Alternate Control Strategy for Dreissinids Using Carbon Dioxide

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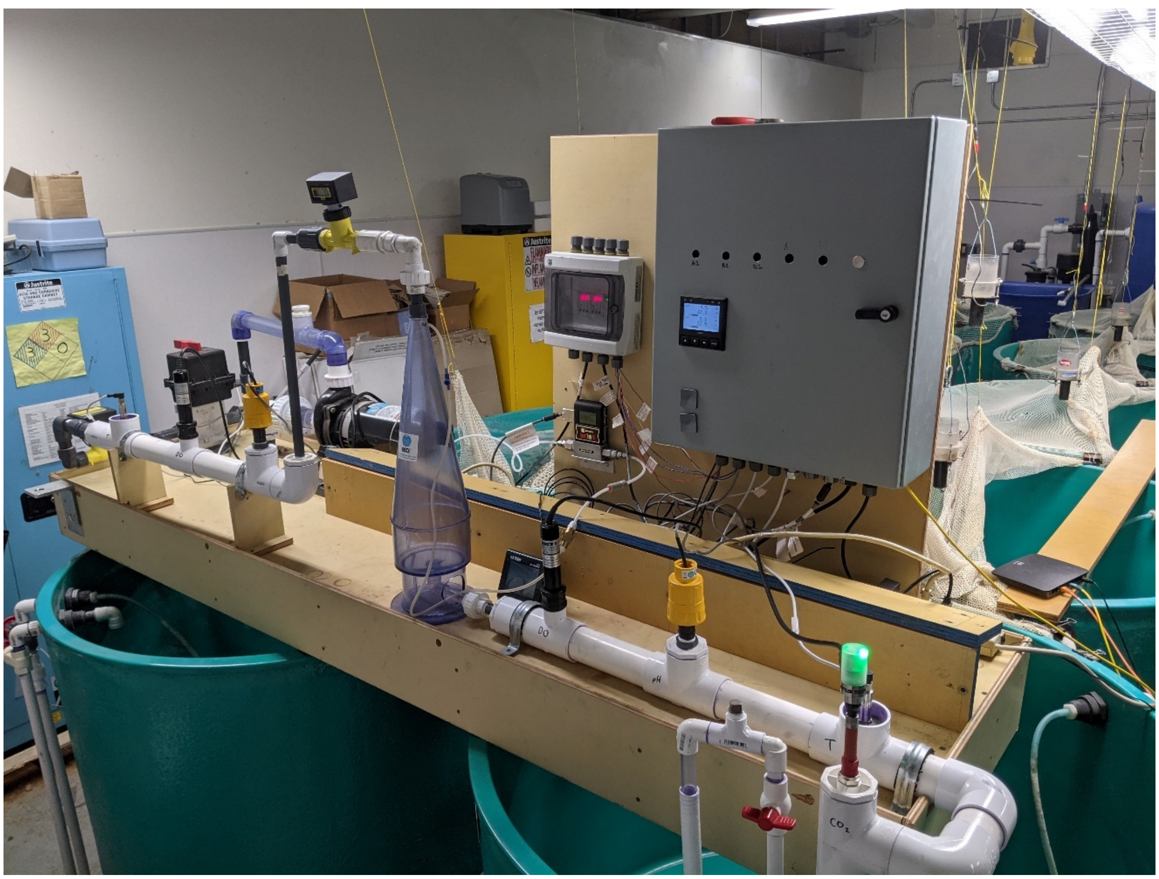

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14. ABSTRACT Zebra and quagga mussels (*Dreissena ssp.*) are major macrofouling species that impact the operations and maintenance of Reclamation water delivery systems. There is a need for an economical and environmentally safe control strategy for these invasive mussels within Reclamation structures. One potential control strategy involves the use of carbon dioxide (CO_2) to prevent the settlement of zebra and quagga mussels inside of Reclamation facilities. To determine the feasibility of using CO_2 for this purpose, this project was split into a two-prong research approach to investigate the parameters required for successful implementation. The first prong will research CO_2 treatment regimens required to prevent dreissenid settlement. A field study is being planned at Davis Dam during spring 2022. This portion of the research project will be conducted using a mobile biotesting laboratory and test on actual Lake Mohave water and quagga mussel veligers to determine effective concentrations of CO_2, minimum effective exposure period and CO_2 treatment efficacy with different water chemistry at different locations. This part of the research project is being done in partnership with UMESC. The second prong of this research project is investigating the use of a Speece Cone as the most efficient method of carbonation of reservoir water at Reclamation dam and powerplant facilities.			
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Acronyms and Abbreviations

APHA	American Public Health Association
$CaCO_3$	calcium carbonate
CO_3^-	carbonate ion
CO_2	carbon dioxide
HCO_3^-	bicarbonate ion
IAA	interagency agreement
Reclamation	Bureau of Reclamation
RISE	Reclamation Information Sharing Environment
S&T	Science and Technology
UMESC	Upper Midwest Environmental Sciences Center
USGS	U.S. Geological Survey

Measurements

ºC	degrees Celsius
h	hour
hr	length of survival time in hours
LC_{50}	median lethal time
LT_{50}	lethal concentration with 50% mortality
mg/L	milligram per liter
min	minute
mm	millimeter
mm Hg	millimeter mercury (pressure)
Pco_2	partial gas pressure carbon dioxide
pH	potential hydrogen
torr	1/760 of one standard atmosphere (pressure)

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Executive Summary

Zebra and quagga mussels (*Dreissena ssp.*) are major macrofouling species that impact the operations and maintenance of Reclamation water delivery systems. There is a need for an economical and environmentally safe control strategy for these invasive mussels within Reclamation structures. One potential control strategy involves the use of carbon dioxide (CO_2) to prevent the settlement of zebra and quagga mussels inside of Reclamation facilities. To determine the feasibility of using CO_2 for this purpose, this project was split into a two-prong research approach to investigate the parameters required for successful implementation. The first prong will research CO_2 treatment regimens required to prevent dreissenid settlement. A field study is being planned at Davis Dam during spring 2022. This portion of the research project will be conducted using a mobile biotesting laboratory and test on actual Lake Mohave water and quagga mussel veligers to determine effective concentrations of CO_2, minimum effective exposure period and CO_2 treatment efficacy with different water chemistry at different locations. This part of the research project is being done in partnership with UMESC. The second prong of this research project is investigating the use of a Speece Cone as the most efficient method of carbonation of reservoir water at Reclamation dam and powerplant facilities. Since water chemistry varies with location, it is necessary to ultimately test out the Speece Cone on site where CO_2 may be used to prevent settlement of zebra and quagga mussels inside Reclamation dam and powerplant facilities. This will be done when COVID-19 travel restrictions are lifted. Since very little information exists on the operations and use of Speece Cones for carbonation, laboratory-scale studies are being performed to determine the relationships between CO_2 dosage levels with key water quality parameters such as alkalinity and pH.

