African Clawed Frog (*Xenopus laevis*) Risk Assessment, Strategic Plan, and Past Management for Washington State Department of Fish and Wildlife.

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EXECUTIVE SUMMARY

African Clawed Frog (*Xenopus laevis*; ACF) occur in at least three disparate locations across the Puget Sound region. Repeated introductions by people, rather than dispersal among these locations, is the most likely explanation for these occurrences. Such repeated introductions may also be more widespread than is currently understood and, as such, future introductions are likely. ACF are voracious predators and vectors of novel pathogens and so pose a risk to native species through direct predation, competition, and disease. Here, we compile information about known ACF introductions, attempted control methods, and potential risks from ACF. We also outline knowledge gaps that are essential for research to address for risk assessment and mitigation. Unsuccessful prior management efforts and a lack of resources underscore the challenges of managing ACF in Washington State and the need for continued research. Without continued commitment from the agency, long-standing partnerships, particularly with local jurisdictions, that support ACF containment in Lacey are tenuous.

Purposeful management of ACF can only happen with an informed risk assessment. Such a risk assessment would ideally happen early in a species' invasion which ACF in Washington presumably are, although data on the extent and timing of their introduction and spread are sparse. To inform a risk assessment, we propose prioritized ideal next steps in ACF management that vary in effort and investment. These include support to maintain and build partnerships that are essential to ACF management and validation of environmental DNA (eDNA) as an important tool to rapidly and affordably monitor ACF spread. This proposal also includes multiple research efforts that would provide the necessary data to inform a risk assessment and ACF management plan. These efforts include surveying to document the true extent of current ACF populations and associated spread and the studies necessary to assess the efficacy of various control methods. Depauperate data on ACF in Washington preclude informed risk assessment and management. When we better define elements of risk, we will collectively understand tradeoffs between future management scenarios, their chance of success, and their costs.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

Invasive species pose a substantial threat to biodiversity and the Washington State Department of Fish and Wildlife's (WDFW) ability to preserve the state's native fish and wildlife and their habitats. The African clawed frog (*Xenopus laevis*; hereafter ACF) is an aquatic frog native to sub-Saharan Africa and a voracious predator that readily acclimates to a wide range of habitats (ACF biology is reviewed in **Appendix 1**; Measey *et al.* 2012). ACF was first identified in Washington state at two locations in western Washington in 2015. A third location was identified in 2020 in Issaquah. The species likely poses a substantial risk by preying on native species and potentially transmitting disease (Robert *et al.* 2007; Tinsley *et al.* 2015a). ACF's ability to rapidly reproduce and spread makes it a significant conservation concern (Vimercati *et al.* 2020). Listed as a prohibited level 3 species in Washington State, ACF are illegal to possess, introduce on or into a water body, or traffic without a permit (RCW 77.135.030(1)(c)). In 2017, WDFW and the City of Lacey were unsuccessful at eradicating the species from a stormwater pond at one of the two known introduced locations. Here, we assess the risk of ACF to Washington ecosystems based on the current status in the state, management history, and relevant literature on life history and other ACF invasions. We identify knowledge gaps and propose a research agenda that will be important for establishing management plans to address this invasive species.

1.1. INVASIVE SPECIES CLASSIFICATION

In 2002, the legislature classified ACF as prohibited, forbidding the purchase or sale of the species in Washington. Currently, ACF are classified as "Prohibited level 3" species under RCW 77.135.030(1)(c) and WAC 220-640-050(1)(c) because they are non-native aquatic animal species that are considered to pose a "moderate to high invasive risk" and which may require management by the Department or other affected landowners. Prohibited level 3 species, under RCW 77.135.040(1), "may not be possessed, introduced on or into a water body or property, or trafficked without department authorization, a permit, or as otherwise provided by rule". WDFW's 2015 Statewide Wildlife Action Plan (Chapters 2 & 3) highlights invasive plant and animal species and pathogens and diseases as a major statewide conservation issue which "constitute a severe and growing threat to Washington's native wildlife, habitat and biodiversity."

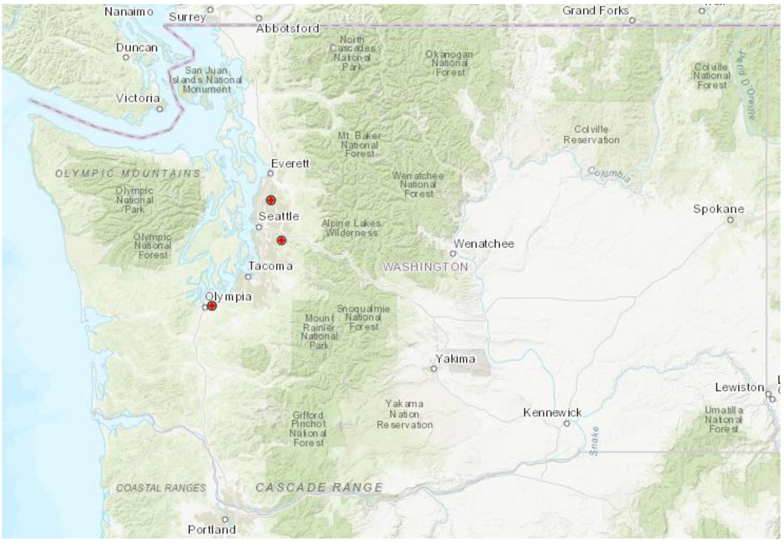


Figure 1. Active ACF introductions in Washington State in Lacey, Issaquah, and Bothell (Basemap ESRI).

2. STATUS IN WASHINGTON STATE

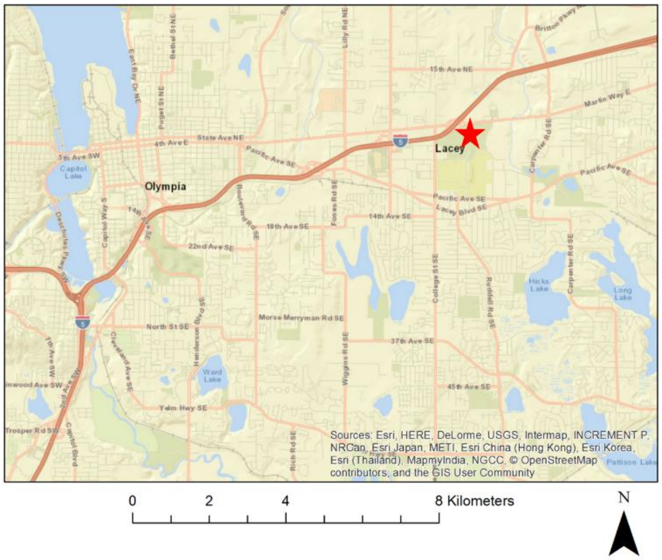
WDFW confirmed ACF present at two locations in 2015 and one location in 2020 (**Error! Reference source not found.**; **Table 1**). In all instances, ACF were discovered by non-agency personal and reported voluntarily. We know of no other ACF locations in the state, however no broad-scale systematic survey to identify additional populations has been conducted in the state. Though there is no direct evidence on how ACF have been introduced in Washington, the department believes the frog populations were initially established as the result of individuals discarding aquarium pets, which is a common practice with other invasive aquatic species (e.g., red-eared slider turtles (*Trachemys scripta elegans*). Importantly, the distribution of the three known ACF populations across the Puget Sound area suggests introductions were done by different individuals and that the possibility that introductions may be more widespread than currently understood.

Table 1. ACF introduction summary information by location.

	Lacey	**Bothell**	**Issaquah**
Site Description:	3 connected stormwater ponds and associated 171-hectare storm sewer network	3 ponds along North Creek including unconfined wetlands	Sediment pond with strong connection to Tibbets Creek
ACF Status:	Active	Presumed active	Presumed Active
First Detection:	July 2015	July 2015	July 2020
Reporting Party:	Department of Ecology staff	Children fishing	Fish removal contractor
Most Recent WDFW Effort:	December 2020	May 2019	None
Most Recent WDFW Detection	December 2020	May 2019	None
Containment provision	Perimeter fences and pipe screens. Annual coordination with City of Lacey during stormwater sewer maintenance.	None	None
***Ranavirus*:**	Undetected in 2020 using qPCR assays	Unknown	Unknown
Batrachochytrium dendrobatidis	Confirmed July 2018	Unknown	Unknown
ACF Removed	> 6,900	75	0 (no effort yet)
Partners	City of Lacey	Business park owners have allowed WDFW access in the past.	None

2.1. LACEY

2.1.1. Location Profile



Sources: Esri, HERE, DeLorme, USGS, Intermap, INCREMENT P, NRCan, Esri Japan, METI, Esri China (Hong Kong), Esri Korea, Esri (Thailand), MapmyIndia, NGCC, © OpenStreetMap contributors, and the GIS User Community

Figure 2. Vicinity map of Lacey Stormwater Ponds (College Regional Stormwater Facility) in relation to Cities of Lacey and Olympia. The ponds are denoted by the red star.

The Lacey Stormwater Ponds site, called the “College Regional Stormwater Facility”, is located near Martin Way and I-5 in Lacey, WA (**Error! Reference source not found.**). The Lacey Stormwater Ponds consists of three constructed stormwater catchment ponds measuring approximately 0.42, 1.46 and 0.46 hectares respectively for Pond 1, 2, and 3 (**Error! Reference source not found.**). Pond 1 is connected to Pond 2 by a valved pipe. There is an overflow that connects Ponds 2 and 3 at high water (e.g., during a storm event). Ponds 2 and 3 drain northward under I-5 and eventually to a forested wetland and Woodland Creek. Pond 3 is longer and shallower than Pond 1 and 2, and has a firmer substrate than Pond 1, which has silty substrate covering a liner. Overland movements of ACF have been observed on the gravel paths adjacent to the ponds and crossing Abbey Way SE ~ 50 m south of the stormwater ponds personal communication, F. Waterstrat, USFWS). A silt fence is maintained around the perimeter of each pond.

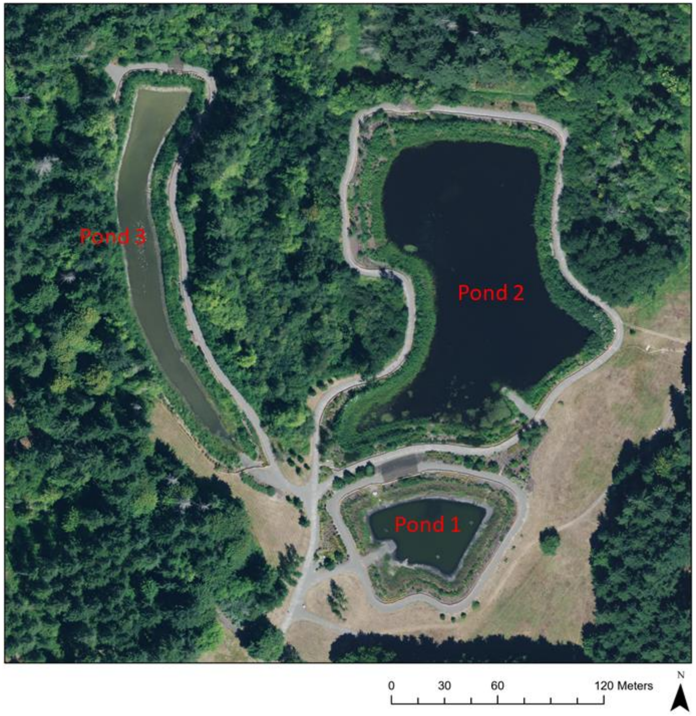


Figure 3. Lacey stormwater ponds with introduced ACF. The star indicates the pond where the first ACF was detected.

