Biological Controls

U.S. ARMY CORPS OF ENGINEERS **Building Strong®**

ANS Control: Biological Control[1,2] –Introduced Predatory Fish Species (Triploid Grass Carp,and Molluscivorous and Piscivorous Fish), Introduced Predatory Insect Species, *Pseudomonas fluorescens* CL 145A, and Targeted Disease Agents.

Targeted Species: The use of predatory species, including insects and triploid grass carp (*Ctenopharyngodon idella*), as biological control agents has been developed for management of both aquatic and terrestrial plants. Dotted duckweed (*Landoltia* (*Spirodela*) *punctata*) and water chestnut (*Trapa natans*) are the only ANS of Concern – CAWS[3] that can be controlled by triploid grass carp. There has been considerable research on the use of insect agents to control water chestnut; however, to date, none of these insect agents have been approved for release in the U.S.

Triploid grass carp are herbivorous fish commonly used to control unwanted aquatic vegetation.

Introduced predatory fish species and targeted disease agents have been considered for controlling silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*). Molluscivorous fish may be effective for control of the greater European pea clam (*Pisidium amnicum*), European fingernail clam (*Sphaerium corneum*), and the European stream valvata (*Valvata piscinalis*).

Pseudomonas fluorescens CL 145A, is under development as a biopesticide for controlling mollusks; possible ANS of Concern – CAWS that may be affected by *P. fluorescens* CL 145A include greater European pea clam, European fingernail clam , and European stream valvata.

Selectivity: Insect biological control agents approved for release in the U.S. have undergone extensive host-specificity testing and are considered selective for their target host. Triploid grass carp are non-selective feeders and will consume most aquatic vegetation. Predatory carnivorous fish species eat small fish, mussels, and other invertebrates, and targeted disease agents are currently being researched for application in the wild to affect the minnow family of fishes. *Pseudomonas fluorescens* CL 145A was developed specifically for control of *Dreissena* mussels but has activity on golden mussels (*Limnoperna fortunei*) as well and may have activity on other mollusk species.

Developer/Manufacturer/Researcher: The use of insects for biological control applications must follow strict procedures and regulations specified by the U.S. Department of Agriculture (USDA). Currently, there are no vendors for insect biological control agents that can be used for control of plant ANS of Concern – CAWS. There are numerous State natural resource agencies and commercial

[1] A common name for the term biological control is 'biocontrol.'
[2] Some forms of biological control have been addressed in separate fact sheets. Please see the fact sheet labeled "Deleterious Gene Spread" for information on genetic methods for biological control of non-native fishes.
[3] For a complete list of the 39 specific ANS of Concern – CAWS, please see Table 1 of the main report.

vendors that could supply live predatory fish. Examples of certified triploid grass carp suppliers include J. M. Malone & Sons (Lonoke, Arkansas) and Keo Fish Farms (Keo, Arkansas).[4] Targeted disease agents are in the conceptual stage of development and there are currently no manufacturers of this control technology. Marrone Bio Innovations (Davis, California) is the developer of *P. fluorescens* CL 145A.

Pesticide Registration/Application: Pesticides, including microbial biopesticides, must be applied in accordance with the full product label as registered by the U.S. Environmental Protection Agency (USEPA). Users must read and follow the pesticide product label prior to each application. The registration status, trade name, and availability of pesticides are subject to change. The listing of a pesticide in this fact sheet or Appendix B does not represent an endorsement by the U.S. Army Corps of Engineers or the USEPA regarding its use for a particular purpose.

Brief Description: Biological control is broadly defined as the planned introduction of one organism (the biocontrol agent) to reduce pest populations of another organism (the target species) to economically acceptable levels (Ross & Lembi 1985; Perry et al. 2000; Cuda 2009a). Biological control rarely results in eradication of the target pest species, but rather, suppresses growth and reproduction of the target species. Schooler et al. (2004) reported that a successful biological control agent will reduce the density of a target species to a desired level and maintain it there with minimal risk of damage to non-target species. Often, biocontrol agents are imported from the native range of the target species (i.e., a "natural enemy"). It is critical that a biological control agent prey specifically on the target species and not on native, non-target organisms. Substantial research, planning, and care are needed to avoid introducing additional pest species (Cox 2004).

