Guidelines for Prevention of Introduction and Spread of Aquatic Threats by Cleaning and Disinfecting Equipment

There are five primary disease/invasive exotic threats to aquatic species within the Arkansas watershed: *Batrachochytrium dendrobatidis*, ranavirus, whirling disease, New Zealand mud snails and Eurasian watermilfoil. While these five are of main concern, it is important to keep in mind that there are numerous possibilities for the introduction of others.

***Batrachochytrium dendrobatidis* (*Bd*) and Ranavirus**

Bd may be responsible for the greatest disease-caused loss of biodiversity in recorded history (Skerratt et al. 2007). Over just the past 30 years, Bd has caused the catastrophic decline or extinction (in many cases within a single year) of at least 200 species of frogs, even in pristine, remote habitats.

Batrachochytrium dendrobatidis (*Bd*) is a species of chytrid fungus that affects amphibians resulting in a disease called chytridiomycosis. Infected individuals suffer damage to their skin which may result in additional infections and impair respiration. This disease if often fatal and can infect populations very rapidly. Chytridiomycosis has been implicated in devastating declines in amphibian populations worldwide and is currently one of the greatest threats to amphibians, second only to habitat destruction.

Ranaviruses may infect insects, fish, amphibians, and turtles. This group includes the largemouth bass virus (LMBV) and *Ambystoma tigrinum* virus (ATV). Ranavirus infection in amphibians typically causes death in larvae or recently metamorphosed individuals and infected individuals often have ulcers on their skin. Turtles typically show signs of skin lesions, respiratory distress, and multiple organ failure.

The transmission of *Bd* and ranavirus is not completely understood but is thought to occur by multiple routes, including contaminated soil, direct contact, waterborne exposure, and ingestion of infected tissues during predation, consumption of carrion or cannibalism. *Bd* and ranavirus are relatively stable in aquatic environments, persisting several weeks or longer outside a host organism.

Bd and ranavirus threaten all amphibians including the northern leopard frog and plains leopard frog which are listed on the Forest Service Region 2 Regional Forester's Sensitive Species List. Also at risk is the boreal toad which is classified as endangered by the State of Colorado and is a candidate species for listing under the Endangered Species Act. These species are declining throughout their range. The primary reason for the decline of the boreal toad is believed to be mortality related to exposure to *Bd*. *Bd* has been confirmed in Chaffee County and the infected populations have suffered severe drops in population.

Whirling Disease (WD)

Whirling disease (WD) is caused by a microscopic parasite that produces a long-lived spore. Young fish are at greatest risk because the parasite attacks their soft cartilage, causing nerve damage, skeletal deformities, and in some cases death.

WD threatens trout populations. Rainbow trout and cutthroat trout appear to be the most susceptible of trout species. Brown trout can become infected with the parasite and may carry the disease, but they are much more resistant to the disease and have not been as greatly impacted as rainbow trout. Greenback cutthroat trout, a federally listed threatened species, is susceptible. In studies at the USFWS National Fish Health Research Laboratory, it was found that even a light exposure to WD resulted in mortality of over 25% of greenback cutthroat trout <1 year old.

Tubifex worms, which inhabit the sediments of lakes and rivers, serve as hosts for the WD parasite. These worms can live up to 14 days in dry mud on equipment and up to 70 days if wetted periodically.

WD has been confirmed in several low elevation waters. See attached map for streams known to be WD positive.

New Zealand Mud Snail (NZMS)

New Zealand mud snails (NZMS) compete with native species, including native mollusks and fish, for space and food resources. Because of their prolific nature, mud snails can occupy up to 80% of invertebrate biomass and can consume more than 75% of the gross primary production in the stream. Adverse impacts to lower levels of the food web may have implications for organisms at higher levels, such as fish, which rely on lower-level organisms as a food source. Mud snails may reduce the availability of native invertebrate prey for fish such as trout and sculpin, and at the same time, are not a viable food source themselves! Their hard shell and resistance to digestion allow them to pass through a fish gut unscathed, thus lending no nutrition or calorie input to the fish.

The invasive snails are nearly impossible to contain once they have invaded an aquatic ecosystem. For instance, they are so small (only up to 6 mm in length) that they cannot be skimmed from waters. Highly resilient, the snails can survive several days out of water and can withstand a wide range of temperatures. Additionally, because they are self-reproducing “livebearers” that give birth to well-developed clones, it only takes one NZMS to start a new colony in a stream or river.

NZMS were recently discovered in the South Platte River in Elevenmile Canyon, below Elevenmile Reservoir Dam. They were first reported in Colorado in 2005 within Boulder Creek. They are also known to be present in the Green River in Utah, and throughout Yellowstone National Park.

Eurasian Watermilfoil

Eurasian watermilfoil is an attractive plant with feathery underwater foliage that is native to Northern Europe and Asia. The plant is typically submersed with stems to 4 m long, becoming emerged only while flowering.

Eurasian watermilfoil spreads most commonly by stem fragmentation and runners.

Eurasian watermilfoil starts spring growth before other native aquatic plants making it very invasive.