1. Introduction

Zebra and quagga mussels (*Dreissena ssp.*) are major macrofouling species that impact the operations and maintenance of Reclamation water delivery systems. There is a need for an economical and environmentally safe control strategy for these invasive mussels within Reclamation structures.

Carbon dioxide is a naturally occurring gas and an alternative to registered molluscicides for control of dreissenid mussels due to low costs, wide availability, ease of application, natural occurrence in freshwater systems, lack of harmful chemical residues or disinfection byproducts, and low risk to human health. Recent laboratory and field studies have shown success using carbon dioxide in causing mortality and a reduction in the rate of byssogenesis (settlement and colonization) in dreissenids. This project proposes investigating the effectiveness and applicability of carbon dioxide under field conditions found in Reclamation facilities. This project also proposes to develop and test the most efficient process for the carbonation of infested flowing raw reservoir water that will prevent veliger settlement and colonization within Reclamation structures.

Much of this research is being done in close collaboration with research scientists from the Upper Midwest Environmental Sciences Center (UMESC) of the U.S. Geological Survey (USGS). An Interagency Agreement was approved with funding obligated by the Research and Development Office to work closely with UMESC scientists to investigate effective treatment strategies using carbon dioxide for reducing dreissenid mussel biofouling at Davis Dam and Powerplant. In parallel with the field testing at Davis Dam and Powerplant, engineers from Reclamation's Water Treatment Group (86-68190) will be testing the carbonation efficiency of a newly constructed Speece Cone and the adaptation of this new technology for the carbonation of flowing raw reservoir water within Reclamation water delivery structures.

1.1 Project Background

There is a pressing need for new economical and environmentally safe control strategies for zebra mussels and quagga mussels in closed water conduits, open channels, and reservoir systems. Aquatic species, in general, are intolerant to increases in dissolved CO_2 concentrations (Pco_2) given its effect on water, blood pH, and hemolymph pH. These species are also sensitive to elevated total dissolved gas pressure. USGS and other research scientists have been exploiting these sensitivities by developing a control method based on manipulation of Pco_2. Water entering or held within the area impacted is supersaturated with CO_2 and held in this condition for a selected exposure period. The water can then be stripped of CO_2 and the CO_2 may be recovered for reuse. Sources of carbon dioxide needed for elevation of Pco_2 include commercial bulk liquid or combustor/furnace exhaust streams.

In an early study, Elzinga and Butzlaff (1994) reported that elevation of Pco_2 induces a narcotizing effect on mussel species such as Asian clams at a concentration of 100 mg/L and toxicity at a concentration of 500 mg/L. The LC_{50} values they established for zebra mussels at 48h and 96h were 196 and 74 mg/L (18ºC) and 290 and 81 mg/L (22ºC), respectively. Effects of Pco_2 treatment were size dependent with the 1-5 mm size class less vulnerable to treatment than the 6-10 mm and 11-15 mm size classes tested. Sub-lethal narcotizing effects were induced after only short (4 h) exposure periods as indicated by a gaping response. McMahon et al. (1995) subjected zebra mussels and Asian clams to hypercapnic, anoxic and normoxic water at 25ºC. Here, a P_{CO_2} of 38 torr resulted in detachment of byssal threads and inhibition of byssal thread formation. Elevating P_{CO_2} to 75 torr resulted in complete mortality of zebra mussels (LT_{50}=78h). Survivorship (hr) was correlated to shell length. Exposure of mussels and clams to a P_{CO_2} of 760 torr (anoxic) reduced the range of LT_{50} values to between 26.0 to 40.3 h. These data suggested carbon dioxide alone or in combination with other gases could be used as an environmentally neutral molluscicide and that required exposure periods could probably be reduced further by elevating P_{CO_2} above atmospheric pressure.

Watten et al. (2005) constructed four replicate test systems that provided hyperbaric chambers needed to test the effect of elevating P_{CO_2} above atmospheric pressure on LT_{50}. Each system provides for control of the test variables P_{CO_2}, exposure time, operating pressure, dissolved oxygen, temperature and pH. Tests performed demonstrated that CO_2 is effective in causing mortality of Asian clams and a variety of other aquatic species. For example, LT_{50} values established for Asian clams decreased from a high of 270 min at P_{CO_2} = 260 mm Hg to a low of just 90 min at P_{CO_2} = 2070 mm Hg. This latter value represents a 27-fold decrease in LT_{50} below that established by McMahon et al. (1995) at a P_{CO_2} = 760 mm Hg. Gas recovery and reuse methods have also been developed that reduce gas requirements by up to 85% making the method economically and environmentally attractive in shipping applications. Further refinement of the method may be required prior to application of CO_2 in facilities operated by Reclamation or industry.