Biological control agents currently used for management of invasive plants include insects, pathogens (bacteria, fungi and viruses), herbivorous fish (triploid grass carp and tilapia), and grazing animals (goats and sheep) (Ross & Lembi 1985; McIntosh et al. 2003; Coombs et al. 2004; Cuda 2009a; Colle 2009). Pathogen biological control agents have not been developed or approved for use on plant species identified as ANS of Concern – CAWS. Grazing animals have not been widely used to control aquatic and wetland plants such as those identified as ANS of Concern – CAWS.

Biological control for fishes includes the introduction of carnivorous fish species (e.g., northern pike (*Esox lucius*), walleye (*Sander vitreus*), largemouth bass (*Micropterus salmoides*)), species in the Salmonidae family (hereafter referred to as salmonids), and the development of targeted disease agents as biological control agents against invasive fish. In addition, microbial biopesticides (e.g., *P. fluorescens* CL 145A) have been investigated and are under development as a biological control alternative for managing invasive mollusks.

There has been considerable research on the development of insect agents to control water chestnut. Pemberton (1999, 2002) conducted extensive surveys for natural insect enemies of water chestnut in Northeast Asia and Europe. Of the insects found, a leaf beetle (*Galerucella birmanica*) was the most common and the most damaging species in Asia, causing complete defoliation of water chestnut plants

[4] Manufacturers and products mentioned are examples only. Nothing contained herein constitutes an endorsement of a non-Federal entity, event, product, service, or enterprise by the U.S. Army Corps of Engineers or its employees.

(Pemberton 1999; Ding et al. 2006a; Ding et al. 2006b). Although water chestnut continues to be a problem in North America, biological control research on this species in the U.S. was suspended in 2002 (Pemberton 2002), primarily due to lack of program funding.

Introduced Predatory Fish Species –

Triploid Grass Carp: Grass carp were initially imported into the U.S. in 1963 by the United States Fish and Wildlife Service (USFWS) as a biological control alternative to chemical control methods for aquatic vegetation. This fish is native to Eastern Asia and has specialized grinding teeth (called pharyngeal teeth) and a long intestine, which allow it to shred and digest aquatic plants as its principal food source (Sanders et al. 1991). Grass carp can survive for up to 25 years and can grow as much as 10 lbs in a year if adequate food is available (Colle 2009). Triploid grass carp have three sets of chromosomes and are incapable of producing viable offspring (Sanders et al. 1991), which minimizes the risk of stocking a non-native fish. They are produced by combining eggs and sperm from diploid grass carp (diploid having two sets of chromosomes) and then shocking the fertilized eggs in the early stages of development, using temperature, pressure, or chemicals (Sanders et al. 1991; Colle 2009). The USFWS offers a National Triploid Grass Carp Inspection and Certification Program for resource agencies in the United States and in other countries to help agencies protect their aquatic habitats (U.S. Fish and Wildlife Service, 2011). This inspection and certification program provides assurance that shipments of grass carp alleged to be triploid do not, within the confidence limits of the inspection program, contain reproducing diploids.

Triploid grass carp are considered general herbivores and will consume almost any plant material (including grass clippings); however, they have preferences for some plants over others (Colle 2009). Once the preferred plants have been depleted, triploid grass carp will consume most other plants, with the exception of Eurasian watermilfoil (*Myriophyllum spicatum*). For a list of the preferred plant species consumed and controlled by grass carp, see Miller & Decell (1984) and Sanders et al. (1991). The only ANS of Concern – CAWS listed as a preferred food source of triploid grass carp are duckweed species (*Lemna* and *Landoltia*). While not a preferred food source, Krupauer (1971) reported that repeat stocking of grass carp controlled 80 to 100% of submersed aquatic plants in Central and Eastern Europe, including water chestnut.

Caution should be used when considering grass carp as a control strategy because of their potential to become a high impact invasive species. Grass carp can be detrimental to native vegetation.

