AMPHIBIAN AND REPTILE DISEASES

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Minimizing the Spread of Herpetofaunal Pathogens in Aquatic Habitats by Decontaminating Construction Equipment

Some problems have relatively simple solutions compared to the cost of neglect. Preventing the spread of invasive species and harmful pathogens clinging to construction equipment is one such solution. Here we explain how resource managers and contractors can decontaminate construction and field equipment by cleaning, disinfecting, and drying, thus minimizing the spread of harmful organisms.

Management of aquatic and wetland-dependent invasive species including pathogens associated with emerging infectious diseases can be a prohibitive expense for natural resource agencies, non-government organizations, contractors, corporations, and private citizens (Horan and Wolf 2005). Preventing the spread of invasive pathogens may not only be a cost-effective way to control their impacts (Leung et al. 2005; Rothlisberger et al. 2010) but it may be the only successful method to preclude establishment in a new area because emerging diseases have rarely been eradicated (Wobeser 2002; Friend 2014; Cunningham et al. 2017). Current preventative measures include: directing boaters to clean, rinse, and dry their hulls of any plant or animal hitchhikers prior to entering non-contaminated waters (Rothlisberger et al. 2010; Bruckerhoff et al. 2015); assigning equipment to categories of work sites (e.g., clean versus contaminated) to minimize cross-contamination; and using disinfectants or mechanical measures to decontaminate equipment, machinery, and work sites (Bruins and Dyer 1995). Within the fish, livestock, and avian food-production industries and within commercial pet and wildlife markets, biosecurity protocols are vital to maintaining healthy populations of both the animals and humans involved.

Here we focus on aquatic invasive pathogens of amphibians and reptiles because the importance of biosecurity protocols to combat infectious disease is a developing priority for herpetofauna (Cheatwood et al. 2003; Green et al. 2009; Marschang 2015; Lorch et al. 2016; Gray et al. 2017, 2018). Numerous pathogens are known to infect or be carried by amphibians and reptiles. Some overlap both classes, some are shared with fishes, and others are species- or genus-specific. More are currently unknown but undoubtedly will be identified in the future because of extreme and long-term climatic events and increases in human–wildlife encounters, invasive species, and urbanization pressures. Three emerging infectious pathogens in amphibians and reptiles include the two aquatic fungal species associated with amphibian chytridiomycosis, *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*), and several species of iridovirus in the genus *Ranavirus* (Rv) that can infect amphibians, reptiles, and fishes. *Bd* is one of the more ubiquitous of these pathogens, infecting over 500 species of amphibians in more than 50 countries, and has been implicated in the decline of at least 501 species (Scheele et al. 2019; Olson et al. 2013). The more recent discovery of *Bsal* (Martel et al. 2013, 2014) has conservationists alarmed due to high mortality rates among naïve species, prompting the U.S. Fish and Wildlife Service to ban the import of 20 genera of salamanders into the United States (USFWS 2016) and Environment and Climate Change Canada to ban all salamander imports to Canada (ECCC 2017). A variety of Rv species have been identified as infecting over 100 species of amphibians across 25 countries and 28 turtle, lizard, and snake species (Duffus et al. 2015). Whereas several lineages of Rv have been observed in amphibians and reptiles, Frog-virus 3 (FV3) is the lineage responsible for the majority of Rv-induced mass mortality events in North America (Price et

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Fig. 1. Equipment and materials used near wetland habitats. A) Installing concrete-coated pipe through a wetland habitat. B) Sediment, plant material, and other debris stuck to the tracks of an excavator.

al. 2017). Because fishes can be susceptible to Rv (41 species of fish over 22 families: Duffus et al 2015), methods proposed here for minimizing potential Rv transmission to fishes may be applicable. While the references related to fishes in this manuscript are not comprehensive, proposed methods could cover additional pathogens (potentially hundreds) that affect fishes if they incorporated disinfectants with an active ingredient (e.g., potassium peroxymonosulfate) that is effective against a broad spectrum of fish pathogens.

Biosecurity protocols have focused on minimizing the transmission of these pathogens by using prophylactic measures (disposable gloves or coverings) and decontaminating personal clothing and sampling gear (Green et al. 2009; Phillott et al. 2010; NEPARC 2014; Gray et al. 2017). Less attention has been given to field vehicles and heavy equipment used in and around habitats that may harbor disease-causing pathogens, including bodies of water where chytridiomycosis or Ranavirus disease may emerge. Construction efforts associated with wetland habitat restoration, dam-building or removal, forestry practices, and energy development (e.g., oil and gas pipelines and well sites, access roadways) result in the use of heavy equipment in and adjacent to wetland habitats (Fig. 1A). The mud, organic debris, and water that collect on the undercarriage, wheels, and tracks of this equipment contain biologic material that may be transported between work sites, thereby potentially introducing pathogens and invasive species into new areas (Fig. 1B). These pathogens remain in the environment (Nazir et al. 2012; Anderson et al. 2014) and can be translocated through the same methods that invasive animals and plants are transplanted overland from one habitat to the next (Johnson and Speare 2003; Anderson et al. 2014; Bruckerhoff et al. 2015).

Organizations that have established decontamination (or "clean-down") protocols for field vehicles, construction equipment, and watercraft have focused on reducing the spread of visible invasive plants and aquatic organisms (Appendix 1). Herein we suggest how and when to modify the foundational protocols of *cleaning*, *disinfection*, and *drying* to address the inadvertent movement of amphibian and reptile pathogens between habitats by construction equipment. We present levels of decontamination for heavy equipment based on the risk of moving pathogens into naïve populations. We provide a decision tree to determine the minimum recommended level of decontamination for heavy equipment and machinery (Fig. 2) prior to starting a project.