1.2 Previous Work Performed

In a white paper submitted to the Research and Development Office and the Lower Colorado Basin (LCB) Region (Kelly 2010), *An Initial Assessment of Carbon Dioxide as an Alternative Control Strategy for Quagga and Zebra Mussel Biofouling*, carbon dioxide was described as having the potential to be used as a molluscicide for quagga mussel control. Like most bivalves, these organisms are susceptible to even relatively modest increases in the ambient concentration of carbon dioxide, since they do not contain oxygen-carrying proteins to buffer blood pH. Inhibition of byssogenesis (attachment) and mortality of adult mussels have been achieved by injecting carbon dioxide-enriched gas mixtures into the water. The white paper recommended carrying out exploratory research to determine the efficacy of carbon dioxide treatment on the byssogenesis and mortality rates of quagga and zebra mussels simulating conditions in an infested pipe structure. To this end, S&T Project ID 1367 (Kelly 2013) proposed a Lake Mead study plan which described how one may perform an investigation on the use of CO_2 on site somewhere on the lower Colorado River in order to obtain experimental results that would be relevant to implementation of CO_2 in one of our facilities along the lower Colorado River.

More recent efforts by Reclamation and UMESC have focused on understanding the effects of carbon dioxide on zebra and quagga mussel settlement rates. Recent laboratory experiments performed by USGS UMESC indicated success using carbon dioxide to prevent juvenile zebra mussel settlement during 72-96 hour exposures in a laboratory with water temperature greater than 12ºC (Waller and Bartsch 2018). A more recent study by USGS UMESC tested the efficacy of CO_2 for the prevention of zebra mussel settlement, the efficiency of their designed CO_2 system, and infusion based on changing water chemistry. Instead of a laboratory-controlled study, this study took place in a mobile field trailer along the Mississippi River in the Dubuque Harbor (Dubuque, WI). This would represent a more "real world" application of CO_2 to prevent zebra mussel settlement. UMESAC (Waller et al. 2021) determined that continuous infusion of 50 and 100 mg/L CO_2 prevented settlement of zebra mussel veligers in a flow-through system during an 11-week settlement period. Levels of CO_2 dissolved in a freshwater that would be required to affect *Dreissenid spp.* settlement or mortality appeared to be dependent on key water quality parameters, including alkalinity, conductivity, pH, temperature, and organic content.

1.3 Research Partnership

Reclamation and USGS are collaborating on this project. UMESC was founded in 1959 as an USGS Science Center with a laboratory dedicated to the development of chemical agents for the control of common carp. Since then, scientists at UMESC have conducted a wide range of ecological research projects that support and investigate ecological and population concerns of the Department of Interior (DOI). Some of these projects performed research on the controls of aquatic invasive species, including zebra and quagga mussels. UMESC's primary contribution to this research project lies in their capability of conducting field research using CO_2 to prevent the settlement of zebra and quagga mussels at various locations where these invasive mussel species have established populations. UMESC and Reclamation entered an interagency agreement (IAA) to collaborate on investigating prevention of quagga mussel settlements using CO_2 at Davis Dam on the lower Colorado River.

To complement UMESC field work, Reclamation shifted its research focus on how best to carbonate raw water within Reclamation dam and powerplant facilities with CO_2. Literature research has indicated that a special aerator called a "Speece Cone" may provide the best gas-water interface for efficient transfer of gaseous CO2 to raw water (Beard et al. 2015). Commercial applications of the Speece Cone focus on increasing dissolved oxygen concentrations in lakes, rivers, harbors, and wastewaters. A basic schematic of how the Speece Cone may be installed on a raw water pipeline in Reclamation dam and powerplant facilities (e.g., powerplant raw-water cooling lines) is shown below in Figure 1. However, very little study has been done to investigate the use of the Speece Cone for carbonating raw water with gaseous CO_2. For this reason, Reclamation's role in this research project to date has been on the development and testing of a laboratory-scale Speece Cone apparatus to determine how best to achieve carbonation of water to the desired levels of pH and dissolved CO_2 concentrations that literature have indicated should provide mitigation control of zebra and quagga mussel settlements within Reclamation dam and powerplant facilities.
Once field data is obtained from the Davis Dam field work to determine the minimum effective CO_2 dosage for preventing mussel settlement, CO_2 parameters may be adjusted on the Speece Cone to achieve maximum effectiveness with minimal requirements.

Using the combined experimental results from both the laboratory optimization of Speece Cone carbonation and Davis Dam field work should lead to the opportunity to design and build a pilot-scale carbonation system for the hydropower cooling lines at Davis Dam Powerplant.

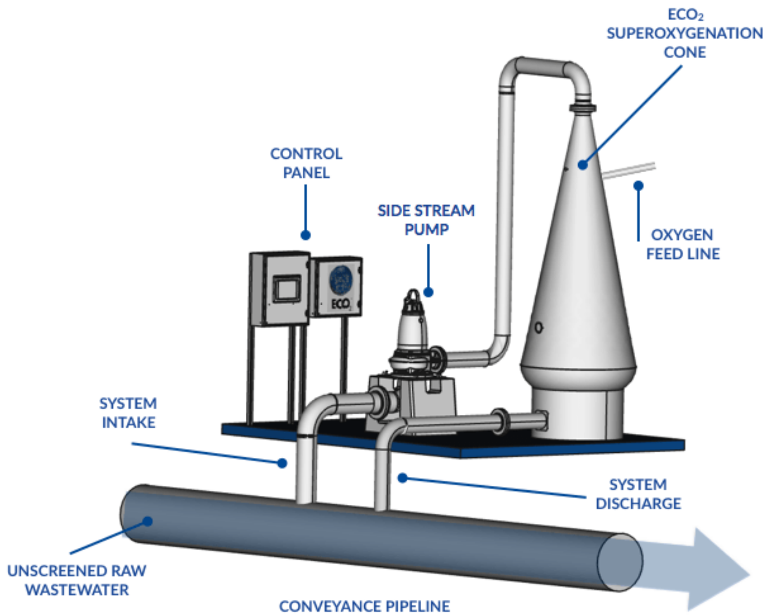

Figure 1 Side-stream Speece Cone system design. Credit: ECO2.

1.4 Objective

The main objective was to investigate the potential of CO_2 to prevent settlement of zebra and quagga mussels inside of Reclamation dam and powerplant facilities. To determine the feasibility of using CO_2 for this purpose, this project was split into a two-prong research approach to investigate the parameters required for successful implementation.