To achieve effective control of nuisance aquatic vegetation, triploid grass carp must be stocked in sufficient numbers such that the rate of consumption by the fish is equal to or greater than the growth rates of plants (Sanders et al. 1991). Stocking proper numbers of fish is important and depends on several factors, including size and age of fish, density and species of plants to be consumed, size of the waterbody, seasonal water temperature, and whether other control practices (herbicide or mechanical treatments) are employed (Sanders et al.

1991; Lewis 1998). Simulation models which consider these parameters are available and can be used to determine suitable stocking rates (Stewart & Boyd 1999). Lewis (1998) reported that proper stocking of triploid grass carp can result in a 75 to 90% reduction of target plant species in 3 to 4 years.

Grass carp can be used in conjunction with other management strategies, such as mechanical harvesting or aquatic herbicides. Typically, herbicide application or mechanical harvesting is utilized prior to stocking of fish (Sanders et al. 1991). Because triploid grass carp are non-selective herbivores, there may be instances where native vegetation should be protected.

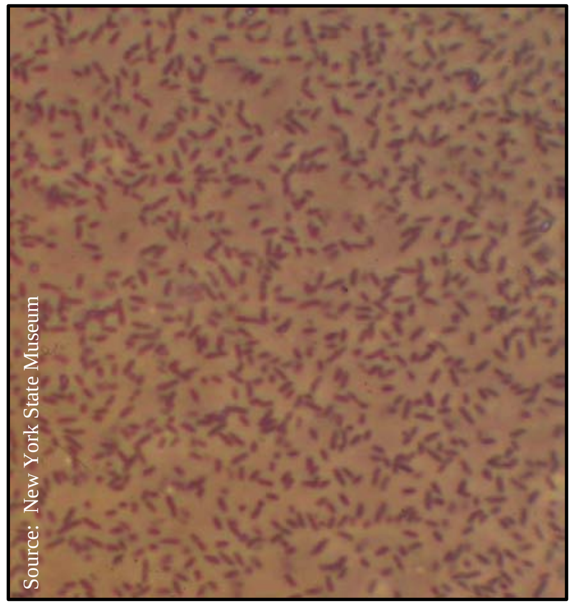
Piscivorous Fish: Natural resource managers have stocked piscivorous fish species in the U.S. for over a century for the purpose of enhancing recreational fishing opportunities (Nielsen 2010). This technique has been used to control invasive fish species in the Great Lakes (Mills et al. 1993). There are two common outcomes of stocking fish predators: replacement of native predators or an increase in predator species richness (Eby et al. 2006). In created aquatic environments, such as the Chicago Sanitary and Ship Canal, there are no native species; however, stocked piscivorous fish may migrate into the tributaries that flow into created aquatic environments, disrupting ecosystems outside of the treatment area.

Molluscivorous Fish: Natural resource managers could increase the abundance of several fish species already present in the CAWS that eat mollusks through stocking propagated fish. Native molluscivorous fish species include the freshwater drum (*Aplodinotus grunniens*) and pumpkinseed (*Lepomis gibbosus*), and non-native molluscivorous fish species include the common carp (*Cyprinus carpio*) or round goby (*Neogobius melanostomus*) (Kirk et al. 2001). If not contained, molluscivorous fish have the potential to migrate to adjacent habitats and disrupt ecosystems outside of the treatment area.

Introduced Predatory Insect Species – Numerous insect biocontrol agents have been developed and approved for release on more than 25 invasive target plants in the U.S. (Coombs et al. 2004). Of these 25 target plants, only eight are aquatic and/or wetland species. The USDA's Animal and Plant Health Inspection Service, Plant Protection and Quarantine is responsible for controlling introductions of species brought into the U.S. for biological control of plants, in accordance with the requirements of several plant quarantine laws, the National Environmental Policy Act, and the Endangered Species Act. Petitions for release of plant biological control agents are evaluated by a Technical Advisory Group, which represents the interests of a diverse set of federal and non-Federal agencies (Cofrancesco & Shearer 2004; Horner 2004; Cuda 2009b).