These guidelines will provide natural resource managers, their employees, contractors, and partner organizations with biologically secure, feasible options for operating equipment in or near aquatic habitats. While the cleaning, disinfecting, and drying practices can be applied to biosecurity protocols for pathogens with stronger ties to terrestrial habitats and host species, updates on guidance for new or more terrestrially linked pathogens are available through organizations, such as Partners in Amphibian and Reptile Conservation (see parcplace.org/resources/herpetofaunal-disease-resources/). The biosecurity protocols and decontamination priorities described herein can be incorporated by regulatory agencies into the permitting, review, or consultation processes as recommendations or requirements for projects, depending on the level of risk and species involved. Resource managers wanting to develop a standardized, detailed decontamination protocol can review the Hazard Analysis and Critical Control Point Plan (or HACCP; ASTM 2009); some concepts from that Plan are incorporated here. Many agencies including state, Federal, and non-government organizations, use the USFWS HACCP template to identify activities that are likely to transfer aquatic invasive species and to address control measures to minimize risks of spread.

Cleaning

Cleaning is the process of physically removing foreign material adhering to equipment (Table 1). Cleaning within the work site or at a wash-down area nearby helps prevent soil and wash-water from entering pathogen-free habitats. An area twice as large as the largest piece of equipment should provide sufficient space to maneuver equipment around foreign material dislodged during a cleaning; directional cleaning or pre-planned loading of clean equipment onto clean towing trailers can reduce recontamination. The careful removal of soil at the work site minimizes the likelihood of vehicles dropping foreign material while in transit to lots or yards. Alternatively, moving vehicles to paved lots and equipment yards for washing is acceptable if these locations keep wash-water and dislodged debris out of storm sewers, aquatic habitats, wetlands, or waterways. On-site cleaning also could be a preparatory procedure before moving vehicles to an offsite area for more attention. Additionally, a commercial carwash is an option for cleaning light debris from smaller automotive vehicles.

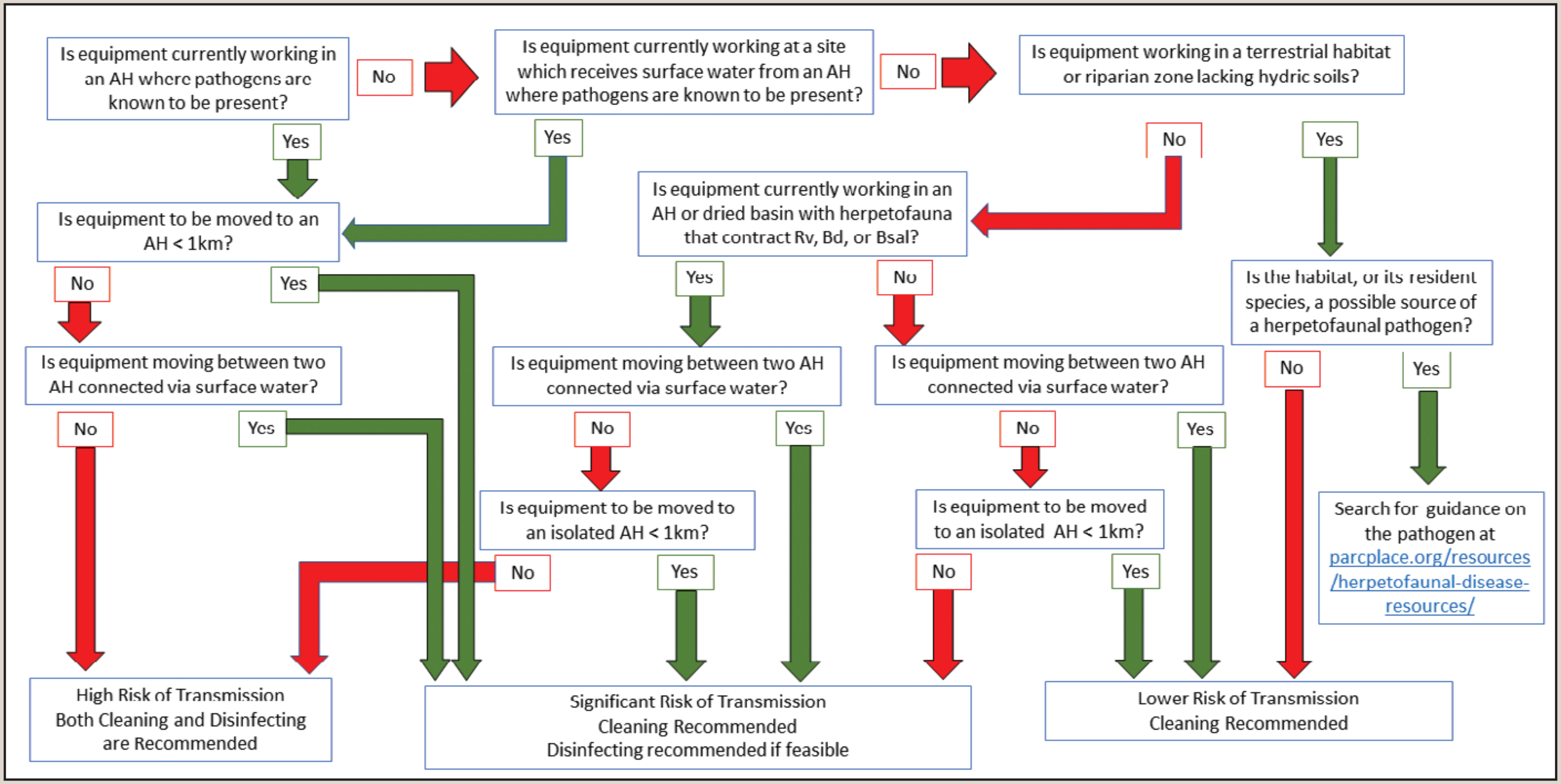

Fig. 2. Decision tree to determine the appropriate level of ranavirus and chytrid decontamination for heavy equipment and machinery (AH = aquatic habitat). The 1-km AH distance guidance is suggested for practical application. When rare, threatened, or endangered species are present, consider enhancing the level of decontamination determined by this figure.