The ponds are fed by a mostly urbanized 171-hectare watershed through a network of stormwater drainage pipes (**Error! Reference source not found.**). In October 2018, ACF were first detected in the drainage network beyond the ponds by the City of Lacey’s vacuum trucks that are used to clean sediment catch basins within the stormwater drainage system. Since then, ACF have been detected throughout much of the drainage system but their full extent is unknown. Groundwater infiltration prevents the stormwater drainage system from drying out entirely despite limited overland flow during drier summer months and a liner in Pond 1 maintains water levels. This hydrology suggests that the sewer drainage system may provide suitable habitat for ACF year-round.

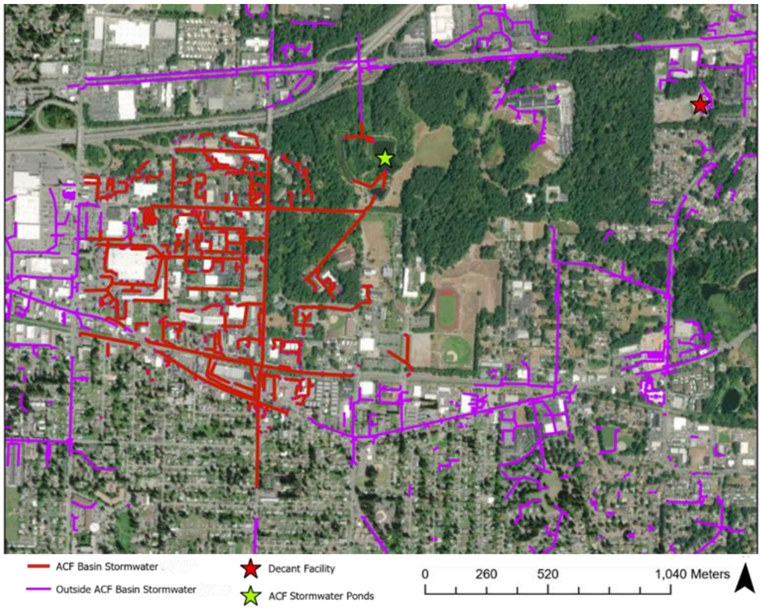


Figure 4. Map of stormwater drainage network that drains to Lacey Stormwater Ponds. Red drainage lines directly drain into stormwater ponds with ACF. Purple lines drain into other stormwater ponds in Lacey

2.1.2. 2020 Lacey Spread Assessment

During the summer of 2020, we evaluated 35 sites near the known focal ACF population to explore the spatial extent of ACF in Lacey. These sites are located within approximately a 1-km radius of the Storm water ponds and were selected based on past survey efforts, GIS aerial imagery, and communication with landowners about potential habitat (Error! Reference source not found.). We set traps at 23 of the 35 sites that had enough water to deploy minnow traps. This effort consisted of setting 10 baited minnow traps at each site for two to three nights for a total of 680 trap nights across the 23 sites. Five of the 680 trap nights used the Mega-trap (large custom designed frog trap; see below 5.1.2. Past Management Actions) instead of minnow traps. During this effort, we removed 13 ACF from the Lacey Stormwater Ponds but found no ACF at any of the other surveyed sites.

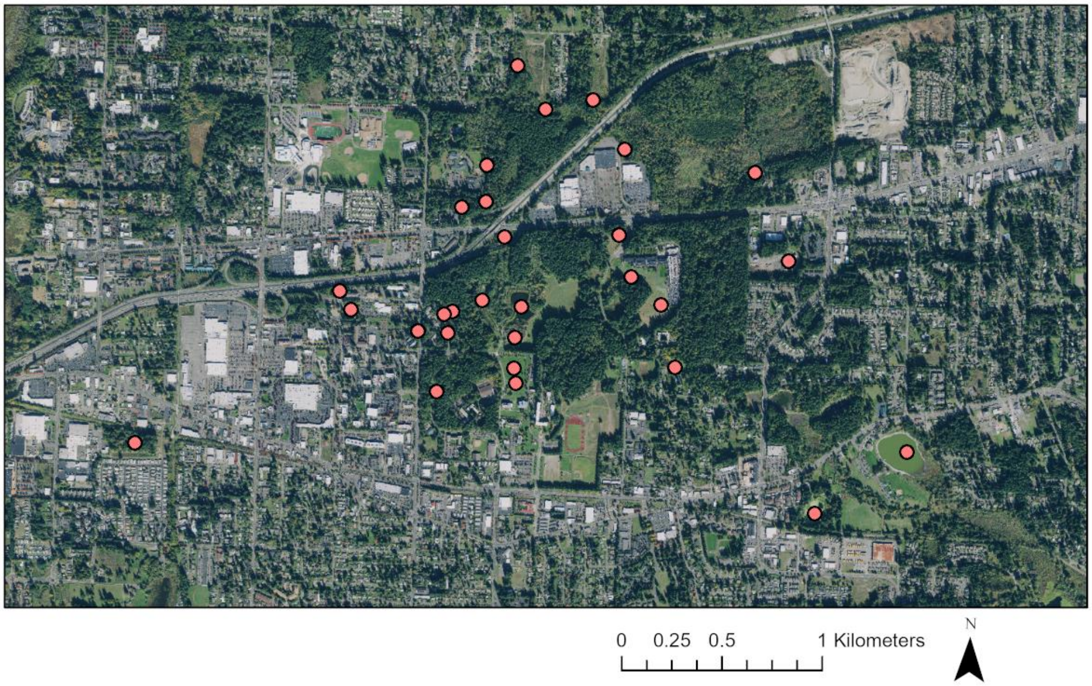


Figure 5. Spread assessment conducted in Lacey in 2020. Circles indicate sites that were evaluated for ACF.

2.2. BOTHELL (NORTH CREEK WETLANDS)

The first ACF in the North Creek drainage was observed by children fishing in the “Ground Zero” stormwater pond (**Figure 6**). Ground Zero pond is 0.6 hectares in surface area and is connected to the main channel of North Creek via 3 outlet pipes. North Creek flows through a large portion of Bothell’s commercial zone and residential areas. Both commercial and residential areas have numerous other stormwater ponds that collect runoff that ultimately empty into North Creek. Two other locations were found to have ACF in a 2016 reconnaissance survey conducted by WDFW. “Richards Pond Large” is 0.03 hectares in size and connected to “Ground Zero” by a culvert. “Twin Ponds” are 44 and 28 m^2 in size, respectively, are 1.5 km downstream from the “Ground Zero” pond and are on the opposite side of North Creek from “Ground Zero” and “Richards Pond Large”.

Figure 6. Map of 2016 Trap Results from North Creek, Bothell.

2.3. ISSAQUAH (TIBBETS CREEK SEDIMENT POND)

A single adult individual was incidentally captured from a sediment pond in July 2020 by a contractor electrofishing during fish capture and relocation efforts prior to removing accumulated sediment. The pond is well-connected to Tibbets Creek near Tibbets Valley Park in

Issaquah (**Figure 7**). Little is known about the status of ACF beyond this single individual at the site due to WDFW's limited capacity to follow up on the report.

Figure 7. Vicinity map (top) and aerial imagery (bottom) of Tibbets Creek ACF observation reported in July 2020. The pond is denoted by the red star in both maps.

3. INSIGHTS FROM OTHER INVASIONS

ACF can spread rapidly and establish under a wide range of habitat conditions.

Case studies from around the world illustrate the ability of ACF to rapidly expand beyond their point of introduction. For example, in France, ACF are believed to have been introduced in the 1980's (Fouquet and Measey 2006) and now occupy at least 2,055 km^2 area in an agricultural landscape (Courant *et al.* 2019; Vimercati *et al.* 2020). A population in Chile that is believed to have established from one or more releases in 1973 has expanded to an estimated 21,200 km^2 (Lobos et al. 2013). ACF can occupy a range of habitat freshwater aquatic habitats including both flowing and nonflowing waters as well as seasonal and permanent waterbodies (Moreira *et al.* 2017).

ACF can harm native amphibians and other species locally.

ACF can impact native amphibians populations through a combination of predation, competition, and disease (Lillo *et al.* 2011; Courant *et al.* 2018). Introduced ACF have been documented preying on native amphibian species (Measey *et al.* 2015; Vogt *et al.* 2017) and Wilson (2018) suggests that native species may leave an area to escape predation pressure (predator avoidance). ACF have toothed jaws, robust hindlimbs, and claws that allow them to consume larger prey items than other similar sized frog species. ACF was found to prey upon both larval and adult

Pacific chorus frog (*Pseudacris regilla*) (Wilson *et al.* 2018b), a common species native to Washington State. ACF have also been shown to reduce populations of invertebrates and zooplankton, which are important food sources for Washington's native aquatic fauna such as fish and amphibians (Courant *et al.* 2017; Courant *et al.* 2018). The extent to which ACF may impact salmonids is unknown.

ACF are cryptic and can spread without detection.

ACF behaviors including, nocturnal activity patterns, traveling along the bottom of waterbodies, and their lack of surface vocalizations makes them difficult to observe (Ringeis et al. 2017). In some cases, long extant populations may have gone undetected for 2-25 years (Measey *et al.* 2012). The Lacey Stormwater Ponds were constructed in 2008 and so the ACF population in these ponds may be over a decade old. To our knowledge, differences in detection probability has not been assessed as a function of survey method or environmental conditions.

ACF may carry diseases, including *Ranavirus* and chytrid fungus.

ACF, like other amphibian species, can act as a vector for *Ranavirus* (Robert *et al.* 2007) and the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd)*. The earliest confirmed evidence of *Bd* is from a 1938 museum specimen of ACF, indicating that *Bd* may be long-associated with this species (Weldon *et al.* 2004). Both pathogens are believed to have caused declines in amphibian populations (Skerratt *et al.* 2007, Lesbarreres *et al.* 2012). The greatest concern is that introduced ACF could spread new more virulent strains (i.e., lineages) of *Bd* (Byrne et al. 2020).

The pet trade is a likely source of new introductions.

Globally, many introduced ACF populations are believed by the research community to have originated from medical laboratories in the early 20th century (Tinsley and McCoid 1996, Measey *et al.* 2012). ACF are still used in laboratory research but are often bred domestically for the pet trade and more recent introductions are thought to originate from discarded pets (Tinsley and McCoid 1996, Measey *et al*. 2012). For example, an albino population of ACF in China almost certainly originated from pet trade given albino ACF are common pets (Wang *et al.* 2019). Additionally, ACF introductions in California and Chile among others locations suggest multiple populations are the result of multiple releases (Measey *et al.* 2012). Over 99% of international trade of ACF to the USA has been for the pet trade rather than for laboratory purposes (Measey 2017), and the lack of regulation on pet ACF breeding highlights the risk of importing novel pathogens.