Pseudomonas fluorescens CL 145A – The bacterium, *P. fluorescens* CL 145A, is under development as a commercial biopesticide (proposed product name, Zequanox™). *Pseudomonas fluorescens*, a common soil microbe with worldwide distribution, is naturally present in all North American aquatic sediments and typically functions to protect plant roots from disease. In laboratory screening trials, Dr. Dan Molloy of the New York State Museum discovered a strain of *P. fluorescens*, CL 145A, with lethal activity against *Dreissena* species (Molloy 1998). Cells of *P. fluorescens* CL 145A contain a natural byproduct that acts as a toxin to destroy epithelial cells in the digestive system of susceptible mussels (Molloy 2001; Molloy & Mayer 2009). As filter feeders, susceptible mussels readily ingest the bacterial cells; exposure causes no adverse reaction to feeding, as opposed to typical chlorination treatments which cause mussels to close their inhalant siphon tube (Molloy & Mayer 2007). As a result of this discovery, Dr. Molloy obtained patents in both the U.S. (Molloy 2001) and Canada (Molloy 2004) for use of *P. fluorescens* CL 145A as a method for controlling invasive *Dreissena* mussels.

Marrone Bio Innovations is currently developing *P. fluorescens* CL 145A as a biopesticide (Zequanox™) for invasive mussel control (Marrone Bio Innovations 2011). The biopesticide formulation will contain dead cells of *P. fluorescens* CL 145A, since it was shown in laboratory studies that dead cells of this bacterial strain were equally lethal against *Dreissena* species as live cells (Molloy & Mayer 2007, 2009). Marrone Bio Innovations received approval (Section 3 registration) for the active ingredient, *P. fluorescens* CL 145A, from the USEPA in July 2011 (Marrone Bio Innovations 2011). USEPA-approval of the commercial formulation of this product (Zequanox™) is pending, but is expected in March 2012.



***Pseudomonas fluorescens* strain CL 145A, active ingredient in Zequanox™, a new biopesticide under development for control of invasive mollusks**

Targeted Disease Agents – There are three different diseases that mainly affect the minnow family (Cyprinidae), which includes silver and bighead carp: spring viremia of carp (SVC), koi herpes virus (KHV), and carp pox. Spring viremia of carp was first reported in North America in 2002 (Goodwin 2002) and confirmed in common carp taken from the Calumet-Sag Channel near Chicago in 2003 (Nelson 2003). Ideally, the proliferation of these diseases in the CAWS could reduce the health and abundance of common, silver, and bighead carp without affecting non-target organisms.

Prior Applications:

Introduced Predatory Fish Species –

Triploid Grass Carp: Triploid grass carp are widely used to control unwanted aquatic vegetation throughout the U.S. Grass carp have been so effective at aquatic weed control that they are now used in 35 different states (Colle 2009). Their primary use is in aquaculture and closed public and private waterbodies, but they are also used in large lakes and reservoirs.

Piscivorous Fish: Stocking predatory fishes (e.g. northern pike, walleyes, and largemouth bass) has been used commonly by fisheries managers in the past to control early life stages of common carp. Several species of non-native salmonids were successfully introduced into the Great Lakes beginning in the mid-1960s to control invasive rainbow smelt (*Osmerus mordax*) and alewife (*Alosa pseudoharengus*) (Stewart et al. 1981). However, these fish control projects have highly variable effectiveness and have rarely included adequate monitoring to determine success (Meronek et al. 1996). A review of manipulative field studies showed that in about 75% of cases, generalist predators, whether single species or species assemblages, reduced pest numbers significantly (Symondson et al. 2002). Programs to stock predators as a means of reducing prey populations must consider the size and abundance of the predator, the size and abundance of the target prey, the size and abundance of alternative prey, and the physical-chemical characteristics of the habitat. Unfortunately, little is known about the susceptibility of bighead, black, grass, and silver carps, to native piscivores (Conover et al. 2007).

Molluscivorous fish: Fishery hatchery managers have used molluscivorous fish to control snails in fish culture ponds (Carothers & Allison 1968). Computer simulation models have been used to estimate the effect of molluscivorous fish as a control for zebra mussels. Bioenergetics modeling suggested that there is a strong correlation between the effectiveness of molluscivorous fish and water temperature. Model results indicate that fishes in southern latitudes consumed up to 100 percent more food than those in northern systems because of increased metabolism. (Eggleton et al 2003).

