gloves                             |
| <input type="checkbox"/> 1 quart quaternary ammonium<br>“decontamination solution” | <input type="checkbox"/> laundry line, preferably located in<br>direct sunlight |
| <input type="checkbox"/> 2 1-quart spray bottles                                   | <input type="checkbox"/> fresh water  |

## Decontamination Procedure

- Prepare decontamination solution in 1 5-gallon bucket, diluting quaternary ammonium solution at a ratio of 1:128 (1 fluid ounce per gallon of fresh water; 1/4 cup of solution for a 4-gallon bucket).
- Prepare decontamination spray in 1 spray bottle, diluting quaternary ammonium solution at a ratio of 1:64 (0.5 fluid ounces per quart of fresh water).
- Fill 3 remaining 5-gallon buckets and one remaining spray bottle with fresh water.
- Unroll plastic tarp, spread on ground, and secure with weights to keep from blowing.
- Place harp trap in center of tarp and remove harp trap from stuff sack. Remove bar supports from harp trap catch bag and take catch bag and stuff sack and soak in decontamination solution for 15-minutes.
- Meanwhile, apply decontamination spray to all parts of harp trap, paying special attention to saturating the Head Piece, Side Bars, and Bottom Bar with solution and allow solution to sit for at least 10-minutes on trap parts before rinsing with spray bottle of fresh water, refilling fresh water bottle as needed. (It is important to thoroughly rinse all solution from trap parts to prevent metal pieces from corroding.)
- After soaking stuff sack and trap catch bag for 15-minutes, wring each out as thoroughly as possible then rinse thoroughly, using each of the three rinse buckets; agitating bag and sack while rinsing and wringing thoroughly after each rinse. Hang bag and sack on laundry line and allow to dry completely before repacking trap parts in catch bag and stowing parts. Dispose of decontamination solutions and rinse water appropriately. Allow buckets to dry before re-packing decon equipment.

# Bat Survey Kit Bag: Contents

## Mist Net Kit Bag Contents

- 1 8-foot (2.6m) Mist Net, in bag
- 4 18-foot (6m) Mist Nets, in bags
- 3 30-foot (9m) Mist Nets, in bags
- 4 42-foot (12m) Mist Nets, in bags
- 1 60-foot (18m) Mist Net, in bag
- 12 tent stakes
- 2-4 pitons
- small rock hammer
- 12 12-15 foot guy ties
- 2 25-foot guy ties
- 12 bat holding bags
- clothespins to hang bat bags
- 2 bat weigh-tubes and/or weigh-bags
- 1 heterodyne bat detector
- 1 kit bag
- 1 pole bag
- 6 pointy poles
- 12 flat poles
- duct tape
- flagging
- reflector tape
- extra batteries for electronic gear
- plastic sleeve for permit(s)
- 1 first aid kit with alcohol wipes
- net-repair kit (main-line string, black nylon thread, needle)

## Data-Taking Clip Board (Tray-style)

- 4 pens/pencils
- 2-3 Sharpie markers
- 5-10 blank datasheets
- laminated ID key(s)
- peel off labels to ID bat bags
- 2 mm rulers and/or calipers
- 2 digital scales and/or spring scales
- 1 GPS unit
- 1 digital thermometer

## Harp Trap Kit Contents

- harp trap
- spool of fishing line (8lb test)
- extra screws, pins, washers, nuts, etc.
- metal file or sandpaper
- small hammer
- Allen wrenches
- 2 25-foot guy ties
- carabiners

## 3H Pole Mist Net Kit Contents

- 3 18-foot (6m) Mist Nets
- 3 30-foot (9m) Mist Nets
- 3 42-foot (12m) Mist Nets
- 1 hand-held sledgehammer
- 2 25-foot "helper ropes"
- extra carabiners
- 2 pole anchors
- extra pole anchors or heavy duty stakes
- 10 main line loop extenders
- 1 kit bag

## Acoustic Survey Suitcase

- bat detector
- recording device (MP3 player, hard drive, CF card, laptop, net book, etc.)
- connecting cables
- instruction manual and/or quick start guide
- extra batteries for all electronics
- zip-line kit (fishing line, stoppers, cyalume, glue-stick, duct-tape, elastic thread, scissors, pliers, etc.)
- spot-light
- straps and/or bungees
- carrying case

# More Summer Survey Gear



Tall Kitchen Trash Bags: White shows up better at night!



Quart Size Ziploc Bags: Excellent for storing small items. Gallon Size Ziploc Bags: Excellent for storing larger items.



Totes: Various sizes for various applications to carry gear to and from the field.



Germicidal Wipes: Be sure they have the same compounds as the quaternary disinfectants.



Quaternary Disinfectant: Be sure % by weight numbers match recommended solutions!



Bleach: Effective, but you will use more and it's harsher on some items.



Spray Bottles: Nozzles with an "off" position are more spill proof.



Measuring Tools: For properly diluting disinfectants.



Hand Sanitizer: Pump bottles can be messy, select lidded options.