Eradication is possible but requires large and sustained efforts.

In at least one case, eradication of ACF was achieved through intensive management in a small isolated wetland site at Golden Gate Park, San Francisco, CA over years of chemical treatment using calcium carbonate (lime) solution (personal communication, E. Larson). In Great Britain, ACF were naturally extirpated by climate and/or fish at one pond (Measey 2012). In Spain, larval ACF were extirpated from a small garden pond by treating it with an anti-algae copper sulfate (Pascual *et al.* 2007). There are on-going eradication efforts for certain populations of frogs in Chile and Portugal but neither have yet achieved full eradication in occupied watersheds (personal communication, R. Rebelo).

4. RISKS TO WASHINGTON STATE RESOURCES

4.1. DISEASE

ACF can be a host for a variety of pathogens, including *Ranavirus* and *Bd* (Robert *et al.* 2007), and can asymptomatically carry these pathogens in the wild and in captivity and then transmit them to native species. ACF may also carry pathogens or pathogen strains which have yet to be discovered and which may harm native aquatic species. To date, *Ranavirus* has not been confirmed at any Washington site, although the presence of *Ranavirus* has not been evaluated at the Bothell or Issaquah sites using reliable sequencing techniques.

4.1.1. Ranavirus

Ranaviruses, or "RAN", an Iridovirus, are on the World Organization for Animal Health (OIE) list of notifiable terrestrial and aquatic animal diseases. They are a risk to the entire suite of the state's native amphibians as they represent one of two globally known and lethal amphibian pathogen groups. ACF have been documented carriers of *Ranaviruses* (Robert *et al.* 2007; Soto-Azat *et al.* 2016). Mortality levels associated with *Ranavirus* epidemics in sensitive (or naïve) species often exceed 95%. Once introduced into an area, *Ranaviruses* can be further spread by fish and turtles, which may act as reservoirs and which also may be susceptible to these pathogens (Brenes *et al.* 2014). In laboratory experiments, *Ranaviruses* can grow in Chinook salmon (*Oncorhynchus tshawytscha*) embryos (Ariel *et al.* 2009). Other Iridoviruses such as the White Sturgeon Iridovirus pose a significant threat to fish and can cause death in up to 95% of juvenile fish (LaPatra *et al.* 1994).

WDFW testing to date of ACF for *Ranavirus* shows no confirmed positive cases. In 2020, 174 samples were analyzed and yielded no positive samples. The WDFW Fish Health Lab used quantitative PCR (qPCR) to detect the presence of *Ranavirus* (using QuantStudio™ 6 Flex Real-time PCR System (ThermoFisher)) by amplifying an 83-base pair region of the *Ranavirus* major capsid protein. This test is a general *Ranavirus* assay that detects many forms of *Ranavirus* (Stilwell *et al.* 2018). For each qPCR run, each sample was run in triplicate (qPCR methods are detailed in Capps and Warheit 2021). Of the 174 samples from Lacey ponds, no samples (0%) were scored as positives and 5 samples (3%) were scored as Below the Limit of Detection (BLD). The remainder of the samples, 171, (97%) were scored as negative. All five BLD samples were sequenced for further *Ranavirus* determination. Two different sequencing reactions were used (Capps and Warheit 2021) including a 359-base pair region that includes the 89-base pair qPCR product (Stilwell *et al.* 2018) and a ~300-base pair region from Mao et al. (1997). None of the BLD samples produced *Ranavirus* sequence from either of the sequencing reactions. In summary, no detectable *Ranavirus* was found in the 2020 sample set submitted from Lacey ponds.

4.1.2. Amphibian chytrid fungus (Batrachochytrium dendrobatidis; Bd)

ACF can be asymptomatic carriers of *Bd*, an introduced fungus that can cause disease known as chytridiomycosis (Solís *et al.* 2010; Wilson *et al.* 2018a) and which is associated with some amphibians declines (Olson *et al.* 2013; Byrne *et al.* 2019). In 2018, USFWS and USGS conducted an analysis of 30 African Clawed Frogs collected from the Lacey Stormwater Ponds. The results showed that 23/30 (76.67%) of swabs were positive for *Bd.* All swabs were negative for *B. salamandrivorans* (*Bsal*), a closely related chytrid fungus that also affects amphibians.

The finding of *Bd* presence is only mildly informative and, on its own, may not be a concern depending on environmental conditions and the lineage or strain of *Bd* present. Many species have been shown to be positive for the pathogen despite showing no clinical signs. However, USGS recommended that the animals at the Lacey Stormwater Ponds should be closely monitored to ensure that no clinical signs of *Bd* develop. Particularly problematic are the diversity of lineages of *Bd* from around the world that vary in their degree of virulence and that can hybridize and create more lethal disease when spread by invasive pets (Byrne *et al.* 2019). Previous *Bd* testing in Washington was not designed to distinguish different *Bd* lineages, thus it remains unclear whether introduced frogs are spreading new, perhaps more lethal, pathogen strains or are simply carrying strains that are native to Washington. Future genomic analyses of *Bd* lineages in Washington are needed, particularly with respect to introduced amphibians like ACF.

Bd assessments Oregon and Washington suggest that *Bd* is relatively widespread in the environment with 16 of 37 (43%) surveyed sites reporting *Bd*-positive amphibians (Pearl *et al.* 2007). *Bd* may decrease survival over longer time scales, even in the absence of mass die-offs, as is suggested for the Washington state- and federally-protected Oregon Spotted Frog (*Rana pretiosa*; Russell et al. 2019). In Washington, we have uncertainty about potential impacts of *Bd* on amphibian populations due to a lack of data on comprehensive morbidity and transmissibility of the pathogen, how *Bd* interacts with other stressors, and knowledge gaps regarding diversity in *Bd* lineages (Pearl *et al*. 2007, Byrne *et al.* 2019). Introduced species have the potential to import new more lethal lineages of *Bd*, highlighting the importance of mitigating the introduction and spread of invasive aquatic species and for genomic monitoring of new *Bd* strains.

4.2. PREDATION AND COMPETITON

ACF can affect community structure through predation and competition (Measey 1998; Lillo *et al.* 2011; Courant *et al.* 2017). Post-metamorphic ACF are generalist predators capable of shredding large prey items with their hind claws. Laboratory predation trials suggest that larval and adult life stages of Pacific Tree Frog (*Pseudacris regilla*), a native species to Washington, avoid ACF which could reduce the direct impacts of predation. However, this behavior may also result in emigration of native amphibians from ACF occupied sites (Wilson *et al.* 2018b). Larval ACF are not considered a predatory threat to native amphibians because they are obligatory filter feeders (Seale 1982), but they may also be capable of modifying food webs. Although adult ACF likely consume native amphibians and invertebrates, diet studies are needed in Washington to understand the extent of this impact, particularly because stormwater ponds can act as critical habitat for a diversity of native amphibian species (Ostergaard *et al*. 2008).

4.3. SPREAD

ACF are cryptic due to their principally aquatic life history, predominantly nocturnal activity, underwater rather than surface vocalizations, and subsurface dwelling behaviors. In France, extensive spread has occurred throughout natural waterbodies (Vimercati *et al.* 2020). In Chile, ACF were more often found in artificial rather than natural waterbodies (Lobos et al. 2013), suggesting that urban areas may be particularly vulnerable to ACF introductions due to the prevalence of artificial waterbodies and the proximity to humans which can facilitate pet releases. All known ACF populations in Washington are stormwater ponds and are in proximity to other artificial and natural waterbodies. The hydrology of stormwater ponds can vary dramatically and include a diversity of seasonal habitats that dry each year and waterbodies that permanently hold water. Dispersal through water and over land are both possible and Gamble (2007) suggest that ACF do not exhibit strong site fidelity, potentially increasing the risk of dispersal and colonization of additional waterbodies.

The possibility of cryptic spread makes it difficult to assess the true extent of invasion in Washington State and to identify the original introduction timing(s) into the state. ACF have been observed on paths and roads near the Lacey Stormwater Ponds (personal communication, F. Waterstrat, USFWS) but, to date, have not been detected in any additional nearby waterbodies since their initial discovery in 2015. The stormwater drainage network also represents a potential pathway for spread that is hard to survey because it is largely subsurface. Large precipitation events that lead to increased surface water connectivity could facilitate escape from the storm sewers. Moreover, the risk of additional releases by the public remains high. For example, in 2016, WDFW Enforcement Officers confiscated four ACF from a pet store. Additionally, without control measures, outreach, and targeted communication, community members may encounter, obtain, and spread ACF

4.3.1. Expansion under Climate Change

Recent process-based species distribution modeling efforts suggest that ACF can tolerate a broader temperature spectrum and geographic extent than previously assumed (Ginal *et al.* 2021). This finding contrasts with prior work by Ihlow et al (2016) that suggested the global range of ACF would likely contract under commonly accepted climate change projections. Regardless, ACF thrive in Mediterranean climates, which are characterized by dry summers and cool, wet winters, conditions like those found along the North America’s west coast. Western Washington’s winters are projected to become wetter and warmer overall (Mote and Salathé 2010), and so will likely favor ACF. Conditions associated with climate change could improve physiological performance, fecundity, breeding success, and increase rates of larval development. It remains unclear how ACF and associated pathogens could expand under climate change.

5. SUMMARY OF WASHINGTON STATE CONTROL ATTEMPTS

There are a large variety of control and extermination options available for invasive amphibians, each with advantages and disadvantages (**Table 2**). As amphibians often have complex life histories corresponding to different habitats (e.g., aquatic habitats for breeding and larvae, terrestrial habitats for juveniles and adults), it is unlikely that a single control method will be effective in controlling the spread or eliminating an ACF population. It is important to consider each method and how they interact with each other for any given situation. Regardless of the methods chosen, adequate time, resources, and labor must be allocated to allow for success.

Table 2. Summary of control techniques.

Technique		**Advantages**	**Disadvantages**
Seining / Dip Net		• Native bycatch minimized • Effectively targets deeper habitats	• Requires multiple personnel • Labor intensive • Cannot access all habitat • Ineffective in deep sediments where ACF bury or where extensive debris clogs nets
Trapping	Minnow	• Successfully removed hundreds of ACF • Effectively targets shallower habitats	• Unknown trap efficiency and detection probability • Unknown efficacy at low densities • Native species bycatch • Trap lines require maintenance
	Mega-trap (described in section 5.1.2)	• Can be checked less often and capture more animals than minnow traps • Effectively targets deeper habitats	• Unknown trap efficiency and detection probability • Unknown efficacy at low densities • Native species bycatch • Trap lines must be maintained • Not suitable for highly vegetated areas or low water levels
Chemical	Salt	• Effective in lentic accessible water body • Other non-native species bycatch	• Expensive • Requires multiple personnel and is labor intensive • Connection to sewer facilitated recolonization • Less effective in flowing water • Native species bycatch
	Other: • Rotenone • CO2 • Calcium Oxide • Copper Sulfate • Clove oil		• Pesticides treatments were initially explored as an option but never implemented due to water quality concerns and the proximity of ESA-listed species • Variable efficacy across life stages • May cause adults to emigrate
Electroshocking (never implemented)		• Can be attenuated to target species	• Requires multiple personnel daily for several weeks • Most effective with specialized "electrofrogger" • Initial testing apparently ineffective for ACF
Water-Level Manipulation	Desiccation and or exposure to freezing temperatures	• Highly effective where applicable	• Refuge areas where water cannot be fully drained or reach freezing temps • Not always applicable • May cause adults to emigrate
Biological Control (never implemented)	Bass and/or Tiger Muskies	• Passive, low maintenance	• Concern of proximity of ESA listed species to the pond.