The plant forms very dense mats of vegetation on the surface of the water that interferes with power generation and irrigation by clogging water intakes. These mats also interfere with recreational activities (e.g. swimming, fishing, skiing, boating, etc.) and creates a mosquito habitat and reduces native vegetation.

Humans unwittingly assist the spread of these organisms by transferring them from one body of water to another on footwear, waders, nets, bicycles, motorbikes, ATV's and other equipment that comes in contact with infected waters. It is expected that forest staff, cooperators, permitted outfitters and guides, special event participants, and researchers working on approved projects will follow the guidelines below. Recreationalists (including forest staff on their days off) are strongly encouraged to follow these guidelines based on recommendations provided by Colorado Parks and Wildlife in 2015.

Best Practices

1. Dedicate specific equipment to each separate site (lake/stream). This may be possible for guides or researchers who only work at one site. If equipment is specific to a site and has not been used elsewhere, guidelines under number 4 do not need to be followed. If equipment will be used at only one site this year but has previously been used in other areas, follow the guidelines under number 4 to disinfect equipment prior to beginning work this season.
2. Do not use felt wading soles.
3. When conducting surveys within a drainage, start at the top of the drainage and work downstream. Aquatic threats are typically located in lower elevation areas and this will minimize the risk of spreading organisms upstream.
4. **Prior** to working in waters clean and disinfect any equipment that has been in contact with soil and water. Examples include boots, waders, nets, bikes, ATV's, trailers etc. Equipment **also** needs to be cleaned and disinfected in the following situations: a) after a visit to any area where whirling disease, *B. dendrobatidis*, or New Zealand mudsnails are known to exist; b) when moving within a drainage to sites that are >4 kilometers (2.5 miles) apart; and c) before visiting different drainages.

Disinfection Protocol

Step 1 (Required): Prior to disinfection, clean debris, mud, and vegetation off of equipment and waders. Muddy disinfectant solution can lose its effectiveness and capacity to kill invasive organisms.

Step 2 (a <u>or</u> b Required):

a. Wash all equipment and tools used to clean equipment with bleach solution. Soak equipment for 10 minutes in a 10% household bleach solution (5,000 ppm hypochlorite) **or** when sensitive equipment cannot be soaked disinfect equipment with a ≥50% contact bleach solution spray.

<u>And</u>

Freeze gear overnight **or** soak equipment for >one minute in >140˚F water (dishwasher cycles will provide adequate heat).

***NOTE* Bleach has not been found to be effective at killing New Zealand mud snails. Completing both the bleach soak AND freezing or heat treatment are necessary.**

b. Wash all equipment and tools used to clean equipment with a Quaternary ammonium compound (QAC) (e.g. Super HDQ, Superquat 256, Quat4, GS 256, Vedco 128 or Quat 128). Soak equipment for 10 minutes in a 6 ounces per gallon dilution; if equipment cannot be soaked it can be sprayed with a dilution of 12 ounces per gallon and must remain wet for the entire 10 minutes.

Step 3 (Recommended): Allow equipment to air dry, preferably in direct sunlight >84˚F for >four hours.

If you believe that you are working with equipment that cannot sustain the rigors of the treatments above, contact aquatic biologist Janelle Valladares at (719)-269-8704.