Table 1. Decontamination terms with examples or definitions.

Term	Examples or definitions
Accessories or attachments	Mudguards, chains, augers, "brush hog" mowers, trailers
Aquatic habitats	Wetlands, marshes, rivers, streams, ponds, springs
Cleaning	Physically removing foreign material
Disinfecting	Applying a treatment (e.g., chemicals, steam, heat) to kill infectious microorganisms
Field vehicles	Cars, trucks, ATVs, watercraft
Foreign material or debris	Soil, water, seeds, plants (including parts), leaf litter
Heavy equipment	Backhoes, bulldozers, excavators, skidders
Personal clothing	Boots, waders, gloves
Sampling gear	Nets, buckets, containers

However, most carwashes do not allow this level of cleaning because they are not equipped to handle the quantity of debris being removed. We caution against cleaning equipment in upland terrestrial habitats that are far from the work site because that could expose terrestrial amphibians and reptiles to contaminated sediments or indirectly introduce pathogens into wetlands by infecting individuals that migrate through those areas.

Before cleaning, detach trailered equipment and mounted accessories (Table 1) to allow access to as much surface area on the heavy equipment as possible. Then physically remove all foreign material, starting with the exterior, progressing from the roof to wheels (or tracks) and undercarriage. Shovels, prybars, and brooms are useful for removing large clumps of sediment (Fig. 3A). Clean vehicle interiors last after removing protective seat covers and floor mats.

This cleaning can be followed by high-pressure air or water hoses and brushes to power-wash exteriors (Fig. 3B). Setting water pressure to 620 kPa (90 lbs/inch^2) or higher should remove sediment from heavy equipment, whereas pressures of up to 20,684 kPa (3,000 lbs/inch^2) have been deemed necessary to remove attached organisms, such as invasive mussel species (DiVittorio et al. 2012). The goal of this level of cleaning is to remove any mud and debris onto which invasive organisms and pathogens (e.g., weeds and their seeds, invertebrates, and microorganisms) may bind. Some chemicals, such as bleach, are ineffective at disinfecting if organics (such as caked-on soil or plants) are not removed. Water temperatures that exceed 40°C (104°F) used to treat watercraft for aquatic invasives are hot enough to deactivate both *Bd* and Rv (Johnson and Speare 2003; USFS 2005; Nazir et al. 2012). These temperatures are also achieved at commercial truck washes intended to prevent transmission across livestock and poultry farms (Thompson 2001). Several sources offer useful diagrams for identifying difficult to clean spots located on heavy equipment (https://www.ontarioinvasiveplants.ca/wp-content/uploads/2016/07/Clean-Equipment-Protocol_June2016_D3_WEB-1.pdf, accessed 14 January 2019).

Fig. 3. Cleaning debris from heavy equipment. A) Debris removed from the treads and undercarriage of track-driven equipment. B) Removing debris with pressurized air using an "air spade."

Disinfecting

In addition to cleaning to remove the foreign material and debris that may contain pathogens, some situations may warrant disinfecting equipment to further protect against the spread of infectious pathogens. Disinfection is the process that treats surfaces to reduce infectious microorganisms through the application of chemicals, secondary compounds, high heat, or a combination of these measures (USDA 2015; Gray et al. 2017). Regulations governing chemical use may vary by city, county, state, or federal jurisdiction, hence warranting consideration in case restrictions or permits apply.

Chemicals are the most commonly recommended disinfection agents used in amphibian and reptile biosecurity protocols, possibly because they are readily available through commercial vendors and do not rely on specialized equipment required by secondary compounds or high heat. Chemical efficacy and concentrations for inactivating herpetofaunal pathogens is reviewed in Gray et al. (2017), and we present chemicals with cost (in its prepared/working solution), availability, and properties that are conducive to the disinfection of construction equipment (Table 2). Chemical selection based on environmental safety (Bruins and Dyer 1995) can be quickly evaluated by checking the Ecological Information (ecotoxicity

Table 2. Disinfectants for use on heavy equipment to minimize the spread of aquatic amphibian and reptile pathogens. AI = Active Ingredient.

	Disinfectant Sodium hypochlorite	Potassium peroxymonosulfate (KPMS)
Brand Name (example)	Clorox Bleach®	Virkon® Aquatic
Concentration of AI	5.25%	20.4%
Cost (as prepared)	US $0.13/gal; US $0.03/L	US $1.22/gal; US $0.32/L
Contact time	5 min	5 min
Effective dilution rate	5%	1% solution
Pros	Readily available	EPA approved and low to no toxicity to aquatic organisms
	Effective fungicide and antibacterial agent	Rapidly broken down in the environment
	Effective disinfection for supplies and personal gear if rinsed thoroughly with water	Safe on fabric
		Acts on a wide range of pathogens
Cons	Toxic to all aquatic organisms	Significant expense
	Corrosive to steel, aluminum, chipped enamel	Irritating to respiratory system and skin; may cause serious eye damage
	Long term use breaks down plastic, vinyl, rubber	May cause pitting on galvanized or metal if not rinsed with water
Shelf life	Concentrated: 3–5 months for max effectiveness; discard at 1 year.	Concentrated tablets: 2 years; powder: 3 years.
	Diluted: can last up to 1 month in opaque container; rapidly inactivated by light in 5 days.	Diluted: can last 7 days when exposed to sunlight and the open air, but longer if sealed in opaque container.

hazards) section 12 on the manufacturer's Safety Data Sheet (SDS), available online. To protect nontarget aquatic life, apply disinfectants at least 50-m away from aquatic habitats (NEPARC 2014). Cleaning crews should follow safety guidelines, such as the use of personal protective equipment, as well as manufacturers' recommended concentrations, application conditions, and contact times for maximum effectiveness (Table 2).