5.1. LACEY – A HISTORY OF CONTROL EFFORTS

Management actions in Lacey have received major contributions from the City of Lacey. Containment and eradication attempts have been supported by their staff time, expert knowledge of infrastructure, and equipment. Future management should prioritize maintaining this vital partnership between the City of Lacey and WDFW.

5.1.1. Containment

Pond containment

A double-layer perimeter silt fence was constructed around the three stormwater ponds to contain ACF and prevent overland dispersal in 2015. Because these stormwater ponds have outflow pipes that exit the facility into uncontained waterways, WDFW and the City of Lacey used seine netting to cover the outflows pipes of Ponds Two and Three. This outflow netting is an attempt to minimize aquatic dispersal of ACF into other waterbodies during high water levels. This netting has been sporadically maintained and repaired as needed. The silt fence was replaced in 2019 by City of Lacey. During winter months, WDFW has periodically checked the condition of the fence and pipe screens due to the risk of damage from heavy precipitation events.

Sewer containment

The City of Lacey detected ACF in the stormwater drainage network within the stormwater pond drainage basin (see **Figures 3** and **4**). This pipe network flows directly into the stormwater ponds. ACF were found when cleaning out the sewer catch basins with vacuum trucks (**Figure 8**). WDFW worked with City of Lacey to determine that all ACF specimens were likely from the stormwater pond basin only. The spoils from the sewer catch basins were manually sifted (**Figure 8**) through when emptying the vacuum truck at the Decant Facility. All ACF specimens were removed and humanely euthanized. To ensure containment, this collaborative work must continue to occur annually when City of Lacey cleans this section of sewers.

Figure 8. City of Lacey storm sewer vacuum truck transports ACF along with debris during annual maintenance.

5.1.2. Past Management Actions

Substantially greater effort has been put into ACF management in Lacey than in Bothell or Issaquah. Since 2015, WDFW personnel have conducted various management actions in attempts to eradicate and minimize the spread of ACF in Lacey, with most effort occurring from 2015-2017. At least 6,911 ACF have been removed from the Lacey sites. This includes 744 adults, 5,320 juveniles, 859 tadpoles, and no eggs. Eggs are laid individually, so the lack of egg observations is not surprising. Control efforts also removed at least 4,074 American bull frogs (*Rana catesbeiana*; another harmful invasive species) and 10,838 invasive goldfish. During these efforts, 748 native amphibians including Pacific Tree Frogs (*Pseudacris regilla*), Newts (*Taricha granulosa*), Northwestern Salamanders (*Ambystoma gracile*) were also encountered.

To date, the WDFW's Aquatic Invasive Species (AIS) unit and Habitat and Wildlife Programs have contributed a total of 2,375 personnel hours into management actions since 2015. Partners such as City of Lacey contributed over 619 personnel hours. Other stakeholders, agencies, and volunteers contributed over 365 personnel hours.

Water levels

Manipulating water levels in ponds can be a critical tool in controlling invasive amphibians. When lowered, ponds can completely freeze or dry out, resulting in eradication of tadpoles. Reducing the volume of water can also help reduce the quantity of chemicals inputs needed to achieve target concentrations needed to lethally remove ACF. However, effective containment nets or fencing must be in place to prevent mass emigration of adults to new areas.

Water levels of the ponds can be manipulated to some extent. Pond 1 has a control valve that can be opened to drain some water into Pond 2. The City of Lacey also used a water pump on Pond 1 and Pond 3 to lower water levels. During the winter of 2015, water levels were lowered to reduce the amount of favorable ACF habitat, enhance control efforts, and increase the ponds' ability to freeze. These ponds are difficult to drain completely due to inputs from groundwater and stormwater runoff. In January 2017 water levels were lowered during a period of cold weather in the hopes that freezing temperatures would kill ACF, but there was no evidence of mortality even though the ponds' surfaces froze. If animals died in the mud, it is possible that they went undetected. In August 2017, water levels were lowered to facilitate salt treatments as detailed below.

Managers have considered discharging water onto the grass fields adjacent to the stormwater ponds to reduce water volumes and desiccate portions of the ponds. However, this could impact a small but persistent Mazama pocket gopher population on site and so this activity would be considered take of a listed species and has not been pursued further (Schmidt et al 2015). Given these limitations, complete drainage of the ponds is not an option, but partial drainage could continue to be used in conjunction with other future efforts.

Seine Net/Dip Net

In August 2015 there was an eradication attempt at Pond 3 using seine and dip nets (**Figure 9**). This effort was implemented in conjunction with lowering the water levels. Seine nets were then dragged along the bottom to herd ACF and dip nets were then used to scoop specimens out. The effort yielded 20 juvenile ACF, 471 bullfrogs, 59 newts, and one unidentified amphibian. The effort required about 283 person-hours.

Figure 9. Seine netting removal effort conducted at Pond 3 in Lacey in the summer of 2015.

Trapping with Minnow Traps

From 2015 to 2020 managers and researchers have used minnow traps to catch ACF, with more efforts in earlier years. Traps are baited with sardines or cat food and placed in shallow waters. The traps are set so that they are not fully submerged allowing native amphibian bycatch to come up for air. Setting traps in this manner limits effort to a depth of no more than 10 inches, the height of the trap. Minnow traps have consistently caught ACF at sites with relatively high density. It has also been used to evaluate spread in nearby waterbodies. It is unknown how successful minnow trapping is at sites with low ACF density.

Mega-trap

A “Mega-trap” (**Figure 10**) was developed by WDFW in 2015 using the minnow trap concept of funneling animals into a holding area from which they cannot escape. This larger trap was designed to be placed in deeper water, hold more specimens, and require less frequent checking than minnow traps. The traps, constructed from PVC and fyke net, have gone through multiple design iterations. Mega-traps can be deployed with minnow traps to trap at multiple depths. It can be baited or deployed without bait. Bycatch of native amphibians has occurred. Mega-traps are responsible for 21% of adult and 8% of juvenile ACF captures at Pond 1 and 42% of adults and 48% of juveniles from Pond 2 from 2015-2020. These traps are also responsible for >99% of the larvae captured.

Figure 10. A mega-trap is a large custom-built trap that can be deployed for multiple days and capture many animals.

Salt treatment

Laboratory research indicates increased sodium chloride (NaCl; i.e., table salt) concentrations are lethal to ACF (Personal communication, J. Gross). Jackson Gross, Smith Root, experimentally determined that 16 parts per thousand (ppt) NaCl results in 100% mortality of both larval and adult ACF (**Error! Reference source not found.**). In August 2017, in partnership with the City of Lacey, WDFW added NaCl to Pond 1. After lowering the water levels in the pond, the City of Lacey applied two treatments using 23.3% NaCl solution two weeks apart. The solution was initially pumped into the stormwater pipe leading to the pond to flush out any ACF residing the pipes. After several flushes with no ACF, the inflow to the pond was blocked using seine nets. Once secured, the brine solution was dispersed uniformly until the salinity was verified to have reached 16 ppt following the pesticide label. Specimens were collected up to eight days after the second treatment. The treated water with elevated levels of salinity was contained until natural rainfall and stormwater runoff filled the stormwater pond and salinity dropped below than 2 ppt.

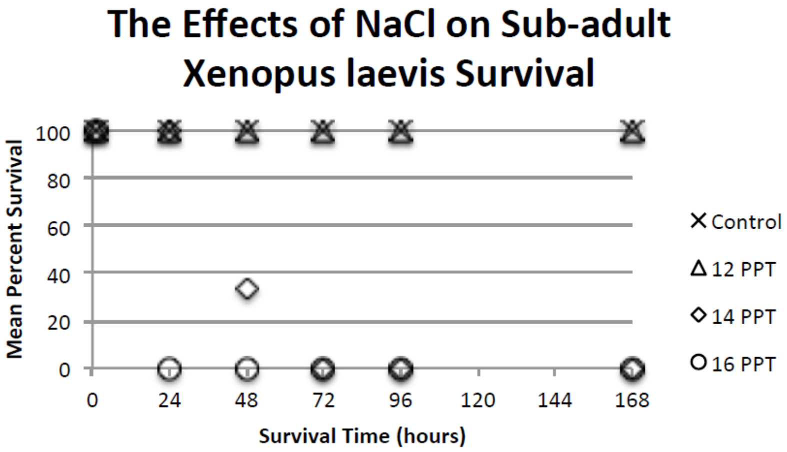


Figure 11. ACF survival under variable sodium chloride (NaCl) concentrations (personal communication, J. Gross).

After the treatment, the pond was visually searched for animals and agitated with rakes over multiple days. In total, 513 ACF, 3 bullfrogs, 2 native red-legged frogs, 258 native newts, and 9,671 introduced goldfish were recovered during the salt treatment. These animals were dead or dying. In February 2018 a large, gravid female was caught in the treated pond. In July 2018, an additional 28 juveniles and 2 metamorphs were captured indicating continued and recent recruitment. In October 2018, stormwater sewer maintenance activities revealed that ACF were more extensively distributed throughout the stormwater network than previously thought and were likely the source of ACF that recolonized the treated pond. The extent of their spread in the stormwater drainage network is still unknown to WDFW and future collaborative work with the City of Lacey is necessary to determine any continued ACF spread in the drainage network.

Other chemical options including copper sulfate, rotenone, and lime were explored but not deemed feasible due to the proximity of ESA-listed salmon.

Electroshocking

Smith Root Inc evaluated the use of electrical fields as a containment barrier and for herding to facilitate ACF capture and eradication. They noted that the frogs would dive to the bottom as soon as they felt the initial electricity. Once the frog came up for air and were immobilized via the second shock, they would either: 1) stay afloat because of air retained in their lungs or 2) release the air and sink. Because of this second scenario there is a limited period for netting frogs and so ultimately electroshocking was not pursued further.

Biological Control

Biological Controls are a possible measure for reducing the impacts of introduced species and were briefly considered for ACF with different predatory fish species (see **Table 2**). However, biological controls have complex positive and negative attributes in their implementation that are challenging to balance. For instance, while biological controls may directly harm the introduced species, they likely also harm native species. Bass have been successfully used in South Africa (personal communication, J. Measey), but ACF may evacuate the waterbody to escape predation. Containment and capture of fleeing ACF would be a necessary component of biological control to mitigate further spread, this approach cannot be effective on its own.

5.2. BOTHELL

5.2.1. Containment

Containment fences at waterbodies with introduced ACF has not been attempted because it is viewed as unfeasible due to the presence of ACF in unconfined wetlands with strong connectivity to North Creek.