Prong 1. Develop the CO_2 treatment regimens required to prevent dreissenid settlement. A field study is being planned at Davis Dam during spring 2022. This portion of the research project will be conducted using a mobile biotesting laboratory and test on actual Lake Mohave water and quagga mussel veligers to determine effective concentrations of CO_2, minimum effective exposure period and CO_2 treatment efficacy with different water chemistry at different locations. This part of the research project is being done in partnership with UMESC.

Prong 2. Investigate the use of a Speece Cone as the most efficient method of carbonation of reservoir water at Reclamation dam and powerplant facilities. Since water chemistry varies with location, it is necessary to ultimately test out the Speece Cone on site where CO_2 may be used to prevent settlement of zebra and quagga mussels inside Reclamation dam and powerplant facilities. However, very little information exists on the operations and use of a Speece Cone for carbonation. Therefore, laboratory-scale studies are being performed to determine the relationships between CO_2 dosage levels with key water quality parameters such as alkalinity and pH.

2. Modes of Action

Carbon dioxide is a natural gas comprising approximately 0.039% (388 ppm) of the Earth's atmosphere. Successful application of carbon dioxide as a biocontrol agent for zebra and quagga mussels requires an initial understanding of its equilibrium behavior between the gaseous and aqueous phases, as well as its carbonic acid equilibrium in the water. Once carbon dioxide becomes soluble in water, a fraction of the aqueous carbon dioxide chemically reacts with water to form carbonic acid. Carbonic acid is a weak acid that dissociates in chemical equilibrium with bicarbonate and carbonate, which decreases the pH of the water. Figure 2 shows the equilibrium distribution of dissolved carbon dioxide in the forms of carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) as a function of pH.

Many of Reclamation's reservoirs are naturally alkaline water bodies. For example, Lake Mead has a total alkalinity of approximately 140 mg/L (as $CaCO_3$) and pH of 8.1. The bicarbonate species is expected to be the predominate species. When Lake Mead water is injected with carbon dioxide at elevated pressures (e.g., intake structures), the resultant pH of the water depends primarily upon the concentration of aqueous carbon dioxide and the alkalinity of the water, as described by the Henderson-Hasselbalch equation:

$$pH = pK_1 - \log [(CO_2) / (HCO_3^-)]$$

Where:

pK_1	=	6.35 (at 25°C)
(CO_2)	=	the concentration of dissolved CO_2
(HCO_3^-)	=	the concentration of bicarbonate, which is usually the alkalinity because it is the predominate form of CO_2 in most naturally alkaline water

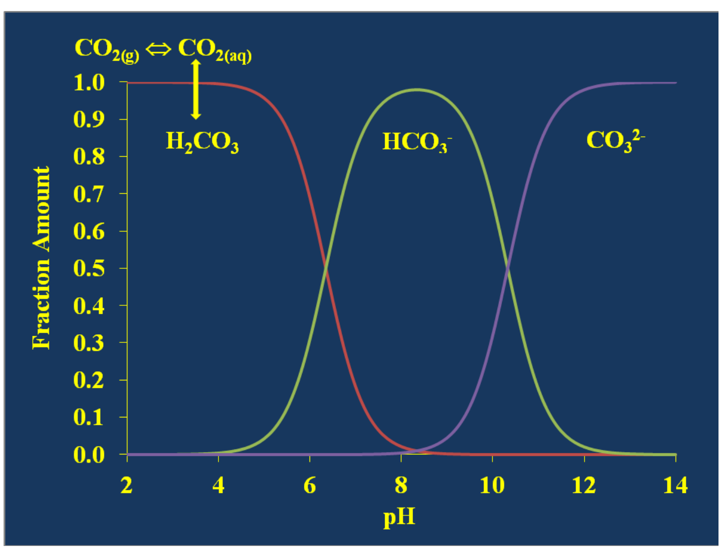

Figure 2 Relative proportions of aqueous CO_2 as a function of pH.

Figure 3 shows the relationship between carbon dioxide and pH at different levels of alkalinity (as $CaCO_3$). It is important to note here that while the basicity of Lake Mead water may be reduced, the alkalinity does not change with the addition of carbon dioxide. Although there may be other minor chemical contributors to total alkalinity in Lake Mead, the carbonic acid – bicarbonate – carbonate system is expected to be the dominant equilibrium system. With a pK_1 of 6.35 (25°C) for carbonic acid, there would be an initial steep drop in aqueous pH and a gradual leveling of the pH as it nears and drops below pH = 6.35. Eventually, adding more carbon dioxide will have little effect on the pH. When Lake Mead water is allowed to re-equilibrate upon exposure to the atmosphere (e.g., discharged from conduit), excess aqueous carbon dioxide is naturally degassed, and the original pH is restored. Therefore, unlike most mussel controls, no residuals or byproducts remain in the water which may impact aquatic ecology and non-target species downstream of the treatment zone.

In the literature, carbon dioxide has been shown to affect the behavior of zebra and quagga mussels through several different mechanisms; as a narcotic, pH adjustment, sub-lethal detachment from the byssus and inhibition of byssogenesis, and bubble gas disease. Each of these modes of action are described here.

2.1 Narcotizing Effect

Elzinga and Butzlaff (1994) first reported that elevation of Pco_2 induces a narcotizing effect on Asian clams and zebra mussels at a concentration of 100 mg/L and toxicity at a concentration of 500 mg/L. The LC_{50} values they established for zebra mussels at 48 h and 96 h were 196 and 74 mg/L (18˚C) and 290 and 81 mg/L (22˚C), respectively. Sub-lethal narcotizing effects were induced after only short (4 h) exposure periods as indicated by a gaping response. More recently, McMahon et al. (1995) subjected zebra mussels and Asian clams to hypercapnic, anoxic, and normoxic water at 25˚C. Three replicate samples of zebra mussels (n = 25-30) were kept in water made anoxic by bubbling with 100% CO_2 or 100% N_2, or in water made normoxic by bubbling with air. Zebra mussels exposed to anoxia bubbled with 100% CO_2 displayed significantly greater mortality rates, with a mean mortality time of 43.6 h, than when exposed to anoxia bubbled with 100% N_2, which had a mean mortality time of 103.7 h. Exposure of zebra mussels and Asian clams to a Pco_2 of 760 torr (anoxic) further reduced the range of LT_{50} values to between 26.0 h to 40.3 hr. Required exposure periods can probably be further reduced by elevating Pco_2 above atmospheric pressure (760 torr). Such hyperbaric levels of CO_2 would require elevation of the mole fraction of CO_2 in the gas phase and the use of gas-liquid contacting pressures (total pressure) exceeding atmospheric pressure. Here, a different form of Henry's Law equation may be used to determine the saturation concentration of CO_2 in water:

$$C^* = K_H \bullet 1000\,(X\,(P_T - P_{H2O})/760)$$

Where C* is the saturation concentration of CO_2 in water, X is the gas phase CO_2 mole fraction, P_T is the total gas pressure that exceeds atmospheric pressure, and P_{H2O} is the partial gas pressure for water. The partial pressure for CO_2 (Pco_2) can be easily determined by the product of total pressure (P_T) and the gas phase mole fraction (X) following Dalton's Law:

$$Pco_2 = P_T \bullet X_{CO2}$$

An example of where hyperbaric application of CO_2 may be useful would be in pressurized on-line systems such as dam fire suppression systems that use the infested reservoir as a source of its water. Such water systems are already pressurized (~120 psi) well over atmospheric pressure.

The difference in zebra mussel mortality rates between 100% CO_2 and 100% N_2 clearly demonstrates that anoxia alone does not account for the increased rate of mortality when zebra mussels are subjected to 100% CO_2. Even at a reduced P_{CO2} of 76 torr (10% CO_2: 19% O_2:71% N_2), three replicate samples of adult mussels (n=25) resulted in complete mortality of zebra mussels (LT_{50} = 78 h), despite the presence of normal levels of oxygen. In addition, rapid death recorded under 100% CO_2 was unlikely to be induced by the reduction of water pH alone. Adult zebra mussels have been found to tolerate the level of pH for far longer than those required to achieve 100% mortality under 100% CO_2.

2.2 pH Adjustment

The threshold pH value for survival of adult zebra mussels appears to be near pH 6.5. For larvae (veligers) and juveniles, the threshold appears to be higher at pH 6.9. Incipient lethal level for larvae begins near pH = 7.4. Like many invertebrates, quagga and zebra mussels have an open circulatory system where blood (hemolymph) is not completely contained within blood vessels and the hemolymph has no specialized oxygen-transporting pigments. The oxygen concentration is near the same as the water taken into the mantle cavity. In addition, like all bivalves, zebra and quagga mussels do not have blood proteins to buffer the blood pH. Instead, they utilize shell carbonate as the main blood buffer, which is a much slower and less efficient process.

When mussels are exposed to elevated levels of CO_2, diffusion of CO_2 into mussel tissues results in decreased body fluid and hemolymph pH. At the same time, hemolymph acidosis would be compounded by the decrease of external water pH associated with elevated levels of dissolved CO_2 added to the water. The acidosis would reduce the ability of mussels to buffer blood pH, since they are forced to utilize the less efficient mobilization of shell carbonate to buffer the hemolymph. Reduction in hemolymph and intracellular pH would then inactivate metabolic enzymes, thus leading to rapid death.

As described above, 100% mortality of zebra mussels was achieved using a hypercapnic, normoxic gas mixture with a Pco_2 of 76 torr (10%) due to a narcotizing effect. However, in naturally alkolytic water, continuous application of Pco_2 = 76 torr may not lower the pH below neutrality or the survival threshold of pH 6.5. If pH adjustment alone is expected to be used as a preventative measure to keep Reclamation facilities free of infestation, it may be necessary to increase the amount CO_2 to achieve the target pH. To determine the effect of CO_2 addition on pH, one can use the Henderson-Hasselbalch equation:

$$pH = pK_1 - \log [(CO_2)/(HCO_3^-)]$$

Where pK_1 = 6.35 (at 25˚C), (CO_2) is the concentration of dissolved CO_2, and (HCO_3^-) is the concentration of bicarbonate, which is usually the alkalinity since it is the predominate form of CO_2 in most naturally alkolytic water (for more explanation, please see above section on carbon dioxide equilibria). The relationship between carbon dioxide and pH at different alkalinity is shown below in Figure 3.

This equation was derived from the carbonic acid dissociation constant equation and is useful for estimating the amount of CO_2 required or the resulting pH at equilibrium when elevated levels of CO_2 are added to the water. In a further attempt to help determine the concentration of dissolved CO_2 that can be achieved when treated at a P_{CO_2} for a given feed gas mixture is detailed in Table 1

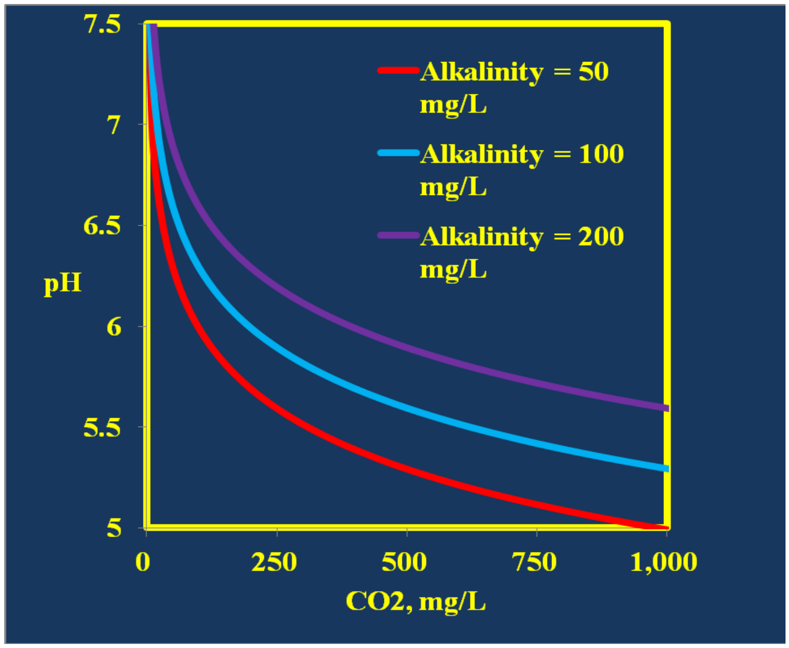

Figure 3 [CO_2] vs pH (Henderson-Hasselbalch Equation).

below (from McMahon et al., 1995), which is based on pure water at neutral pH 7.