To disinfect emergent pathogens, a 1% potassium peroxymonosulfate (KPMS; commercially available formulations as Virkon® Aquatic, Oxone™) with a 5-minute contact time will deactivate Rv and *Bd* (Gray et al. 2017), although contact times as short as 1-minute can be effective against Rv (Bryan et al. 2009) and *Bd* (Johnson et al. 2003). KPMS products that contain no dyes nor perfumes and formulated to degrade quickly in the aquatic environment, such as those recommended for aquacultures, exert little or no effect on amphibians (Schmidt et al. 2009; Hangartner and Laurila 2012). There are little or no data available for effects on reptiles (Gray et al. 2017). Concentrated granulated formulation can have a shelf-life that exceeds 24–36 months however, once diluted to 1%, the product may break down in a week when open to the air and exposed to sunlight. As with most oxidizing agents KPMS is effective longer if tightly sealed and minimally exposed to sunlight and certain organic compounds (https://syndel.com/wp-content/uploads/2019/01/new-virkon_aquatic_general_instructions-Combined.pdf; accessed 28 April 2020). Products containing chlorhexidine as the active ingredient (e.g., Virisan™ and Nolvasan®) have a long history of use in veterinary practices and are effective at deactivating Rv (Bryan et al. 2009), however, chlorhexidine can be toxic to aquatic life "with long-lasting effects"(GHS code H410-H411; https://pubchem.ncbi.nlm.nih.gov/compound/Chlorhexidine-diacetate#section=Safety-and-Hazards; accessed 13 January 2020).

Bleach is widely used for disinfecting, with a recommended concentration of 1,750 ppm sodium hypochlorite (NaClO) and a contact time of 5 minutes (NEPARC 2014). This concentration is equivalent to a 3% solution (i.e., 1-part bleach to 32-parts water) of a bleach formulation with 6% NaClO. Bleach is readily available in retail stores with formulations of 5% to 8% NaClO, or a commercial aquaculture formulation of 12% NaClO. We provide dilution curves for several bleach formulations in Fig. 4, and chlorine dilution calculators are also available online to ensure that solutions are prepared with an effective concentration of NaClO (https://www.publichealthontario.ca/en/health-topics/environmental-occupational-health/water-quality/chlorine-dilution-calculator; accessed 13 April 2020). All formulations must be diluted with water before being applied to increase the toxicity to microbes. An open bottle of bleach concentrate, even when kept tightly sealed and away from sunlight, is effective only for 30 days; once diluted, it is effective only for 5 days. Bleach is less desirable than other disinfectants because the volatile nature of its active ingredient makes it difficult to maintain consistent concentrations. Additionally, long-term use can corrode metals, plastics, and equipment, and it is environmentally harmful if applied within 50 m of wetlands due to its toxicity to nontarget aquatic organisms (Schmidt et al. 2009). Limiting bleach to the

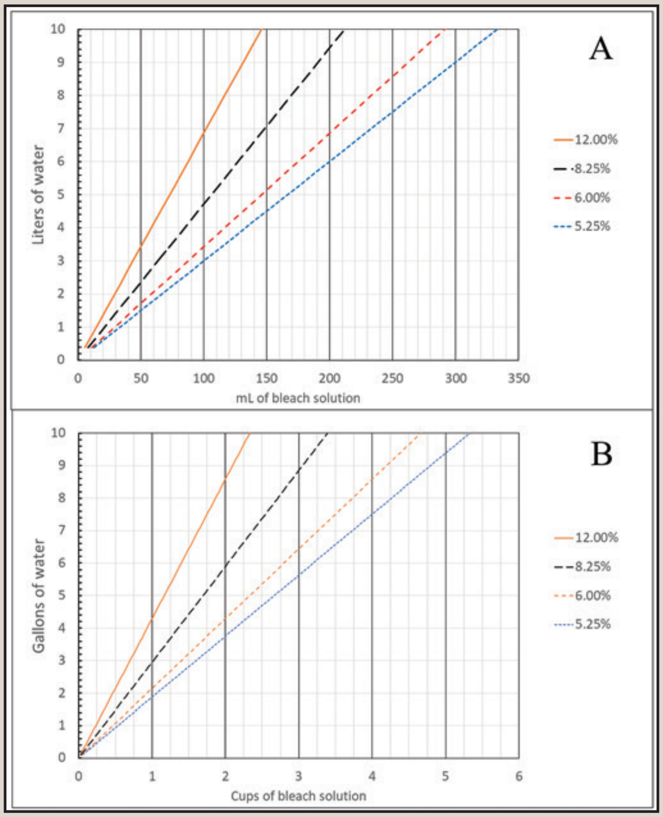

Fig. 4. Dilution curves in A) metric and B) standard English units for volumes of bleach solution (X-axis) added to volumes of water (Y-axis) to achieve a 1750 ppm concentration of hypochlorite for bleach formulations with different active ingredient (AI) concentrations. Solid line: 12.00% AI; Long dashed line: 8.25% AI; Short dashed line: 6.00% AI; Dotted line: 5.25% AI.

disinfection of personal equipment can ensure that only small amounts of this caustic disinfectant enter the environment. There may be restrictions on the quantity of bleach entering local sewer systems per time period, hence adherence to local bleach disposal guidance is needed if it is done in an area where wastewater is linked to sewage systems. Note, SDS are available from product manufacturers and should be consulted prior to being applied to ensure proper handling and safe disposal.