Introduced Predatory Insect Species – The use of insects as biological control agents for aquatic nuisance plant species has yielded mixed results. Currently, alligatorweed (*Alternanthera philoxeroides*), purple loosestrife (*Lythrym salicaria*) and melaleuca (*Melaleuca quinquenervia*) are being successfully controlled using insects released as biocontrol agents (Cuda 2009b). Gangstad (1976) reported that the total acreage of alligatorweed controlled by the USACE was significantly reduced over a 10-year period as a result of releasing the alligatorweed flea beetle (*Agasicles hygrophila*). In addition, the cost of using herbicides to control this weed was reduced by 75% after agent release. Insect biological controls are commonly utilized in combination with other control technologies in an integrated pest management (IPM) approach.

Pseudomonas fluorescens CL 145A – Preliminary laboratory and facility trials showed that a 6-hour exposure of 50-100 parts per million (ppm) dry bacterial mass per unit volume consistently provided > 90% mussel mortality (Molloy & Mayer 2007). Pilot-scale facility treatments and field demonstrations are ongoing at: Davis Dam, Bullhead City, AZ (Nibling et al. 2010); Ontario Power Generation; and DeCew II Generating Station near Niagara Falls (Van Oostrom et al. 2010). To date, the results of these trials showed 53.5% adult mortality (2+ year-old mussels) and 82.5-100% mortality of pediveligers and other juvenile life stages of *Dreissenid* mussels following treatment with *P. fluorescens* CL 145A. To date, this control technology has not been evaluated over a wide variety of field conditions, such as open water systems.

Targeted Disease Agents – The introduction of targeted diseases has been widely discussed, however, natural resource agency managers are reluctant to introduce a disease that cannot be controlled in the wild and whose effects are not fully known. Targeted diseases would likely be highly regulated and only considered on an experimental level.

General Effectiveness:

Introduced Predatory Fish Species –

Triploid Grass Carp: Triploid grass carp consumption rates, which are measured as the daily percentage of body weight eaten, are affected by the size of the fish and environmental factors such as water temperature, oxygen content and salinity levels (Colle 2009). Colle (2009) reported that large grass carp (> 15 lbs) consume up to 30% of their body weight daily, whereas smaller fish (< 10 lbs) can consume as much as 150% of their body weight in one day. Maximum consumption occurs when water temperatures are at 78 to 90 °F and is greatly reduced below 55 °F (Colle 2009). Similarly, consumption of plants by grass carp is reduced by 45% when oxygen content in the water falls below four ppm (Colle 2009). Specific to duckweed species, Miller and Decell (1984) reported that a 35.2 gram (g) grass carp can consume 436 to 700 g of duckweed per day. Grass carp consumption rates for water chestnut have not been reported.

Piscivorous Fish: Experience in aquaculture indicates that bighead and grass carps are highly susceptible to predacious fishes, but little is known about which native predators will prey effectively on bighead, black, grass, and silver carp, at what sizes, and the effects of environmental factors or habitat types on this relationship (Conover et al. 2007). Research is needed to determine which native predator fish can effectively prey on Asian carps, the vulnerability (sizes and life stages) of Asian carps to predation, and the stocking size and density of predators required for effective population control. Bioenergetics models have shown that some predatory fish are very good at controlling prey populations. Simulations of alewife consumptions by stocked salmonids suggest that as much as 20 to 33% of the annual alewife production in Lake Michigan may be consumed annually (Stewart et al. 1981).

However, Asian carps grow very rapidly, achieving a length of almost 12 inches by the end of the first year of life (Williamson & Garvey 2005). The size of the predatory fish's mouth, also known as its gape-size, restricts the predator's ability to consume larger fish. A prey fish is too large to be consumed by a predatory fish when the width of a prey fish reaches the predator gape-size limit (Nilsson & Brönmark 2010). Stocked piscivorous fish would only have a short window when their prey would be small enough for them to consume, therefore they would have to survive on smaller native species for most of the year.