5.2.2. Past Management Actions

In September 2015, 40 ACF were removed from “Ground Zero”. In August 2016, two of seven additional locations revealed ACF. During Winter 2016, an additional 29 ACF were removed from “Ground Zero”. In July 2017, six minnow traps were set overnight at “Ground Zero” yielding 2 additional animals. In May 2019, USGS researchers trapped an additional 4 animals. No work has been conducted at the North Creek sites since 2019. Reconnaissance surveys to revisit ACF occupied and other nearby waterbodies was proposed for 2020 but, due to COVID-19 impacts to fieldwork, these surveys were not conducted.

6. NEXT STEPS TO INFORM A MANAGEMENT PLAN

Future management recommendations for ACF will rely on building and maintaining partnerships in addition to insights gained from targeted research. Because ACF pose a risk to native species through predation, competition, and disease there is an urgent need to study the extent to which invasive ACF can be managed. Based on our current limited understanding of ACF in Washington State, we cannot assess the risks ACF pose to the state’s native species relative to other invasive species. These limitations severely hinder our ability to determine if invasive ACF are contained to several urban stormwater ponds in disparate regions of Puget Sound, if ACF has the potential to significantly spread throughout the region and negatively impact a broader suite of aquatic habitats and species, and the extent to which management options – including eradication – are feasible. We emphasize that existing data gaps preclude developing a robust management plan for ACF.

A top priority for WDFW is continuing partnering with City of Lacey and cultivating additional partnerships to monitor and control ACF. Containment at the Lacey Stormwater Ponds relies on a partnership with the City of Lacey and cross-program ad-hoc efforts. Funding has come from different agency programs to fill this critical need. Without ACF designated funds, a cross-program commitment may be necessary to maintain current containment measures. Such a commitment could support a sustained partnership with the City of Lacey and build new partnerships to evaluate the threat of and management potential for ACF beyond Lacey.

Aquatic invasive species that pose threats to non-game species and terrestrial and semi-aquatic invasive species are not currently addressed by WDFW. Given ACF have multiple similar attributes to another serious invasive amphibian – the American bullfrog (*Rana catesbeiana*) – there is an urgent need to reconsider how the state manages species like these amphibians which are not strictly aquatic. Doing so may require a more comprehensive management strategy that includes but is not restricted to the Aquatic Invasive Species Unit (AIS). Beyond having sufficient knowledge to manage ACF, AIS currently does not have the financial capacity to effectively manage aquatic invasive species and "implement the findings and broad authorities provided by the state Legislature under Chapter 77.135 RCW"[1]. These limitations extend to the study and management of ACF. ACF management has largely relied on ad-hoc cross-program support to maintain the vital partnerships with City of Lacey. However, no control work has taken place since 2017. A 2021 legislative budget request of $2.8 million for AIS did not include support for ACF. Although AIS was ultimately allocated $6 million, it is unclear whether some of that funding may be allocated towards ACF given European green crabs and Zebra and Quagga Mussels remain top management priorities by Fish Program. Because of this and with diverse expertise across the agency, there may be opportunities for a broader wildlife invasive species coordinator to support management of ACF, strengthen cross-program collaboration, and build the partnerships that are necessary to manage introduced species.

Our literature review suggests that released aquarium pets are a major cause of ACF invasions. Based on the limited known occurrence of ACF in the state we suggest that there may be value in enhancing education and outreach. For example, education campaigns like "Don't let it loose" by RCO's Invasive Species Council could help reduce new introductions. In addition to maintaining partnerships, policies and outreach that prevent introductions should be a top priority for WDFW. Furthermore, because it is unknown if eradication of the three currently identified populations is feasible, early detection of new populations will be essential to a rapid response and mitigation efforts. The AIS reporting system[2], which is managed by the Washington Invasive Species Council, relies on voluntary reporting and does not provide a comprehensive status of the species' extent. The AIS unit maintains a rapid response program as part of the Agency's Policy 5310 Managing Invasive Species. Even so, currently AIS does not have enough capacity to respond to new or existing ACF reports. Further investment into

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[1] 2021 Legislative Session: Budget Information, Funding for emerging issues in 2021-2023 (https://wdfw.wa.gov/about/administration/budget/update#)

[2] https://invasivespecies.wa.gov/report-a-sighting/invasive-animals/

community engagement that helps report new populations will be an essential component of future management efforts.

6.1. KNOWLEDGE GAPS AND RESEARCH NEEDS

The largest data gaps with respect to invasive ACF ecology in Washington are 1) not knowing the full extent of ACF's distribution, 2) uncertainty about the novel pathogens ACF may carry, and 3) whether ACF cause local extirpation of native amphibians or fish and whether coexistence between invasive ACF and native species is possible. Although ACF represent likely risks to aquatic ecosystems in Washington, addressing these three unknowns is essential to addressing the extent of ACF's threat and developing a management plan. Targeted surveys adjacent to areas with known ACF introductions would inform the extent of spread and the means by which ACF spread (i.e., via stormwater drainages and/or by natural means like creeks). Additionally, a broader series of surveys across habitat throughout the Puget Sound region will be essential in understanding whether ACF have been introduced or have spread to other habitats beyond what is currently known. Identifying the strains of *Bd* and *Ranaviruses* will be critical in determining the disease risks ACF poses to Washington's native aquatic species.

Beyond knowledge gaps pertaining to the ecology of ACF in Washington, we are limited in our understanding of the efficacy of various management tools for ACF. In particular, we do not know which methods are most suitable for detecting and managing ACF, how effective trapping is for determining occupancy of ACF especially at sites with low densities, or how detection probability is related to environmental variation such as water temperature, depth, and seasonality. Additionally, non-permanent waterbodies may facilitate movement or continue to host ACF that burrow into muddy substrate where they can survive during extended periods of drought. Understanding how these factors influence detection probability could inform when and how to trap ACF for detection and control efforts. A variety of detection and removal methods are suitable for experimental testing and should be rigorously evaluated before being deployed at scale. Furthermore, it is likely that multiple control measures will be needed to manage ACF, although this needs to be experimentally determined.

Addressing the efficacy of management tools and of eradication is important because incomplete eradication of invasive species can have unintended consequences. For instance, in some invasive species scenarios, attempts to remove the invader without complete eradication can result in a compensatory response (known as the "hydra effect") where the invasive species' population quickly recovers (Grosholz *et al.* 2021). WDFW should assess the extent to which control and eradication attempt may exacerbate the threats ACF pose or be a costly problem to address but never solve.

WDFW also needs resolved our data management and information sharing approach within and outside the agency. We lack a consistent data management structure that documents our efforts. Having an information sharing approach would facilitate a more efficient analysis of attempted efforts and success. For instance, a Microsoft Access database that enforces data structure would help facilitate summarization and cross-program utilization of data. Additionally, the occurrence of ACF in Washington State is not documented in peer-reviewed scientific literature and therefore is underreported in international research. The substantial effort put into salt treatments and trapping could be published in the peer-reviewed literature to help inform

ACF management issues. Importantly, publishing this work would make this issue better understood by the broader research and management community which could help foster new collaborative partners. Currently, the financial support to publish this work is missing.

6.1. RECOMMENDED NEXT STEPS

We propose prioritized next steps in ACF management that vary in effort and investment (**Table 3**). These include support to maintain and build partnerships that are essential to ACF management (Priorities 1 & 2) and validation of environmental DNA (eDNA) as a tool to rapidly and affordably monitor ACF spread (Priority 3). This proposal also includes multiple research efforts that would provide the necessary data to inform a risk assessment and ACF management plan (Priorities 4-7). These efforts include surveying to document the true extent of current ACF populations and associated spread and the studies necessary to assess the efficacy of various control methods. Purposeful management of ACF can only happen with an informed risk assessment. Such a risk assessment would ideally happen early in a species' invasion which ACF in Washington presumably are, although data on the extent and timing of their introduction and spread are sparse. Depauperate data on ACF in Washington preclude informed risk assessment and purposeful management and the outlined priorities would help meet these data needs.

Table 3. Critical Research Needs and Proposed Next Steps.

Priority	Action	Estimated Cost
1	**Maintain Partnership with City of Lacey** Funds for WDFW to participate in routine maintenance of stormwater sewers to make sure that ACF are not transported away from the occupied site within the debris.	$10,000 annually
2	**Build and Maintain New Partnerships** Pursue new partnerships with faculty at Pacific Lutheran, St. Martin's, and other universities. Invest in better understanding the management of stormwater ponds in Bothell and Issaquah and seek willing partners in the control of ACF in those areas. Bolster public outreach efforts to minimize further ACF releases and increase reporting of ACF observations.	$10,000 annually
3	**eDNA Pilot Study** Evaluate the utility of eDNA as a tool for rapid assessment of ACF populations in Washington.	$45,000 total; one-time expense
4	**Assessment of ACF extent** Includes eDNA and conventional trapping methods to determine the likely extent of ACF at three known ACF locations.	$100,000 total; repeat at 3-5 year interval
5	**Testing Various Eradication Measures** Experimental tests of different approaches to eradicate ACF and whether eradication is feasible.	$100,000 total; one-time expense
6	**Pathogen (Ranavirus and Chytrid fungus) Assessment** Surveys at all three known ACF locations.	$40,000 total may be repeated at 5-year intervals
7	**Spread Assessment at New Localities** Random sampling of stormwater ponds with eDNA and conventional methods to evaluate cryptic spread of ACF and other AIS.	$100,000 total may be repeated at 3-5 year intervals

6.1.1. Maintain Partnerships

The agency's current partnership with City of Lacey is at risk under the current ad-hoc approach. There is a consistent need each Fall to sift the debris removed from storm sewer catch basins which is a labor-intensive task but one which provides essential data on ACF presence, abundance, and location. Maintaining partnerships will require sustained commitment from WDFW. Estimated annual cost: $10,000.

6.1.2. Build New Partnerships

A commitment to ACF management may be required to leverage participation by external organizations. We recommend pursuing new partnerships with faculty and staff at nearby academic institutions including St. Martin's as well as other universities. Given ACF's association with stormwater facilities, a better understanding of the design and management of stormwater ponds in Bothell and Issaquah (and other possible future sites) would inform the ecology of ACF in Washington and what mechanisms may facilitate ACF management. Seeking willing partners like regional water resources engineers and stormwater managers will be pivotal in developing this cross-disciplinary insight. Additionally, bolstering public outreach efforts to minimize further ACF releases and increase reporting of ACF observations is key to the agency's management of ACF and identifying partners that can facilitate public engagement will be valuable (Section 7). Additional potential partnerships are outlined below in the Partnership section. Estimated annual cost: $10,000.

6.1.3. Environmental DNA Pilot

Environmental DNA (eDNA) is a cutting-edge monitoring tool that involves sampling for DNA shed from target organisms into their environment. It has been used to assess spread of ACF (Secondi *et al.* 2016; Vimercati *et al.* 2020) and *Ranavirus* (Miaud *et al.* 2019). ACF have been detected using eDNA at a density as low as 1 individual/100 m^2 (Secondi *et al.* 2016). An eDNA approach could be scaled for different objectives and budgets that range from sampling in waterbodies adjacent to invades sites to broader efforts to identify additional ACF invasions. eDNA of an additional three species (including an array of native and exotic fishes, amphibians, and other aquatic species) could be assayed simultaneously with ACF to identify co-occurrence. We propose a pilot project in **Appendix 2** that includes a sampling design and cost estimate for materials. Estimated total cost: $45,000.