Drying

Equipment can be air-dried for 24 h after being cleaned; drying without disinfecting does not deactivate pathogens as effectively. Nazir et al. (2012) found that dried surfaces inoculated with *Ranavirus* spp. took 9–11 days to lose 90% of its transmissibility (T90) at ambient temperatures, while T90 in moist sediments took 13–22 days at 20°C and 30–48 days at 4°C. Brunner et al. (2007) found that pond sediments inoculated with Rv were no longer able to transmit the virus after they were dried completely at 20°C over a four-day period. Given the ability for Rv to remain transmissible in moist sediment, it is essential to ensure that foreign material is removed from each vehicle's undercarriage where it is unlikely to be exposed to sunlight or drying. Standard drying procedure for boating equipment is 5 days in warm, dry weather after a thorough cleaning to prevent the transmission of invasive plants and animals (USFS 2005; NOAA 2018). Having heavy construction equipment idle for more than one day can put contractors behind schedule and significantly increase project costs. Given the large range of costs associated with equipment use relative to disinfectants, the contractor may need to consider whether the chemical disinfectants (followed by a shortened drying time of 24 h) are more cost effective than cleaning and leaving the equipment to dry over multiple days.

Decontaminating Priorities

It is always important to clean equipment before moving it to an aquatic habitat that may harbor amphibians or reptiles, and the need for disinfecting increases when that equipment has been recently exposed to areas with pathogens, recently exposed to species known to harbor pathogens, or is to be moved to a distant or hydrologically isolated aquatic habitat (Fig. 2). A thorough decontamination (cleaning, disinfecting, and drying) also may be considered when equipment may have been exposed to a pathogen and is expected to be used next within an aquatic habitat that is unlikely to have that pathogen. These considerations are addressed in Haman et al.'s (submitted) risk assessment to determine the need for enhanced biosecurity measures for herpetofaunal pathogens. Haman et al. also weigh the importance of enhancing biosecurity measures when working at sites that harbor rare, threatened, or endangered species. As we indicate in Fig. 2, resource managers should also consider enhancing the level of decontamination in situations when equipment is moved into habitats that contain rare, threatened, or endangered species.

The presence of pathogens at an aquatic habitat can be confirmed by having either live animals or water samples tested with molecular techniques. Laboratories can analyze tissue residues or swab samples from live animals (Gray et al. 2017), whereas water samples can be tested for the presence of environmental DNA of pathogens (Kirshtein et al. 2007; Hall et al. 2016). If testing is not feasible, a natural resource professional with expertise in herpetology can help assess the risk of pathogen exposure by using on-line resources that map the occurrence of herpetofaunal pathogens, such as The Amphibian Disease Portal (https://amphibiandisease.org/; accessed 13 April 2020) for *Bd* and *Bsal*, and the Global Ranavirus Reporting System that is maintained by The Global Ranavirus Consortium (https://www.ranavirus.org/) for Rv. An experienced herpetologist can cross-reference online herpetological atlases with the databases of pathogen mapping sites to help determine whether species within a specific aquatic habitat have the potential to harbor pathogens. If multiple species are observed at the site but no information is available on whether pathogens are present, it may be safer to disinfect equipment as a precaution; pathogen occurrence, prevalence, and load have been positively associated with amphibian species richness (*Bd*: Olson et al. 2013; Hydeman et al. 2017; Rv: Tornabene et al. 2018).

Disinfecting may be of lesser priority if equipment is moved between two nearby sites that are known to contain the same pathogen species. As a practical assessment, this is more likely when habitats are connected by channelized surface water or are close enough to each other that amphibians may commonly move between them. In these situations (connected wetlands, or those < 1 km apart), cleaning and drying (without disinfection) could suffice for decontamination protocols (Fig. 2). Investigators have considered less aggressive decontamination protocols when equipment is moved between wetlands within the same watershed (watershed delineations, Phillot et al. 2010; Fig. S5 in Olson et al. 2013). Tornabene et al. (2018) found a wetland had a lower probability of being infected with Rv the farther it was from another wetland that contained Rv, but the distance needed to exceed 2 km to reduce this probability by 20%. The added measure of disinfection could be reserved for movements between watersheds (Olson et al. 2013), or movements between hydrologically isolated wetlands that exceed ~1 km. For heightened vigilance, distances greater than ~350 m can serve as a criterion to initiate disinfection protocols (Gray et al. 2017), but this distance is based on home ranges of amphibian species that conduct annual breeding migrations. These distances (distinct watersheds, 1 km, and 350 m) are offered as practical operational guidelines, but at this time, do not have specific support relative to pathogen transmission.

Cleaning and drying without disinfecting may be sufficient for equipment used in riparian zones lacking hydric (wetland) soils (Fig. 2) and in wetlands that have amphibian and reptile species not known to carry pathogens (refer to https://amphibiandisease.org/ for *Bd* and *Bsal*; www.ranavirus.org/ for Rv). Equipment operating in these habitats would have a reduced (but not minimal) risk of encountering pathogens. It would be prudent to decontaminate equipment that operates in the dried basins of seasonal wetlands (e.g., vernal and autumnal pools, playa wetlands) with disinfection protocols until we have a better understanding of the role that pathogens in dried pool basins play in infection dynamics. The basins of these pools may harbor infectious Rv because mass mortality events are often reported in these communities (Harp and Petranka 2006; Brunner et al. 2011; Hoverman et al 2011). Therefore, when in doubt as to the habitat or species at risk, it is best to plan for both cleaning and disinfecting protocols (see Scheele et al. 2017).