Many invasive fish species can tolerate poorer water quality and higher water temperatures (USEPA 2008); consequently, the survival requirements of stocked piscivorous fish must be considered.

Molluscivorous fish: The long-term reduction of invasive mussels by natural predators has yet to be demonstrated (Molloy et al. 1997; Molloy 1998).

Introduced Predatory Insect Species – There are no insect biological control agents currently approved for release in the U.S. for use against ANS of Concern – CAWS. Insects such as *G. birmanica*, however, are known to be important pests to cultivated water chestnut in China and India (Pemberton 1999, 2002).

Pseudomonas fluorescens CL 145A – The bacterium, *P. fluorescens* CL 145A, is effective for controlling veligers and adult life stages of zebra and quagga mussels (*Dreissena polymorpha* and *D. bugensis*, respectively) and golden mussels. The product has not been tested on the three (3) invasive mollusk species identified as ANS of Concern – CAWS (greater European pea clam, European fingernail clam, and European stream valvata); it is possible, however, that these species are also sensitive to *P. fluorescens* CL 145A.

Targeted Disease Agents – Spring viremia of carp (SVC) can be highly fatal in young fish with mortality rates up to 70%. In Europe, where this disease has been endemic for at least 50 years, 10 to 15% of one-year-old carp are lost to SVC each year (U.S. Department of Agriculture, Animal and Plant Health Inspection Service 2003). Koi herpes virus (KHV) is a highly contagious disease that may cause between 80 to 100% mortality in susceptible populations, with signs of disease most commonly being expressed when water temperatures are between 72 and 81 °F (22 to 27 °C) (Ornamental Aquatic Trade Association 2001). Carp pox is closely related to KHV but is less fatal. This disease weakens the fish with infection and lesions, leaving it susceptible to secondary infections by other microorganisms.

Operating Constraints:

Introduced Predatory Fish Species – Triploid grass carp are best used in waterbodies with no outflow; this ensures fish will stay in the area where they are needed and minimizes potential impacts to downstream vegetation. To prevent loss of triploid grass carp during flooded conditions, containment devices such as mesh fence or screens are needed at all potential overflow points. Restocking may be necessary due to predation or offsite migration in unconfined systems. The use of triploid grass carp or piscivorous fish requires continuous surveillance and manipulation (restocking) to assure effectiveness and to minimize unwanted side effects (denuding the waterbody of all vegetation or native fish).

Introduced Predatory Insect Species – Once regulatory approval has been obtained for release, the success of a predatory insect species' introduction and the subsequent impact on the target or host species will depend on the establishment of a viable population. Factors that can influence successful establishment include climatic extremes, host incompatibility, predation, competition, parasites, and disease (Coombs 2004).

Pseudomonas fluorescens CL 145A – Performance of *P. fluorescens* CL 145A in flowing water systems and effects on native mussel species are unknown, however, evaluations are ongoing to determine suitability of product use as a treatment in open waters such as lakes and reservoirs (Heilman et al. 2010). Current success with this product is largely based on results of in-line pipe treatments at power generating facilities. There is some evidence that product performance can vary under certain environmental conditions, such as: soft waters with pH less than 7.4; low O_2 levels (< 2 ppm); and highly turbid waters can all reduce efficacy (Molloy & Mayer 2007). In addition, susceptibility of mussels to *P. fluorescens* CL 145A increases with water temperature (> 90% mussel mortality at 23 °C) (Molloy & Mayer 2007).

Targeted Disease Agents – The use of Targeted Disease Agents would require additional research and development prior to implementation of this technology. Unintentional consequences such as the impact of disease agents to non-target organisms, the risk of disease transfer to other waterbodies, and the environmental factors that affect disease performance and proliferation are unknown. There is some evidence that disease incidence for the koi herpes virus is best expressed when water temperatures are between 72 to 81°F (22 to 27°C) (Ornamental Aquatic Trade Association 2001).

Cost Considerations:

Introduced Predatory Fish Species – The considerations below apply to triploid grass carp and other piscivorous and molluscivorous fish.