# WNS Gear Checklist

Note: The list below will allow for manning one (1) each: mist net/trap site, processing station, roost visit station, and decontamination/disinfection station. To add mist net/trap sites, additional gear for that site and the processing station will be required. Consult the checklists in each of those activity descriptions to purchase sufficient additional quantities of gear.

## One-time Purchase Items

- 4 5-gallon, lidded buckets
- 1 electric spin-dryer
- 1 extension cord
- 1 AC/DC adapter
- 1 digital camera
- 1 light box
- 1 quilting ruler grid
- 1 folding aluminum table
- 2 folding camp chairs
- 1 1-cup measuring cup
- 1 1/8-cup measuring cup
- 1 1-tsp measuring syringe
- 3 24-ounce spray bottles
- 1 lidded shoe-box size tote
- 2 lidded foot-bath size totes
- 3 long-handled brushes
- 2 plastic cups
- 24-48 mesh style net bags
- 2 large mesh style delicate laundry bags
- 3 black Sharpie markers
- 1 pair scissors
- 24-48 cloth bat holding bags
- 5 12-15 foot lengths of rope for hang lines
- 1 pair elbow-length rubber gloves
- 2 2-way radios

- 1 12'x12' plastic tarp

## Disposables

- 2 gallons quaternary ammonium solution
- 1 gallon bleach
- 3 canisters sanitizing wipes
- 3 bottles hand sanitizer
- 3 50-ct. boxes latex gloves
- 3 bags 50-ct. paper bags
- 2 boxes gallon size Ziploc bags
- 2 boxes quart size Ziploc bags
- 3 boxes 50-ct. tall kitchen trash bags (white, drawstring variety)
- 50 spring-style wooden or plastic clothespins
- 24 AA batteries
- 2 gallon jugs fresh water

## Optional Items

- 4 5-gallon jugs, for water
- 1 camp stove
- 3-6 canisters stove fuel
- 2 12-quart stock pots
- 1 box "strike anywhere" matches
- 3 pair Tyvek coveralls



# Vendor Resources



Electric Spin Dryer  
by "The Laundry Alternative"

Amazon.com  
\$100



Battery-operated Light Box  
by "Art-o-Graph"

Amazon.com  
\$20



Handy Bag for Mist Nets  
by Bat Conservation & Mgmt

batmanagement.com  
\$6

# Definitions

For purposes of this document, the terms used herein are defined as follows:

**affected** – a location is considered to be “affected” if WNS symptoms were observed and confirmed by toxicology reports; as of May 2010, WNS affected states include: New Hampshire, Vermont, New York, Massachusetts, Connecticut, New Jersey, Pennsylvania, West Virginia, and Virginia.

**air-dry** – placement of items, preferably outdoors and in the sun, where all moisture can be naturally eliminated

**border area** – a location is considered to be a WNS “border area” if it abuts a WNS affected area or is within bat migratory distance of a WNS affected area; as of May 2010 WNS affected areas include: the states of Maine, Rhode Island, Delaware, Maryland, Kentucky, Ohio, Tennessee, Oklahoma, Missouri, and North Carolina in the United States and the provinces of Ontario, Quebec, New Brunswick and Nova Scotia in Canada

**containment** – physical means of preventing accidental release of fungal spores into non-affected areas

**cross-contaminate** – transfer of fungal spores from one object to another, from one object to a person, from a person to a person, from one bat to another, from a bat to an object, or from a bat to a person

**decontamination** – reduction or removal of WNS spores from an item, person, or animal

**disinfection** – use of an anti-microbial agent to decontaminate an item, person, or animal

**fungus** – a multi-cellular, microscopic organism living in a symbiotic relationship with plants, animals, or other fungi; fungus is most noticeable when “fruiting” as microscopic fungal filaments reproduce

**handling** – direct human-bat contact including, but not limited to netting, trapping, removing from nets or traps, and physically manipulating with or without the use of gloves or other barriers

**non-porous** – a material having no void spaces to allow penetration by microbial agents (e.g., metals, glass, stone, hard plastic) pathogen – any organism, especially a microorganism, capable of causing disease

**porous** – a material with varying degrees of void spaces that microbial agents can colonize (e.g., wood, masonry, cardboard, leather, cloth, vinyl, soft plastic)

**rinse** – thoroughly submerge in a fresh water bath, agitating to dislodge embedded matter and/or disinfectant, wringing and repeating in a fresh bath as recommended

**roost** – any natural or man-made location where one or more bats rest during the day or night (e.g., caves, mines, barns, attics, bridges, rock-shelters, trees, cavities, etc.) sanitize – cleaned and/or disinfected to be free of microorganisms spore – single-celled reproductive body of a fungus that can germinate into a new individual user – any person participating in or entering onto a BCI-sponsored event or location

**visit** – physical presence in or near a designated area (i.e., a bat roost)

**wash** – physically remove coarse debris from an item by scrubbing, soaking, rinsing, or mechanically laundering

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