Heavy equipment that has been used in terrestrial habitat like forested uplands should still be brushed and hosed off before it is redeployed. Some terrestrial-breeding species of salamanders can carry *Bd* and Rv (Hamed et al. 2013), and terrestrial habitats can serve as hibernacula for pond-breeding amphibians and reptiles. Although *Bd* has not been shown to be viable in the soil within their hibernacula, some anurans have been found to harbor *Bd* on their skin while in their hibernacula. Unlike *Bd*, *Bsal* has an encysted spore life stage that can adhere to inert surfaces, potentially promoting transmission (Stegen et al. 2017), increasing the need for considering cleaning and disinfecting equipment used near water bodies. *Ophidiomyces*, a fungus related to snake fungal disease, can be found in the soil as well (Allender et al. 2015). If there is reasonable suspicion a terrestrial habitat either contains a herpetofaunal pathogen or a species that is highly likely to contract one, land managers should search for guidance on appropriate biosecurity measures that need to be taken. A current list of resources on herpetofaunal diseases can be found at webpages managed by Partners in Amphibian and Reptile Conservation at https://parcplace.org/resources/herpetofaunal-disease-resources/.

Factors Affecting Preventative Measures

A contractor's ability to follow preventative measures will vary depending on the scope and duration of the project, the project management, the level of regulatory oversight (permitting) of a project, and the availability of information on decontamination. Natural resource organizations are expected to have the greatest control over accidental transmission of pathogens when a project involves only a single habitat or job site under their supervision, such as stream or wetland restorations and stream bank erosion controls. Ideally, these stakeholders would maintain unimpeded, transparent lines of communication with their contractors regarding considerations including, but not limited to:

1) Stating the importance of decontaminating early in the planning or request for proposal process. The applicable time, materials, equipment, and costs need to be clearly addressed in the bidding process and during all pre-construction communications and meetings. Discussing the topic on-site at a construction kick-off meeting will be too late in the process to implement many preventative measures.
2) Confirming whether the contractor is working on other projects in the area and if equipment might be shuttled between different projects.
3) Bringing equipment on-site only after it has been cleaned, disinfected, and dried at a level appropriate to the risk as identified above.
4) Introducing equipment to a site only once, if possible, by leaving it on-site until all work is completed.
5) Cleaning and pressure-washing equipment prior to leaving the site or planning to clean it at a designated facility or lot.
6) Limiting access to the job site through approved roads and points of entry for all personnel, including inspectors, to minimize the potential for pathogen transmission.

Preventative measures can become increasingly difficult to control as the scale of the project increases. Larger-scale projects, such as infrastructure repair, urban development, commercial development, commercial solar farms, mining, and resort construction, may include multiple job sites within the same watershed, land management area, or region. The largest projects may include numerous work sites within multiple watersheds, counties, ecoregions, or states. Examples of such projects include public utilities with transmission lines and natural gas pipelines, transportation infrastructures, and wind farms. Due to the linear nature of many of these projects, a single piece of equipment is often used at multiple sites in the same week or even on the same day. In these cases, equipment has the potential to carry pathogens between sites. However, in some of these situations, it may not be feasible to decontaminate equipment when moving between job sites on the project. For example, there may be limited access to the equipment and water for pressure washing once it has been transported to the site.

Resource managers working with permitting agencies ideally can address the wetland pathogen and invasive species concerns for large projects during the early planning stage. Landowner easements, approved access roads, species-specific timing restrictions, and erosion control measures are among the items that are considered in the planning process. With guidelines minimizing the risk of pathogen transmission and equipment decontamination added to the project plans at this time, contractors bidding on a project will know what is expected. Stakeholders can also work with contractors to implement the above bulleted considerations whenever feasible.

Fig. 5. Materials used within and near wetland habitats. A) A long series of construction mats used to drive equipment on. B) Wooden timbers laid down to build a construction mat. C) Stone and rip-rap used in a wetland crossing.

Regardless of the scale of the project, the following preventative measures can help minimize the risk of transmitting pathogens. These measures address the types of equipment used at construction sites and considerations for the decontamination of each.

Drivable Equipment.—Construction activities involve the use of a wide variety of drivable equipment ranging from such large steel track machines as excavators and bulldozers, to wheeled machines like log skidders, loaders, dump trucks, and pickups. Disinfecting such machinery can present a challenge and may need to be limited to careful cleaning techniques between wetland sites. Linear utility construction may present a greater potential for the spread of pathogens than some other types of construction sites due to the distances covered involving multiple, non-contiguous wetlands. Debris on track machines collects between the rollers, idlers, sprocket and tracks. Larger sediment pieces can be removed with shovels, whereas an air spade or pressure washer can be used to remove the smaller material. This technique can be used around the rims and hubs of tires, articulating joints, and wheel wells. The remainder of the machine should be inspected for other areas where debris can accumulate.

Attachments and Implements.—Removable attachments for machinery, such as buckets, rock hammers, and bore drills, can be cleaned either manually or with an air spade and pressure washed. If available, a disinfectant can be applied. Walk-behind equipment, such as a rock drill or ditch-witch, can be cleaned in the same manner as drivable equipment.

Personal Gear and Hand-Operated Equipment.—Personal gear and equipment that have been exposed to wetland debris should be cleaned and disinfected daily or more frequently if moving between watersheds or wetlands of known pathogen presence. Cleaning and disinfecting can be done at the worksite if the appropriate materials are present. For some items, such as wetland boots, it may be more practical to carry multiple pairs and place soiled ones in a plastic bag for thorough cleaning and disinfection off-site. Porous items that retain water should be cleaned, disinfected, and allowed to air dry. Avoid using uncleaned gear and equipment at off-site properties. These can be cleaned, disinfected, and dried before storing long term.

Construction Mats.—Construction mats are extensive ground platforms used as temporary protective cover for heavy equipment on job sites (Fig. 5A). Traditional wooden timber mats are used primarily in wetland areas to minimize the disturbance of the wetland structure and vegetation while driving equipment across them. Multiple layers of timber mats are sometimes used in deep sediment wetlands to create a drivable "bridge" across the wetland (Fig. 5B). Hundreds of reused wooden mats may be trucked to a larger project, often by outside contractors.