Table 1 Solubility of CO_2 in pure water at pH = 7.

Temp ˚C	0.033% Normocapnia PCO2 = 2.51 torr	5% Hypercapnia PCO2 = 38 torr	10% Hypercapnia PCO2 = 76 torr	100% Saturation PCO2 = 760 torr
		CO_2 solubility in mg/L		
0	1.10	167.35	334.7	3,347
5	0.92	139.10	278.2	2,782
10	0.77	115.95	231.9	2,319
15	0.65	98.80	197.6	1,976
20	0.56	84.45	168.9	1,689

25	0.47	71.50	143.0	1,430
30	0.41	62.50	125.0	1,250
35	0.36	55.30	110.6	1,106

2.3 Byssal Detachment and Inhibition of Byssogenesis

In the same study conducted by McMahon et al. (1995), they also noted that zebra mussels exposed to hypercapnic, normoxic gas mixtures of 5% CO_2:19% O_2:76% N_2 did not exhibit complete mortality but tend to become detached from the byssus and, once detached, were unable to reform a byssal attachment. This is an important consideration for Reclamation facilities, since the goal is to not necessarily kill quagga and zebra mussels, but rather to get rid of their infestation and their impact on operations within the water system.

In a separate experiment to determine the percentage of samples of adult zebra mussels that detached, two separate group of mussels were monitored, with one group exposed to a hypercapnic, normoxic gas mixture of 5% CO_2:19% O_2:76% N_2 and another group exposed to normal air gas mixture. In the test group exposed to elevated levels of 5% CO_2, individuals were induced to detach from their byssal holdfasts and did not form reattachments, despite a normoxic level of O_2 (19%). In the air-bubbled control, some individuals also detached from the byssus, with only 31.6% remaining attached to the byssal holdfasts they occupied at the beginning of the exposure period. However, the other individuals who detached moved to other locations and byssally reattached, which is a behavior commonly observed in laboratory aquaria of healthy stocks of captive mussels.

To quantify the rates of byssogenesis when exposed to hypercapnic levels of CO_2, attached zebra mussels were removed from their byssus by cutting their byssal threads at the byssal shell gape with razor blades. The detached mussels were then placed on clear plastic plates and allowed to byssally reattach over a 12-h period in water bubbled with air. New byssal threads were counted through the underside of the plates using a 30x dissecting microscope. Over an 11-day period, byssal threads of all attached mussels were examined and counted daily to determine the daily rate of byssal thread production for each individual mussel, during which mussels were exposed to air-bubbled controls or bubbled with a hypercapnic, normoxic gas mixture of 5% CO_2:19% O_2:76% N_2. The number of individual mussels that detached during the same 11-day period was also recorded. After 24h, the mussel group in the hypercapnic, normoxic medium had the byssal thread production rate greatly reduced and completely inhibited by the seventh day of exposure. In contrast, the mussel group in the air-bubbled medium continued to produce byssal threads daily throughout the exposure period, with the production rate declining to 1.67 threads per day on the eleventh and final day of exposure. Although this study did not involve larvae (veligers), since larvae may experience 100% mortality prior to settlement, it is likely that larvae would be similarly affected, and CO_2 may be used as a pro-active preventative measure to keep pipes and other structures free of infestation.

2.4 Bubble Gas Disease

When hyperbaric levels of CO_2 are being applied (as described above), it is also possible to induce bubble gas disease or bubble gas trauma by inducing excessive short-term change in the P_T. The gas can still be CO_2 for this purpose during the course of applying CO_2 biocontrol treatment. Very little information is available in the literature demonstrating this mode of action on quagga and zebra mussels. However, the pathology of this disease is well understood and involves the formation of gas emboli and emphysema in blood and tissues, with associated physiological dysfunctions such as homeostatis. Rapid changes in the P_T within a man-made confined water system (e.g., pipes, ballast water) that is infested by undesirable species such as quagga and zebra mussels may be a feasible concurrent method of inducing mortality of quagga and zebra mussels without affecting other species that exist outside of the confined water being treated.

3. Description of Methods

2.1 Speece Cone

A Speece Cone is a downflow bubble contactor invented by Dr. Richard Speece for efficient gas dissolution in water. The Speece Cone is conical in shape, with the water and gas entering at the top of the unit, and the resulting water stream exiting through the bottom. The downward velocity of the water flow counters the buoyancy of gas bubbles to entrain the bubbles for near complete gas transfer. While entrained, the gas bubbles decrease in size over time as the gas diffuses into the liquid. A simple overview of the system is shown in Figure 4.

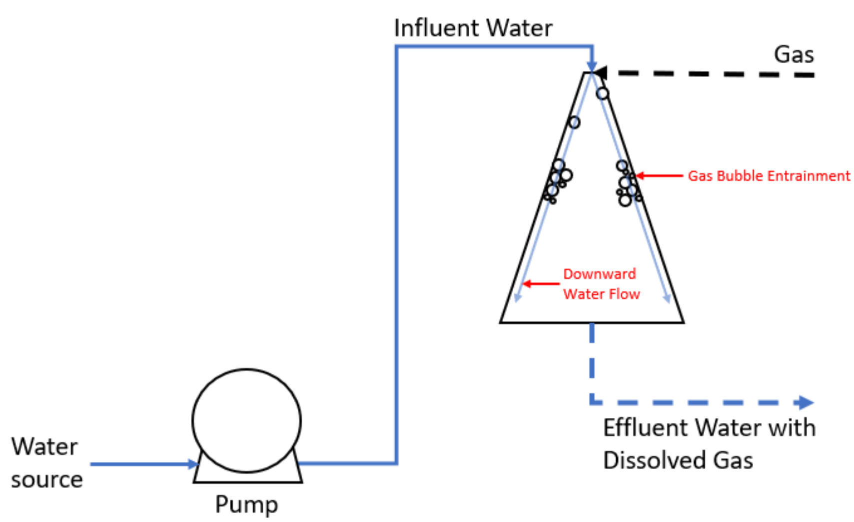

Figure 4 Simplified Speece Cone system overview.