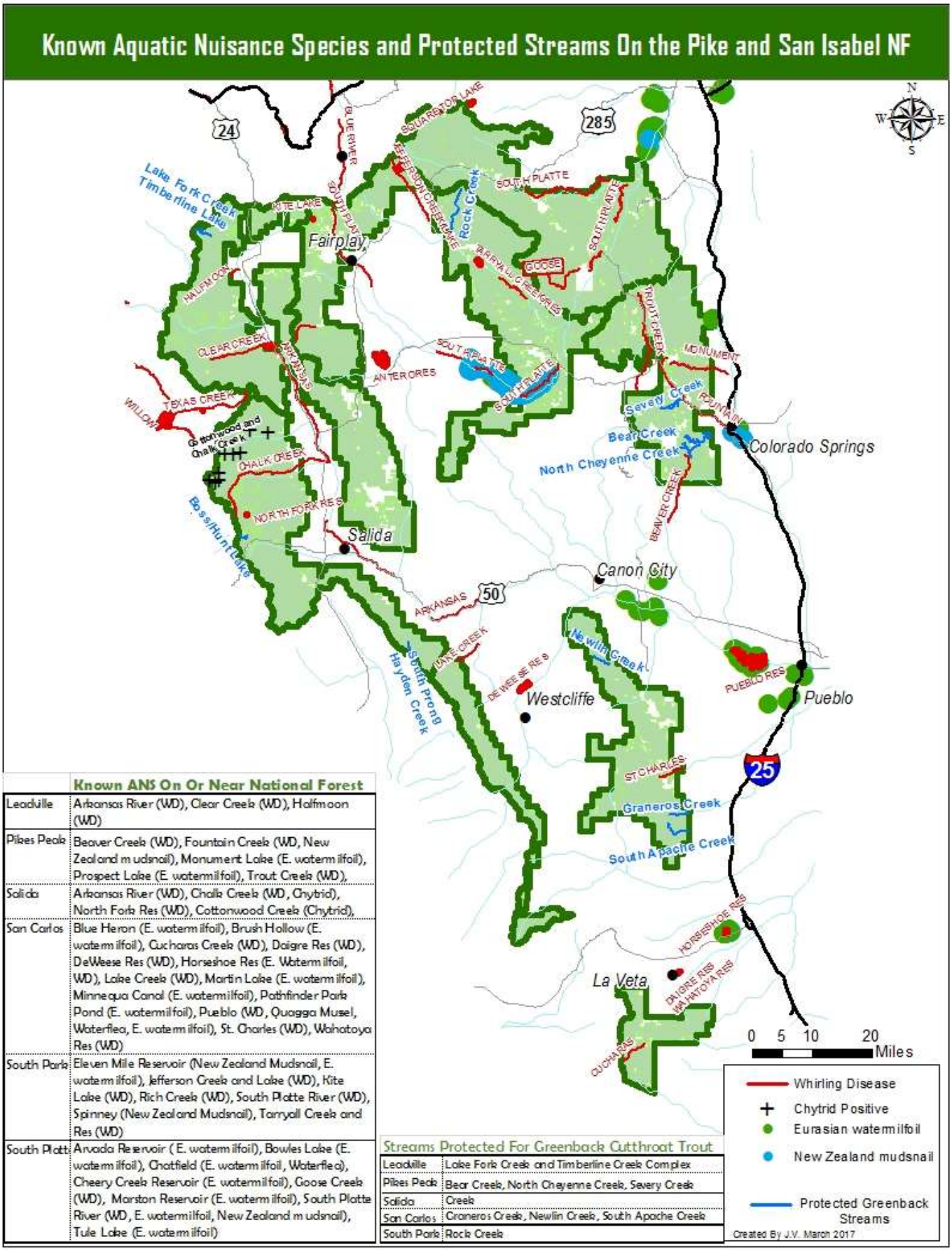
<u>Disposal</u>

Wastewater treatment plants are capable of processing water containing small amounts of QACs in solution. Therefore, rinsing used solutions of QACs down a sanitary sewer is a safe method of disposal. However, QACs should be kept out of storm sewers and other waterways. Always dilute old product before rinsing down sanitary sewers directly from the container, and follow MSDS and label recommendations regarding rinsing and disposal of empty containers. QACs become tightly bound to organic matter in soils and sediments (Owens et al. 2000) and are degraded by aerobic bacteria (Tezel 2009). Therefore, small amounts of QAC from spray or bath disinfection may come in contact with the environment with few negative effects. However, it is not recommended to dump large amounts of QAC solutions directly on the ground.

<u>Disinfection Chemical Suppliers</u>

Waxie Sanitary Supply – In Colorado Springs (719-632-7827) and Denver
Super HDQ - Gallon $34.50; Case of 4 Gallons $119.99 (as of 11/15)

Known Aquatic Nuisance Species and Protected Streams On the Pike and San Isabel NF



	Known ANS On Or Near National Forest
Leadville	Arkansas River (WD), Clear Creek (WD), Halfmoon (WD)
Pikes Peak	Beaver Creek (WD), Fountain Creek (WD, New Zealand mudsnail), Monument Lake (E. watermilfoil), Prospect Lake (E. watermilfoil), Trout Creek (WD),
Salida	Arkansas River (WD), Chalk Creek (WD, Chytrid), North Fork Res (WD), Cottonwood Creek (Chytrid),
San Carlos	Blue Heron (E. watermilfoil), Brush Hollow (E. watermilfoil), Cucharas Creek (WD), Daigre Res (WD), DeWeese Res (WD), Horseshoe Res (E. Watermilfoil, WD), Lake Creek (WD), Martin Lake (E. watermilfoil), Minnequa Canal (E. watermilfoil), Pathfinder Park Pond (E. watermilfoil), Pueblo (WD, Quagga Musel, Waterflea, E. watermilfoil), St. Charles (WD), Wahatoya Res (WD)
South Park	Eleven Mile Reservoir (New Zealand Mudsnail, E. watermilfoil), Jefferson Creek and Lake (WD), Kite Lake (WD), Rich Creek (WD), South Platte River (WD), Spinney (New Zealand Mudsnail), Tarryall Creek and Res (WD)
South Platt	Arvada Reservoir (E. watermilfoil), Bowles Lake (E. watermilfoil), Chatfield (E. watermilfoil, Waterflea), Cheery Creek Reservoir (E. watermilfoil), Goose Creek (WD), Marston Reservoir (E. watermilfoil), South Platte River (WD, E. watermilfoil, New Zealand mudsnail), Tule Lake (E. watermilfoil)

Streams Protected For Greenback Cutthroat Trout	
Leadville	Lake Fork Creek and Timberline Creek Complex
Pikes Peak	Bear Creek, North Cheyenne Creek, Severy Creek
Salida	Creek
San Carlos	Craneros Creek, Newlin Creek, South Apache Creek
South Park	Rock Creek