6.1.4. Assessment of ACF extent

Key to informing future management recommendation is understanding whether ACF are currently restricted to three known localities or whether ACF has spread to or been subsequently introduced to other locations. We recommend surveys that join eDNA and conventional trapping methods to determine the likely extent of ACF at three known ACF locations. The status of ACF spread in the North Creek and Tibbets creek areas are entirely unknown. The 2020 spread assessment activities in Bothell were postponed due to COVID-19 delays and prior efforts in

Bothell in 2017 and 2019 were conducted only at the known site and simply confirmed continued occupancy. Adjacent waterbodies in Bothell have not been trapped to assess spread since 2016. No effort has been made by WDFW in Issaquah. Estimated total cost: $100,000.

6.1.5. Testing Various Eradication Measures

The current state of knowledge on various control measures (e.g., chemical or trapping) prohibits adequately developing an ACF management plan. Targeted, controlled experiments of various control measures are essential to understanding the extent to which ACF can be contained and the feasibility of eradication. Estimated total cost: $100,000.

6.1.6. Pathogen (Ranavirus and Chytrid fungus) Assessment

Given pathogens are a concern for aquatic invasive species generally, including ACF, an investigation of the pathogens known to harm amphibians would inform how much of a risk ACF pose to native amphibians with respect to disease. Currently, the status of *Ranavirus* and *Bd* in Bothell and Issaquah is entirely unexplored and data from Lacey is limited. Estimated total cost: $40,000.

6.1.7. Spread Assessment at New Localities

Beyond exploring the spread of ACF near known localities, assessing spread more broadly in the region would inform the extent of risk and management needs associated with ACF. A random sampling of stormwater ponds using eDNA and conventional methods is recommended to evaluate cryptic spread of ACF and other aquatic invasive species. To date, all known ACF populations are in urban stormwater ponds and prior work around the Puget lowlands suggests that stormwater ponds may help propagate another aquatic invasive amphibian, the American bullfrog (Ostergaard et al. 2008). Given the close affinity of ACF and invasive bullfrogs with urban stormwater ponds, there is an important need to invest further research in understanding the roles these engineered waterways play in the spread of these and other aquatic invasive species and what design plans may be most helpful in minimizing their use by aquatic invaders. Estimated total cost: $100,000

6.2. LONG-TERM MANAGEMENT: LACEY STORMWATER PONDS

Containment- Maintaining containment barriers around each pond and screening overflow outlets is the least expensive management options and requires relatively low staff hours except for during and after storm events. However, containment requires long-term extensive coordination and cooperation by landowners. Regularly conducted stormwater maintenance activities (e.g., vacuuming catch basins) also has a high risk of moving ACF among locations. Even, so, such activities can help monitor for ACF as the species has been detected in the sediment spoils deposited from the trucks.

Control – These activities require a high cost of long-term capture and disposal activities with uncertainty surrounding how much effort is needed to diminish a target population and prevent spread to new sites.

Eradication – The complexity of stormwater drainage networks imposes a high cost and high uncertainty of success for ACF eradication. More thorough and extension survey and monitoring data as to ACF presence and abundances throughout the drainage system would help inform how extensively they are distributed and where to target eradication efforts. The addition of molecular or capture-mark-recapture data may also help inform whether there are ACF "hubs" in the network which produce a high number of individuals that disperse. Identifying these hubs would be critical for targeting management efforts. Rigorous data on the efficacy of various control measures are also essential to confirming eradication success in Lacey.

6.3. LONG-TERM MANAGEMENT: BOTHELL

Containment – Thorough containment requires developing a strategic plan. The "Ground Zero" pond is unlikely to be containable due to its urban setting and direct outlets to North Creek. "Richards Pond Large" and "Twin Ponds" may be suitable for containment but doing so requires research into the suitability of these sites for these activities.

Control – Adequate controlling in Bothell means a high cost of long-term capture and disposal activities with uncertainty as to the effects on the target population preventing spread to new sites.

Eradication –The strong surface water connection to North Creek means that eradication has a high uncertainty of success.

6.4. LONG-TERM MANAGEMENT: ISSAQUAH

Containment, Control, and Eradication cannot be evaluated at this point. WDFW has not had capacity to verify the extent of ACF at the site.

7. PARTNERS

To date, ACF management has been achieved through partnerships with other entities. Partner roles range from active management to helping reduce introductions of additional populations through outreach and education.

7.1. PRIMARY PARTNERS

City of Lacey, Public Works has been an active cooperative partner in supporting eradication and containment efforts but has significant resource limitations to address the ACF issue. Their specific contributions are outlined in the section Summary of Control Methods in Washington

State. Maintaining this partnership is vital for containing ACF at the Lacey Stormwater Ponds and for informing detection, monitoring, and management elsewhere in Washington.

Washington State Recreation and Conservation Office, Washington Invasive Species Council (WISC)

The WISC can help WDFW disseminate messaging surrounding the presence, spread, and possible impacts of ACF. Doing so could help prevent their release and would inform the public of how to report sightings. Additionally, WISC manages the “Report a Sighting” reporting system that includes an Invasive Animals webform, mobile app, and associated field guide. The system routes citizen reports of ACF to WISC staff, which then push the notification through to the appropriate staff in WDFW’s AIS unit. It also publishes confirmed reports to the Early Detection and Distribution Mapping system managed by University of Georgia. The mapping system shares data through publicly available distribution maps contributing to a nationwide citizen science network.

WISC also develops regional messaging campaigns to raise public awareness about invasive species. ACF management can benefit from the “Don’t Let It Loose” campaign to help prevent new releases of ACF into waterbodies in Washington State (Ex. **Error! Reference source not found.**). This outreach campaign is primarily disseminated through social media platforms. WISC also has an educational curriculum targeted at middle schoolers that raises awareness about invasive species including the impacts of released aquarium pets such as crayfish and goldfish that could be expanded to include ACF.

WA Invasives
@WAinvasives

African clawed frogs can be found in two watersheds in #WashingtonState. The #Invasive frogs harm ecosystems by competing with native species, and potentially introducing harmful pathogens. More: invasivespecies.wa.gov/priorityspecie…. #InvasiveSpecies #DontLetItLoose

CAN BE UP TO 5 INCHES LONG, NOT INCLUDING THEIR LEGS. THEY HAVE SMOOTH SKIN CLAWS ON THEIR HIND FEET.

ACF IS A PREDATORY AQUATIC FROG THAT IS HIGHLY ADAPTABLE, AND CAN SURVIVE MANY ENVIRONMENTAL CONDITIONS, INCLUDING FREEZING TEMPS AND DROUGHTS.

AFRICAN CLAWED FROGS ARE ABLE TO REPRODUCE AND BECOME ESTABLISHED IN AN ECOSYSTEM VERY QUICKLY.

11:38 AM · Jan 17, 2020 · Twitter Web App

Figure 12. Washington Invasive Species Council’s “Don’t Let it Loose” Campaign includes messaging about African Clawed Frog in Washington State.

City of Olympia, Stream Team, Michelle Stevie coordinates an annual amphibian egg-mass identification workshop and surveys (January-March). They routinely provide data to WDFW and could be encouraged to train volunteers about ACF identification and reporting. Experienced volunteers could be trained to conduct ACF surveillance at a subset of high-risk sites in Lacey using minnow trapping techniques

St. Martins University, Biology Department and/or Facilities

St. Martins University did not grant access to conduct surveys in 2020. They requested assurances that they will not be held financially liable if ACF are found on their property.

7.2. SECONDARY PARTNERS

7.2.1. Natural Resource Agencies

Natural resource agencies with offices near the Lacey Stormwater Ponds have shown an interest in being kept appraised of ACF activities. They have knowledgeable staff that can help share information about ACF and management activities. Suggested contacts include:

Department of Ecology, Senior Wetland Ecologist, Amy Yahnke

U.S. Fish and Wildlife Service, Teal Waterstrat

United States Geological Survey, the Nonindigenous Aquatic Species (NAS) information resource.

7.2.2. Other Potential Opportunities

Citizen science groups that have active amphibian egg mass monitoring groups present an opportunity for outreach and education. For example, the iNaturalist and Woodland Park Zoo's Amphibians of Washington project (https://www.inaturalist.org/projects/amphibians-of-washington). Additionally, natural history museums like the University of Washington's Burke Museum can play an active role in facilitating research on this issue and educating the public.

7.3. KNOWLEDGE SHARING PARTNERS

Domestic- California, Colorado, Florida, Massachusetts, North Carolina, Virginia, and Wisconsin have all reported ACF invasions. These states have a range of local and state agencies cooperatively managing ACF.

International – John Measey, Stellenbosch University, South Africa is the hub of an international research group with partners in multiple countries. This network may be an avenue to share knowledge about ACF invasions and management.

7.4 CHALLENGES

Efforts in 2020 to assess if there had been a spread of ACF from the Lacey stormwater ponds or drainage system were impacted by a denial of access by one landowner. The landowner was averse to the potential economic risk (management costs) of WDFW detecting AIS on their property. Recent policy advances outline protocols for entry when access is denied (POL-5310 Invasive Species Management and RCW 77.135.170), but more collaborative approaches could be explored. Uncertainty about commitment to ACF management also makes it more difficult to cultivate collaborative partnerships.

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8. APPENDIX 1 – SPECIES DESCRIPTION

ACF (also called the Platanna) are an aquatic frog and intrepid predator in the family Pipidae. They are highly divergent from native amphibians in morphology and behavior. They have been used extensively in research and widely traded as pets. Their extensive uses, in combination with the ability to easily adapt to diverse aquatic habitats, has led to their establishment in numerous aquatic ecosystems around the globe.

8.1. IDENTIFICATION

Post-metamorphic adults (frogs) – ACF have olive to brown skin, often with blotches or spots in a variegated pattern (**Error! Reference source not found.**). These frogs lack eye lids, tongues, and vocal sacs. Their front feet have relatively long unwebbed fingers that lack claws whereas their back ones are fully webbed and the outer three digits have sharp, black claws. Dimorphism exists in body size. Females average larger and reach larger maximum sizes than males, growing to larger than an adult human fist. ACF adult body size and mass range from 50 to over 140 mm (Vogt *et al.* 2017). In their native range, only females attain body size >100 mm SVL, but long-established invasive populations in California produce even larger females >140 mm SVL (Crayon 2005). We have size information for one of the two infested locations in Washington. At the Lacey location, ACF averaged 60 mm SVL (range: 41-90 mm) and all ACF >65 mm SVL were females.