Following removal from a wetland, mats should be shaken off and scraped to remove large chunks of debris. The porous nature of wood, however, makes mats extremely difficult to clean and disinfect because they become saturated and covered in mud. After cleaning mats, stacking them with spacers will allow better air circulation for drying and reducing the risk of housing viable pathogens. Consider discarding timber mats that are used in a pathogen-contaminated wetland. In wetlands with rare, threatened, or endangered species, it is safest to use new mats.

Lightweight resin mats and composition timber-style mats are more expensive alternatives to timber mats, but they can be reused for longer periods and are easier to clean and disinfect. Resin mats are lightweight interlocking mats with light-duty applications because they cannot support larger, steel tracked equipment. Composition timber-style mats do not splinter or wear under the weight of steel track machines, giving them an extended re-usable lifetime. While composition mats accomplish all the tasks of timber mats, they are not as readily available to rent or purchase.

We see the reuse of poorly decontaminated timber mats as a significant biosecurity threat, and we realize that replacing them will be a challenge given their history as an industry standard. In places like right-of-ways, where maintenance work and upgrades are performed frequently, permanent access roads (e.g., gravel beds and culverts) could be installed as an alternative to repeated mat installations and removals at wetlands. This option requires approvals and permitting from the regulatory agencies but eliminates the potential spread of pathogens through the installation, removal, and reinstallation of matting every couple of years.

Materials Incorporated into the Work Site.—Some materials brought into a construction work site will have little risk of containing pathogens, while using materials native to the work site will minimize the risk of introducing pathogens. The following materials brought into a work site have a low inherent risk of introducing pathogens: crushed stone used for roadbeds and mud-pad construction entrances to prevent soil from tracking onto roadways, temporary stream crossings and other activities (Fig. 5C); manufactured sand or rock dust used to pad underground pipes and to fill sandbags; and kiln-dried hay bales for erosion control (if they have not been used at a previous work site). Native materials include topsoil that is stockpiled from the initial excavation of a site, logs and wood chips from on-site trees, streambed rocks (e.g., those used in stream restoration projects), shrubs and woody debris that can be used for erosion control barriers instead of hay or straw, and clay that can be mined in one location of the site then moved elsewhere to line created-wetland basins or build the core of earthen berms and dikes.

Materials Reused Between Sites.—Very few disposable materials used at a construction site can be reused at another site. Plastic construction fencing and traditional silt fencing subjected to the elements, wildlife crossings, and equipment encounters generally become unsalvageable and are discarded. When plastic fencing is reused, however, it rarely accumulates any debris by nature of its smooth surface. The smooth surface of newer, reusable silt fencing also tends to shed debris easily and can be pressure washed as needed. Steel T-posts used for fence installation can be reused multiple times. They can be easily pressure washed on a storage pallet if they were used in a wetland area. Sheets of plywood are often used by welders to lie on, for shoring in conjunction with trench boxes, to cover equipment and for storage. Although it is porous, the smoother surface of plywood lends itself to brushing away debris. Plywood that is contaminated with wetland debris can be discarded.

Incorporating Decontamination Into Resource Projects

Land management organizations will have the best opportunity to adhere to biosecurity protocols when they operate their own heavy equipment and use designated, trained staff to clean equipment. They may further benefit from designating permanent washing stations that are isolated from aquatic habitats or by identifying portable washing stations (see review of stations in USFS 2008) so that equipment can be washed at work sites. Resource managers who rely on contractors and other organizations to operate heavy equipment may find it more effective to communicate all biosecurity concerns and decontamination protocols during the project proposal process.

Applying the guidelines and references presented here and taking this more progressive approach can be an opportunity for applicants and contractors submitting requests for proposals to distinguish their company from competitors by articulating their commitment to biosecurity and the measures they are willing to take to achieve a higher standard. Resource managers can further clarify their expectations for biosecurity during pre-bid or post-bid site visits with contractors while they in turn can communicate how their current biosecurity practices can be modified to meet expectations. Once a project begins, the onus of monitoring biosecurity compliance likely will fall upon resource managers and designated personnel to visit active project sites, especially when new equipment or materials enter or leave the project area. In this way, agencies agreeing to incorporate biosecurity recommendations into the permitting process share the responsibility of monitoring compliance.

The following decontamination steps, summarized from the sections above, provide a useful format to incorporate into a request for proposals:

Cleaning

1. To minimize pathogen spread after the work is completed at a work site, plan to clean within the work site or at pre-identified wash-down areas nearby.
2. Detach parts and accessories to access all surface areas.
3. Physically remove all foreign material and any sediment using shovels, prybars, and brooms from exterior; seat covers and floor mats from interior.
4. Clean the exterior first, starting from the roof down to the wheels and undercarriage, then clean the interior.
5. If needed, power-wash exterior, water pressure ≥ 620 kPa (90 lbs/inch^2); temperatures ≥ 40°C (104°F) to deactivate *Bd* and Rv.

Disinfecting

1. Select chemicals based on environmental safety.
   a. Check "Ecological Information" and "Ecotoxicity" hazards on the manufacturer's SDS, available online.
   b. Follow safety guidelines (personal protective equipment, manufacturers' recommended concentrations, application conditions, and contact times).
2. Apply ≥ 50 m away from aquatic habitats.
3. Disinfect personal clothing and sampling gear.
   a. Brush, disinfect, and rinse with water (see NEPARC reference).
   b. Between work sites as preventative: 1% KPMS solution or 3% solution of a 6% NaClO bleach formulation (1,750 ppm NaClO), 5 min.