2.1.1 Principle of Gas Diffusion

Gas diffusion to the liquid phase is modeled using Henry's Law, shown in Equation 1. The saturation concentration of the gas, C_S, is dependent on the partial pressure, P_G, and Henry's constant, k_H, of the specific gas. Additionally, Henry's constant is temperature dependent.

$$C_S(\frac{mol}{m^3}) = P_G \times k_H(T) \qquad \text{Equation 1}$$

Mass transfer of gas across the surface of a bubble to the water is calculated using the flux equation shown in Equation 2. The mass transfer coefficient, K_{BL}, is dependent on the gas bubble radius.

$$J\,(\frac{mol}{m^2 \times s}) = K_{BL}(C_s - C) \qquad \text{Equation 2}$$

2.1.2 Traditional Use of Speece Cones

Traditionally, Speece Cones have been used for aeration of water bodies to increase the dissolved oxygen concentration. Newman Lake, Washington, was studied and shown to be undergoing eutrophication with near anoxic conditions in the hypolimnion (Thomas et al., 1994). A Speece Cone was later installed to aerate the lake, and dissolved oxygen concentrations had an observed increase to an average 5.5 mg/L. In the Camanche Reservoir, California, low dissolved oxygen concentration in the sediment was producing hydrogen sulfide (which is toxic). A Speece Cone was installed (submerged) and anoxic water in the reservoir was pumped through the apparatus, resulting in dissolved oxygen concentrations ranging between 3 and 7 mg/L (Horne et al., 2019). Additionally, ammonium and phosphorous concentrations were observed to significantly decrease.

2.1.3 Comparison with Other Gas Infusion Methods

As reported by Gafsi et al. (2009), Speece Cones have primarily been utilized for hypolimnetic oxygenation so as not to disturb the temperature gradient of the lake or reservoir. Two common hypolimnetic aeration systems include the airlift hypolimnetic aerator and bubble-plume oxygenator. McGinnis and Little (1997) performed a techno-economic analysis on the three hypolimnetic oxygenation systems, showing that the bubble-plume oxygenator and Speece Cone were able to achieve oxygen transfer efficiencies of 93 and 94%, respectively. The partial lift hypolimnetic aerator only achieved 16% oxygen transfer efficiency. Researchers noted, however, that the Speece Cone use requires sustained, high water velocities to ensure bubble entrainment and efficient diffusion, whereas bubble-plume oxygenators only require consistent pumping of the air/oxygen.

Since CO_2 has a greater solubility in water than oxygen, CO_2 gas transfer efficiency is expected to be greater than the 93-94% traditionally achieved when dissolving oxygen using the Speece Cone.

2.1.4 Advantages of Using Speece Cones

A Speece Cone is being used in this experiment due to the specific application for which it is intended. The goal of the research is to implement rapid gas dissolution into a side stream passing through the Speece Cone setup and returning to a primary water line. Specifically, this research is attempting to reach near saturation of carbon dioxide to provide a sufficient dissolved CO_2 concentration into the primary water line to prevent veliger settlement on the inside of the water line pipe. A Speece Cone can provide highly efficient gas diffusion at a constant rate.

The Speece Cone has numerous advantages from an operations and maintenance standpoint. The continued water pumping costs for sustained, high water velocities is mitigated in this application, as the side stream to be saturated with CO_2 relies on the backpressure of the primary water line and will be sized to provide the optimum flow velocity. As a result, the major energy cost driver associated with utilizing a Speece Cone is eliminated. The Speece Cone is a physical process solution, rather than a chemical process solution that would likely incur large operational expenses and could have the potential for by-product formation. The Speece Cone has no moving parts to break or restrict flow, eliminating any maintenance needed for the Speece Cone itself. The lack of moving parts and the open flow through construction also eliminates the potential for clogging and doesn't require any treatment of the liquid upstream of the cone. The high gas transfer efficiency results in much lower consumption of gas per gallon treated than any of the currently accepted, widely used gas injection methods. A Speece Cone is a closed type of oxygenation technology. Closed-type oxygenation requires greater pumping energy than an open-type of oxygenation unit which operates at atmospheric pressure. Open-type units such as multi-staged low-head oxygenators (LHO™) need less pumping energy, but the open to the atmosphere arrangement wastes injection gas to the atmosphere before the oxygenated water is used for its intended purpose. A Speece Cone with a properly tuned gas injection system will not introduce entrained bubbles to the water stream. This will prevent the potential for reduced heat capacity in the cooling lines caused by bubble entrainment.

Since CO2

2.1.4 Future Plans for Speece Cone Laboratory-Scale Apparatus

Future lab experiments will be designed with independent variables of water flow rate, gas pressure, and mass flow rate of carbon dioxide. The dependent variable will be the measured carbon dioxide concentration. The parameters that will be measured both upstream and downstream of the Speece Cone will include pH, DO, and temperature. The dissolved carbon dioxide (DCO_2) downstream of the Speece Cone will also be measured. The pH and DCO_2 measurements will be used to create a calibration curve to allow pH to be used as a surrogate for DCO_2 as a control measurement. Alkalinity will be measured as well to see how extra carbon dioxide injected potentially interacts with dissolved carbonate species. The experiments will be set up to test direct injection of carbon dioxide to achieve the target range of CO_2 concentration that has the desired effect on mussel settlement, as well as full saturation to test performance at full saturation. Figure 5 show the current set up of the laboratory-scale Speece Cone apparatus in Reclamation laboratories.

Figure 5 Current set up of Speece Cone Apparatus.