Implementation: Implementation costs of this Control would include purchase, delivery and stocking of certified predator fish (i.e. triploid grass carp, walleye, northern pike, and redear sunfish). Stocking rates of triploid grass carp vary with density and acreage of vegetation to be controlled; however, in general terms, the stocking rates for grass carp in southeastern reservoirs ranges from 15 to 20 fish per vegetated acre. The benefits of stocking grass carp can extend more than 7 years (Sanders et al. 1991; Colle 2009). The stocking rate, stocking size, and density of piscivorous and molluscivorous fish predators required for effective population control should be modeled to determine the feasibility and the potential impacts to native species.

Planning and design activities in the implementation phase may include research and development of this Control, modeling, site selection, site-specific regulatory approval, plans and specifications, and real estate acquisition. Design will also include analysis of this Control's impact to existing waterway uses including, but not limited to, flood risk management, natural resources, navigation, recreation, water users and dischargers, and required mitigation measures.

Operations and Maintenance: Operations and maintenance cost considerations include an ecosystem monitoring plan and restocking.

Mitigation: Design and cost for mitigation measures required to address impacts as a result of implementation of this Control cannot be determined at this time. Mitigation factors will be based on site-specific and project-specific requirements that will be addressed in subsequent, more detailed, evaluations.

Introduced Predatory Insect Species – The development of insect biological control is time consuming. On average, it takes 11 to 13 years of research to develop a classical biological control program for a single weed species (Andres 1977). This includes overseas surveys for agents, research on host specificity, clearance/approval of the most promising control agents, and full-scale release programs. While considerable research has been conducted to develop insect agents for water chestnut, these insects have yet to receive approval for release in the U.S. Without the availability of agents, the cost of implementing insect biological control agents against water chestnut cannot be realized.

Implementation: Implementation factors specific to this Control are unknown at this time. Planning and design activities in the implementation phase may include research and development of this Control, modeling, site selection, site-specific regulatory approval, plans and specifications, and real estate acquisition. Design will also include analysis of this Control's impact to existing waterway uses including, but not limited to, flood risk management, natural resources, navigation, recreation, water users and dischargers, and required mitigation measures.

Operations and Maintenance: Operations and maintenance factors are unknown at this time.

Mitigation: Design and cost for mitigation measures required to address impacts as a result of implementation of this Control cannot be determined at this time. Mitigation factors will be based on site-specific and project-specific requirements that will be addressed in subsequent, more detailed, evaluations.

Pseudomonas fluorescens CL 145A – The cost of the product is unknown at this time; until registration of the commercial formulation is approved by the USEPA (expected in March 2012), this biopesticide is unavailable for sale.

Implementation: The implementation of this Control would include planning, design, and application of the product. Planning and design activities in the implementation phase may include research and development of this Control, modeling, site selection, site-specific regulatory approval, plans and specifications, and real estate acquisition. Design will also include analysis of this Control's impact to existing waterway uses including, but not limited to, flood risk management, natural resources, navigation, recreation, water users and dischargers, and required mitigation measures.

Operations and Maintenance: Operations and maintenance costs would depend on dosage and application, and would include effectiveness monitoring.

Mitigation: Design and cost for mitigation measures required to address impacts as a result of implementation of this Control cannot be determined at this time. Mitigation factors will be based on site-specific and project-specific requirements that will be addressed in subsequent, more detailed, evaluations.

Targeted Disease Agents – The cost of introducing a disease agent is unknown. Though certain diseases have been found in the CAWS already, the widespread introduction of an infectious disease would require careful deliberation by regulatory and public health agencies.

Implementation: Implementation factors specific to this Control are unknown at this time. Planning and design activities in the implementation phase may include research and development of this Control, modeling, site selection, site-specific regulatory approval, plans and specifications, and real estate acquisition. Design will also include analysis of this Control's impact to existing waterway uses including, but not limited to, flood risk management, natural resources, navigation, recreation, water users and dischargers, and required mitigation measures.

Operations and Maintenance: Operations and maintenance factors are unknown at this time.

Mitigation: Design and cost for mitigation measures required to address impacts as a result of implementation of this Control cannot be determined at this time. Mitigation factors will be based on site-specific and project-specific requirements that will be addressed in subsequent, more detailed, evaluations.

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