Drying

1. Ensure all dirt is removed from the vehicle, undercarriage, and hard-to-see areas.
2. Dry for 24-h if equipment is disinfected; dry for 5 days if vehicle is not disinfected

Conclusion

Many cleaning protocols for large equipment proposed by organizations are primarily focused on effects on warm-blooded wildlife (e.g., avian influenza), visible invasive species (e.g., zebra mussels), or nonnative vegetation (e.g., watermilfoil and *Phragmites*). Here we relate guidelines from effective small-scale cleaning and disinfecting of personal field equipment to ensuring the larger scale equipment used for major outdoor jobs are not facilitating the spread of pathogens to herpetofauna. We present a decision tree to assess relative priorities for cleaning (Fig. 2), alternative steps for cleaning and disinfecting large equipment as needed, and suggestions for incorporating these methods into a preplanning process. Given the anticipated increase in large environmental projects (e.g., dam removal, conservation landscaping) and improvements to infrastructure, the methods we provide, when shared as widely as possible with managers of construction and habitat restoration projects, will be a critical step in helping to minimize the spread of pathogens in wildlife.

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Appendix

Existing protocols for cleaning and disinfecting heavy equipment, vehicles, and machinery. Note: Cleaning measures in this table are recommended for cleaning off sediments and mud, rather than as disinfectants for amphibian and reptile pathogens.

Source	Equipment covered	Target organism(s)	Cleaning measures	Available at
Australian Government Department of the Environment. 2015. Arrive Clean, Leave Clean: Guidelines to help prevent the spread of invasive plant diseases and weeds threatening our native plants, animals and ecosystems. 22 pp.	All vehicles and machinery	Invasive plants, seeds	Car wash or other wash-down facility to remove weed seeds, mud, soil and organic matter	https://www.environment.gov.au/system/files/resources/773abcad-39a8-469f-8d97-23e359576db6/files/arrive-clean-leave-clean.pdf
California Stormwater Quality Association. 2003. California Stormwater BMP Handbook.	Construction equipment/vehicles	Nutrients, sediments, pollutants	Phosphate free, biodegradable soaps; High-pressure sprayers	https://www.escondido.org/Data/Sites/1/media/pdfs/Utilities/BMPVehicleEquipmentCleaning.pdf
Canola Council of Canada. Managing Clubroot: Equipment Sanitation Guide.	Farm equipment and vehicles (construction and recreational)	Clubroot disease	Rough cleaning, pressure washing (2,000–3,000 psi) using a turbo nozzle; 1% bleach solution for 15–20 min	http://www.canolawatch.org/wp-content/uploads/2011/12/11CCC2791-Clubroot-Sanitation-Guide_r3_LR.pdf
Minnesota Sea Grant. 2004. Aquatic Invasive Species – Hazard Analysis and Critical Control Point Training Curriculum (MN SG–F11 and MSG-00-400). 91 pp.	Boats, trailers, and water equipment	Aquatic invasive invertebrates, fishes, plants, pathogens, parasites	Hot water (60°C) tap water for 20–30 min; drying for 10 days before reuse; freezing for 2 days before reuse; 250 mg/L chlorine bleach (5% sodium hypochlorite) for 60 min	http://www.seagrant.umn.edu/downloads/ais-haccp_manual.pdf
Biosecurity Queensland, Queensland Department of Agriculture and Fisheries. 2019. Vehicle and machinery clean down procedures 31 pp.	Trucks, 4WD vehicles, compactors, excavators, harvesters, tractors, PTO rotary hoes, track-vehicles, and wheeled loaders	Invasive plants and their seeds, soil-borne pests and disease	Remove visible material and apply high-pressure water rinse	https://www.daff.qld.gov.au/__data/assets/pdf_file/0011/58178/IPA-Cleandown-Procedures.pdf
U.S. Department of Agriculture, Forest Service. Preventing Accidental Introductions of Freshwater Invasive Species. Accessed March 2019.	Boats, seaplanes. and recreational equipment	Invasives and chytrid fungus	60°C for 5 min, or 47°C for 30 min, then dry for 48 hours at less than 70% relative humidity; chlorine bleach (4% solution) for 3 min	http://www.fs.fed.us/invasivespecies/documents/Aquatic_is_prevention.pdf
U.S. Bureau of Reclamation. 2012. Inspection and Cleaning Manual for Equipment and Vehicles to Prevent the Spread of Invasive Species (Technical Memorandum No. 86-68220-07-05).	Wheeled and tracked land vehicles, construction equipment, and watercraft	Invasive plants and zebra mussels	High-pressure compressed air and water rinse 1% table salt solution; diluted household bleach for a minimum of 1 hour; potassium permanganate solutions; various quaternary ammonium and polyquaternary ammonium compounds	https://www.usbr.gov/mussels/prevention/docs/EquipmentInspectionandCleaningManual2012.pdf
U.S. Department of Agriculture. 2018. Foreign Animal Disease Preparedness and Response Plan.	All vehicles and heavy machinery	Viral and bacterial pathogens, primarily warm blooded	Detergent. When below freezing temps add up to 40% propylene glycol in water. Entire EPA disinfectant list	https://www.aphis.usda.gov/animal_health/emergency_management/downloads/sop/sop_cd.pdf
U.S. Fish and Wildlife Service. 2017. Stop Aquatic Hitchhikers.	Motor boats, non-motorized boats, seaplanes, and scuba gear	Non-native aquatic plants and organisms	Saltwater: 5% dishwashing liquid solution (624 ml/L). Freshwater: 3.5% salt solution (312ml/L)	http://stopaquatichitchhikers.org/
Wisconsin Department of Natural Resources. 2010. Wisconsin DNR Watercraft Inspector Handbook. Section 10: Aquatic Invasive Species Monitoring.	Boats and trailers	Aquatic plants and organisms, zebra mussels, invertebrates	Chlorine 200 ppm for 10-min contact time; Virkon® Aquatic 1:100 solution for 20- to 30-minute contact time; Sodium thiosulfate	http://dnr.wi.gov/lakes/forms/protocols/Disinfection_Protocol.pdf