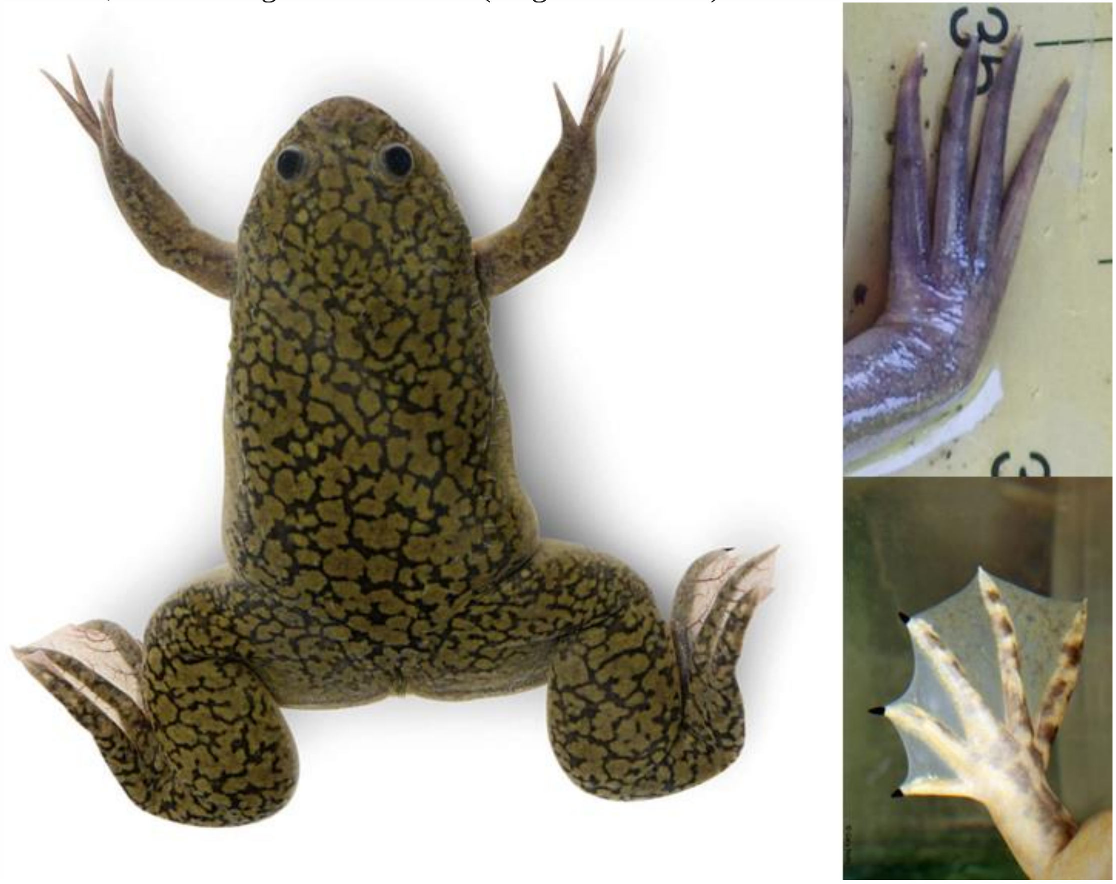

Figure 13. African Clawed Frog. A. Dorsal View. B. Front foot. C. Hind foot with webbing and claws (adapted from: CaliforniaHerp).

Larvae (tadpoles) – Larvae superficially look like a small catfish. The most prominent feature of all but the youngest tadpoles are a pair of long thin barbels that extend from each side of their chin (Error! Reference source not found.).

Figure 14. ACF tadpole with distinctive whiskers (adapted from: CaliforniaHerp).

Eggs – Eggs are deposited singly or in small clusters and are attached to hard structure in fresh waterbodies (**Figure 15**) (Ringeis *et al.* 2017). Clutch sizes are believed to be large based on ovarian egg counts that range in the thousands of eggs, but field observations are scarce, which may reflect their highly scattered presumably concealed nature. Developmental stages have been photographed and characterized in laboratory settings (see Nieuwkoop and Faber 1994).

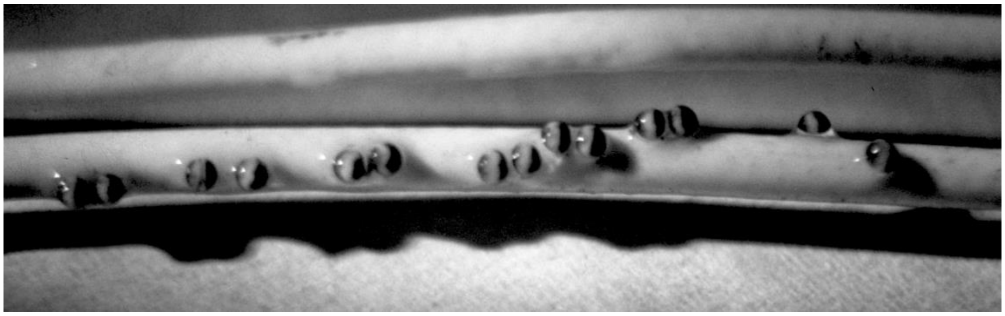

Figure 15. Small clusters of ACF eggs laid on an underwater cable in a pond (Photo: Ringeis *et al.* 2017).

8.2. NATURAL HISTORY

Reproduction – ACF has a short generation time, long life span, an extended breeding season, and prolific reproduction (Measey and Tinsley 1998; Green 2002). Females deposit many small eggs in freshwater. At about the body size when ACF first produce eggs, a 67-mm SVL female had ~2,700 eggs (McCoid and Fritts 1989). However, egg production increases exponentially with size, for example, a 104-mm SVL female contained ~17,000 eggs (McCoid and Fritts 1989). Eggs are scattered in the aquatic habitat because they are laid singly or a few at a time on varied substrates (aquatic plants, rocks and other structures)(Crayon 2005). Introduced ACF in California have been recorded reproducing nearly year-round (January-November), but reproduction typically occurs in spring (March to June)(Crayon 2005). Year-round reproduction is likely in introduced environments in western North America. Like other frogs in the family Pipidae, both their vocalizations and hearing are adapted to underwater conditions. Courtship calls are made by contracting the intrinsic laryngeal muscles and are composed of a series of clicks that differ in repetition rate and frequency (Tobias *et al.* 1998; Ringeis *et al.* 2017).

Development – Development is rapid. Field and laboratory populations reach metamorphosis in about two months, and development from egg to maturity in females requires only about eight months (Gasche 1943; McCoid and Fritts 1989). For a frog, ACF is relatively long-lived. It has been recorded to survive 15-16 years in captivity (Flower 1937; Wager 1965) and at least 23 years in feral populations in Wales, UK (Measey and Tinsley 1998; Tinsley *et al.* 2015b).

Diet –Larvae filter-feed largely on unicellular phytoplankton: alga, diatoms, protozoans and bacteria (Crayon 2005), and have even been known to filter virus-sized particles from water (Wassersug 1996). In contrast, while post-metamorphic ACF are omnivorous (Prinsloo *et al.* 1981), they are predators, with a diet similar to that of American bullfrogs (*Rana catesbeiana*) in that they will eat any live prey that they can successfully overpower that is not too distasteful (including other frogs, fish, birds and snails). Also, like bullfrogs, ACF are opportunistic, with a diet that varies with condition and location. However, ACF diet is more plastic than that of bullfrogs due to scavenging carrion. Benthic invertebrates and large zooplankters dominated the diet of ACF introduced in a South Wales pond (Measey 1998). The diet of introduced ACF in stream habitats in Portugal were also dominated by benthic prey (in this case water snails), but native fishes and amphibians were also consumed (Amaral and Rebelo 2012). In a focused feeding trials, ACF was found to readily prey upon both larval and adult Pacific chorus frog (*Pseudacris regilla*) (Wilson *et al.* 2018b) a common species native to Washington State. ACF have also been observed consuming species of true frogs (*Rana)* and toads (*Bufo = Anaxyrus)*. When present, tadpoles make up the majority of the diet of large adult ACF (Vogt *et al.* 2017). Among amphibians, Crayon (2005) reported the strongest evidence for a negative effect of predation on native amphibians is for Western toads, keeping in mind that the Western toad taxon in California may not be equivalent to that in Washington State. Lafferty and Page (1997) reported consumption of the small federally Endangered tidewater goby in California. ACF have the capacity to find and eat immobile fish eggs (Crayon 2005). ACF commonly cannibalize their eggs and larvae (Measey 1998; Vogt *et al.* 2017).

Habitat – In their native African range, ACF is known to utilize a variety of waterbodies including seasonal rain pools. Over their very broad introduced range, the species has been observed using a much broader array of habitats in both still- and flowing-water, including small streams (Amaral and Rebelo 2012; Moreira *et al.* 2017) and rivers (Crayon 2005; Torreilles and Green 2007). Larval ACF occur in habitats ranging from permanently flowing, air-saturated streams (Moreira *et al.* 2017), to temporary ponds with very low values of dissolved oxygen, and even stagnant pools in buffalo wallows (Hastings and Burggren 1995). Introduced populations have repeatedly shown plasticity in habitat characteristics such as food availability, vegetation, substrate, turbidity, salinity, water temperature, hydrology and food availability (Crayon 2005). The highest densities are reached in permanent, eutrophic, fish-free waters that have soft substrates and submerged vegetation, and do not freeze over but remain above 20°C for most of the year (Crayon 2005, Hill *et al*. 2017). Further, ACF introduced to stream and rivers systems in California have migrated both up- and downstream using human-created waterbodies as "stepping stones" to invade new habitats (Van Dijk 1977). The scavenging ability of ACF enables them to use a broader range of habitats, particularly those of lesser quality.

Thirty years after their introduction in California, Crayon (2005) made five key generalizations about patterns of ACF distribution:

1) Stream systems are vulnerable to complete colonization.
2) Some barriers (climatic and biological) seem to retard the spread of ACF.
3) Desert wetlands can sustain ACF populations.
4) Few freshwater aquatic habitats are not at risk of colonization.
5) Most populations are derived from independent introduction events

These generalizations are likely to apply to ACF in Washington State, though we would expect the first to be less absolute because of climate gradient differences. We underscore the fourth generalization as particularly important, especially in Western Washington due to the milder Mediterranean climate. We also note that the relatively short development time to metamorphosis, combined with their aestivation abilities, allow them to occupy non-permanent aquatic habitats. However, there are potentially limits to these abilities, though they remain poorly understood.

Surfacing activity is greatly diminished during colder months in California populations. Nevertheless, frogs in water bodies that ice up at the edges during the winter remain active enough to come to baited traps (Crayon 2005).

Movement – ACF are often referred to as "purely aquatic", but overwhelming evidence suggests extensive overland movement (Measey 2016; Courant *et al.* 2019). Reports of moves vary from 40 m to over 2 km (De Villiers and Measey 2017), and stream network corridors appear to be commonly used as migration corridors. In their native range, opportunistic migrations to other water sources have been observed when ponds dry up (Crayon 2005; Ringeis *et al.* 2017).

Similar behavior has been observed in their introduced range, but plasticity in behavior is indicated under different local conditions. For example, mass migration was observed during the draining of San Joaquin Reservoir in Newport Beach, California in 1984 (Crayon 2005). At a critical but unspecified low water level, the resident ACF population migrated in mass from the reservoir in one night and were seen traveling over nearby roads. This reservoir has a solid asphalt bottom that precludes frogs digging down to avoid desiccation. Overland movements do not appear confined to wet seasons or conditions, but midday is avoided in movement timing (Measey 2016). Overland dispersal had to be the mode of colonization of many ponds in France (Fouquet and Measey 2006; Courant *et al.* 2019). Movement rates reported some introduced populations appear larger that values reported for native populations (DeVilliers and Measey 2017), and greater dispersals have been observed at the edge of the range in at least one.

Predators - ACF produce a large amount of an extremely slippery mucus from skin glands when harassed (Crayon 2005). Dogs attempting to eat ACF foam at the mouth in response to these skin secretions (Hey 1949). Data are lacking on native North American non-fish predators that might prey on ACF. Larvae are weak swimmers and school in deep waters making them especially vulnerable to predation (Crayon 2005). A variety of birds, warmwater fish and garter snakes are known to prey on ACF (Crayon 2005). Largemouth bass (*Micropterus salmoides* have been used as a biological control in fish ponds in South Africa (Prinsloo et al. 1981).

Environmental Tolerance – ACF may be well suited to adapt to the stressors of increasing temperatures (as anticipated with climate change) and decreasing water depth during the warm season in different ways (Crayon 2005). Having evolved in a Mediterranean climate, they tolerate severe drought via cocoon-like aestivation in fine substrates (Wager 1965; Balinsky *et al.* 1967) and a wide range of water temperatures, ranging from <4C (<39.2F) under ice (Prinsloo *et al.* 1981) up to 28C (82.4F) (Brown 1970). They can survive at least 8 months of starvation in this state (Hewitt and Power 1913), but the actual limits of survivability in the cocooned condition are unknown. In southern California, ACF dug 30-40 cm deep pits in the mud of evaporating ponds where water remain 10°C below surface water temperatures (McCoid and Fritts 1980). ACF can also alter body fluid concentrations via retention of urea, and in this hypertonic state, minimize water loss to the surrounding substrate (Balinsky *et al.* 1967). This ability makes them one of the most saltwater tolerant frog species and facilitates their invasion of brackish water habitats (Munsey 1972, Romspert 1976).

8.3. USES

ACF are common in the pet trade and are used extensively in laboratory research. By 1970, ACF was the world's most widely distributed amphibian (Van Sittert and Measey 2016). The ability to obtain eggs in all seasons, a relatively short lifecycle and the ability to resist disease and survive in captivity helped their rapid proliferation in laboratory use (Gurdon and Hopwood 2000). The widespread global exportation of ACF date as far back as the early 1930s, when ACF were used for pregnancy testing (Shapiro and Zwarenstein 1934), but with the advent of a chemical pregnancy test in the 1960s that use rapidly declined. Simultaneously, after World War II, ACF were adopted as a "model organism" and used for a large range of research including the widely adopted toxicology methodology, frog embryo teratogenesis assay: Xenopus (FETAX) (Dumont *et al.* 1983). Interestingly, ACF were the first vertebrate to be cloned (Gurdon *et al.* 1958) and have been studied in space (Snetkova *et al.* 1995). ACF continue to have major importance in biomedical research. ACF have also been extensively used in teaching for a wide range of educational purposes from classroom pet to dissection (Gurdon and Hopwood 2000). The pet trade has emerged as a major potential source of ACF introductions into non-native range. In the early 2000's approximately 99% of imports were for the pet trade, the majority of these were declared to be captive bred (Measey 2017). Unwanted pets being released to the wild is a likely source of some invasive populations (Measey *et al.* 2012).

8.4. GEOGRAPHIC RANGE

African clawed frogs are native to sub-Saharan Africa and were originally imported to the United States for laboratory use and as pets (Measey *et al.* 2020). ACF are believed to be the most widespread invasive amphibian on the planet (Measey 2017) and can be found in at least 48 countries on four continents. Infestations of the species has been reported in at least nine states across the contiguous United States (**Figure 16**).

Figure 16. Cumulative locations of reported ACF in the Nonindigenous Aquatic Species Database managed by USGS (Basemap ESRI).

Outside of North America, invasive populations have also been reported in South America, Europe, and Asia (Measey et al 2012). Establishment of invasive populations has been most successful in areas with a Mediterranean climate resembling environmental conditions of the southwestern African Cape region of ACF origin (Ihlow *et al.* 2016), but the persistence of populations for decades in cooler environments suggests a capacity for long-term adaptation (Measey and Tinsley 1998). Moreover, recent research emphasizes that the global invasion potential of ACF has been severely underestimated, with vast areas being potentially vulnerable to invasion (Measey *et al.* 2012). It has also been suggested that climate change could enhance this species' invasion potential in regions where the climate is marginally suitable (Tinsley *et al.* 2015b). However, both recent disappearance of some populations in some areas regarded as marginally suitable climatically (Tinsley *et al.* 2015b) and recent modeling efforts contradict this conclusion (Ihlow *et al.* 2016).

9. APPENDIX 2 – EDNA PILOT PROPOSAL

Environmental DNA (eDNA) is an emerging tool that can facilitate surveying for aquatic species in a cost- and time-efficient manner, especially when species are at low density. We propose an eDNA pilot project to validate the efficacy of established protocols in known ACF populations in Washington and to use this tool in other locations to assess ACF spread. Contamination is a potential risk when sampling for low-quality DNA samples, such as eDNA (Goldberg *et al.* 2016). We propose to utilize the rigorous quality control protocol developed by the Asian Carp Monitoring program's Quality Assurance Project Plan, both in the field and laboratory to ensure samples are not contaminated (USFWS 2015). Before sampling begins at a site, all equipment (peristaltic pump, water bottles, etc.) will be either wiped down with or submerged in a 50% bleach solution (Kemp and Smith 2005; Champlot *et al.* 2010), and subsequently rinsed with DI water. Water sampling will be conducted with a peristaltic pump. At each site, we will collect 200 ml in 10 locations around a body of water and combine samples into two 2 L whirlpaks (**Figure 17**). Each 2 L water sample will be filtered through a 1.0 µm pore size filter. At the end of each sampling day, we will filter 500 ml of sterile water for an equipment control to monitor potential contamination from the filtering equipment. Filters (stored in 15mL of desiccant beads) will be stored at room temperature until DNA extraction.

All laboratory work will be performed in AirClean 600 Work Stations (ISC Bioexpress, Utah, USA), which are equipped with HEPA air filters and UV lights. All work surfaces will be wiped down with 50% bleach and exposed to UV light for at least one hour before work begins. We will test for the presence of ACF (Secondi *et al.* 2016) and at least two other aquatic species of interests (e.g., Chinook salmon ,*Oncorhynchus tshawytscha*; Coho ,*Oncorhynchus kisutch*; bullfrog, *Rana catesbeiana*) using species specific qPCR primers and probes (Ostberg, unpublished).

Filter samples will be cut in half, and one of the halves will be used per extraction. eDNA filter subsamples will be extracted with Qiagen DNeasy Blood & Tissue and Qiashredder kits (Qiagen, Inc.), as per Pilliod and colleagues (2013). DNA samples will be processed through OneStep PCR Inhibitor Removal kits (Zymo Research), to remove inhibitors that are typically abundant in Lake/Pond samples. Each filter sample will be qPCRed in triplicate. Each qPCR will include a 10-fold serial dilution of the species of interest (e.g. ACF, Chinook salmon, Coho) amplicons (gBlock from IDT). Additionally, qPCRs will include an extraction blank and a negative template control (water) to assess for laboratory contamination. We will consider samples positive for detection when two out of three qPCR triplicates result in a positive amplification (Cycling threshold, $C_T \leq 40$), as per Turner and colleagues (2014). In order to rule out field contamination leading to false positives in the lab, we will extract the equipment control associated with paired samples.

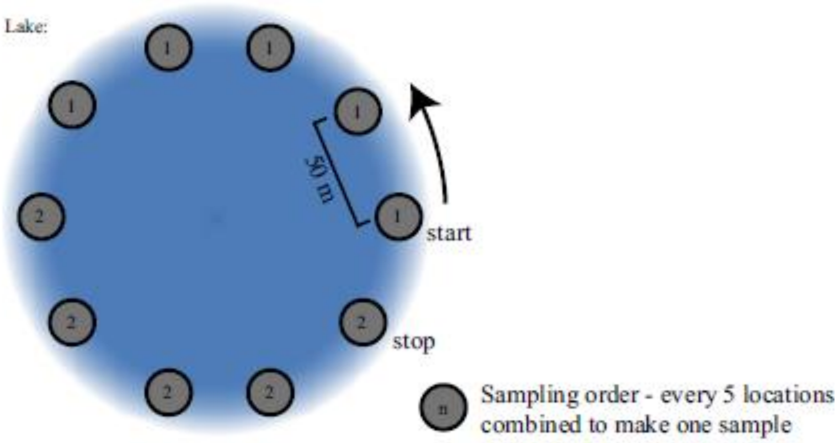

Figure 17. Schematic of pond/lake sampling. Adapted from Bedwell et al. 2020

In addition to utilizing environmental DNA to detect the presence of salmonids or amphibians of interest, eDNA has increasingly been used to detect aquatic pathogens (Chestnut et al. 2014; Miaud et al. 2019; Vilaça et al. 2020; Hall et al. 2016; Hall et al. 2018). Ranaviruses (family Iridoviridae) are double stranded DNA viruses that commonly infect amphibians, fish and reptiles (Gray and Chinchar 2015). Ranaviruses are especially lethal to amphibian tadpoles, in some cases causing 90% mortality. Environmental DNA monitoring for the detection of Ranaviruses allows for the rapid identification of a potential outbreak, in comparison to surveys for clinical signs or histological changes (which involve lethal sampling) (Miaud et al. 2019; Brunner et al. 2004; Hall et al. 2016). Recent studies utilizing eDNA samples to detect Ranavirus have shown that viral DNA titers increased in the environment prior mass mortality events (Miaud et al. 2019; Hall et al. 2016).

Water samples collected above will be analyzed additionally for Ranavirus eDNA detection. Samples will be amplified using primers and probes from Leung et al. (2017). Each qPCR will include a 10-fold serial dilution of the species of interest (e.g. African clawed frog, Chinook salmon, Coho) amplicons (gBlock from IDT). Additionally, qPCRs will include an extraction blank and a negative template control (water) to assess for laboratory contamination. We will consider samples positive for detection when two out of three qPCR triplicates result in a positive amplification (Cycling threshold, CT ≤ 40), as per Turner et al. (2014). In order to rule out field contamination leading to presumptive positives in the lab, we will extract the equipment control associated with paired samples.

We propose a pilot project that would sample 50 sites (ponds/lakes/streams) near known ACF occupied waterbodies.

Materials:

Option 1 Backpack Sampler Method

Smith-Root ANDe backpack= 79.43/filter = $7,943 (50 sites, two filters per site)

The estimates do not include cost of an ANDe backpack. Genetics lab has two, and the AIS team has one. Alternatively purchasing one would cost about $6,000.

Option 2 Peristaltic pump method

Peristaltic pump = 68.26/filter = $6,826 (50 sites, two filters per site)

$493 one-time purchases.

eDNA Sampling- One-time Purchases	Reactions per unit	Unit	Company	Catalog #	Price per case/ unit
Peristaltic pump head	1	Each	Cole-Parmer	EW-07518-12	$278.00
Tubing		1 each	Cole-Parmer	Ew-96410-24	$103.00
Cordless Drill		1 each	Target	LDX120C	$50.00
Rubber stopper (#8)	12	pack	Fisher Sci	14135M	$62.00

The remainder of the budget in Table 3 is for travel and staff time to collect samples, analyze data, and write up the results.