The lab testing of the Speece Cone will provide data to help evaluate the effectiveness of the Speece Cone as a carbon dioxide injection system for closed-pipe aqueous systems. The data obtained will provide the basis for system sizing and cost estimates for installing and maintaining a Speece Cone injection system for mussel mitigation in hydroelectric turbine cooling lines. The data will provide insights into how the system would potentially impact the water stream and how different water qualities will affect the efficiency of the injection system. The full saturation testing will provide information that will inform the use of a fully saturated side stream that will be blended into the main cooling line, while the information on the direct injection to the concentrations relevant to mussel mitigation will inform design decisions on the overall process.

For field testing, the apparatus may be installed in a trailer to create a mobile pilot testing center that can be used to run the same experiments previously run in the laboratory. The trailer will be outfitted to allow 8-12 hours of continuous remote operation with no access to power. It will also contain bioboxes to allow for veliger settlement testing onsite. The mobile Speece Cone trailer will be taken to several locations to test waters with different chemistries. The first tests will be done in the local Denver area to both gather data and optimize the operation of the apparatus in a trailer onsite. Any testing requiring a discharge of carbon dioxide enriched water will be fully permitted with all required local authorities.

The data gathered from the mobile pilot testing will be used in conjunction with the laboratory testing to design a pilot-scale or full-size system for use in Reclamation facilities. The final step before permanent adoption would be to build a mobile version of the full-size system and run it for a long-term study at a Reclamation facility.

2.1.5 Simulation of Lake Mohave Water Chemistry

Since travel to Davis Dam was restricted, deionized water in the laboratories was used and adjusted to simulate the water chemistry found in Lake Mohave, the reservoir impounded by Davis Dam.

2.1.5.1 Water Recipe Writeup

Lab scale testing was conducted to mimic the water quality conditions at Lake Mohave, near Davis Dam. Water quality data from Lake Mohave is shown in Table 2.

Table 2 Lake Mohave Water Quality Parameters

Parameter	Minimum	Average	Maximum
Alkalinity, mg/L (as CaCO3)	122	136	142
pH	7.95	8.40	8.58

The water was artificially prepared in the Water Treatment Laboratory by using deionized (DI) water. Water quality for the DI water is shown in Table 3. Using the dechlorinated fish water from the laboratory was another alternative. However, due to the daily variations in the water quality, specifically alkalinity, this option was not pursued further.

Table 3 Water Treatment Lab DI Water Quality Parameters

Parameter	Minimum	Average	Maximum
Alkalinity, mg/L (as CaCO3)	1.8	1.85	1.9
pH	5	5.5	6

The alkalinity and pH values of the DI water from the lab are significantly lower compared to the water quality values at Lake Mohave. Therefore, to mimic the water quality conditions of Lake Mohave, the addition of sodium carbonate and sodium bicarbonate to the DI water is necessary to increase both the alkalinity and pH levels. The Henderson-Hasselbalch equation was used to determine the amount of sodium carbonate and sodium bicarbonate needed to increase the average alkalinity of the DI water from 1.85 mg/L to 136 mg/L.

The amount of sodium carbonate and sodium bicarbonate needed to increase the alkalinity was first practiced in a 1-L solution of DI water. From laboratory experiments, the amount of sodium carbonate and sodium bicarbonate required to increase the alkalinity is shown in Table 3. After determining how much sodium carbonate and sodium bicarbonate is needed to increase the alkalinity to 136 mg/L in a 1-L solution, those values were scaled to a 700 L water solution, which is the total volume of water that is in the fish tubs.

Table 4 Alkalinity and pH Values for Water Volumes 1 L and 700 L

Water Volume (L)	NaHCO3 (sodium bicarbonate) (g)	Na2CO3 (sodium carbonate) (g)	Alkalinity (mg/L as CaCO3)	pH
1	0.208	0.0031	133	5.0
700	145	2.2	137	5.3

Based on the results, the alkalinity value is similar to that of Lake Mohave. However, the pH is lower than anticipated. As a result, sodium hydroxide was added to increase the pH value to 8.4.

2.2 Davis Dam Field Trials

The overall goal of the field trials planned for 2022 at Davis Dam is to develop CO_2 treatment regimens that will prevent dreissenid settlement within the raw-water cooling lines of the Davis Powerplant. To achieve this overall goal, four intermediate objectives will be investigated:

Objective 1. Determine the minimum effective concentration (EC99) to prevent settlement of dreissenid early life stages in a flow-through system.

Objective 2. Determine the minimum effective exposure period, including continuous and intermittent, to prevent settlement of dreissenid early life stages in a flow-through system.

Objective 3. Conduct laboratory trials to develop a model of CO_2 efficacy with different water qualities (alkalinity, conductivity, temperature, organic matter).

Objective 4. Evaluate field application trials of CO_2 at 2-3 field sites with different water qualities and control objectives (e.g., intake lines, trash screen, open water deterrent).

Description given below of the experimental methods planned for the Davis Dam field trials was provided by UMESC scientists.

2.2.1 Test System

The test system will be contained within a mobile laboratory trailer (24' long x 8' wide) that is parked on the top of Davis Dam adjacent to the forebay. Reservoir water will be drawn from the forebay through a submersible electric trash pump to a continuous flow-through system. The water will be pumped first into a head box (total volume ~ 100 L, 20 gal) and the headbox will deliver the water to three distribution tanks (DT; total water volume ~ 100 L). Each DT will deliver water to four replicate settlement tanks (ST) (water volume ~50 L). DT1 will deliver untreated river water to control tanks and DT2 and DT3 will each deliver CO_2-infused water to Low CO_2 tanks and High CO_2 tanks, respectively. Carbon dioxide will be infused from 50-lb compressed gas CO_2 cylinders through air lines and into air stones to DT2 and DT3 at a flow rate to achieve a pH of 6.9 (~25 mg/L CO2, low treatment) and 6.6 (50 mg/L CO_2, high treatment), respectively (Figure 4). The flow rate to each ST will be adjusted to produce ~ 1 to 1.5 tank exchange per hour (550 – 833 mL/min).

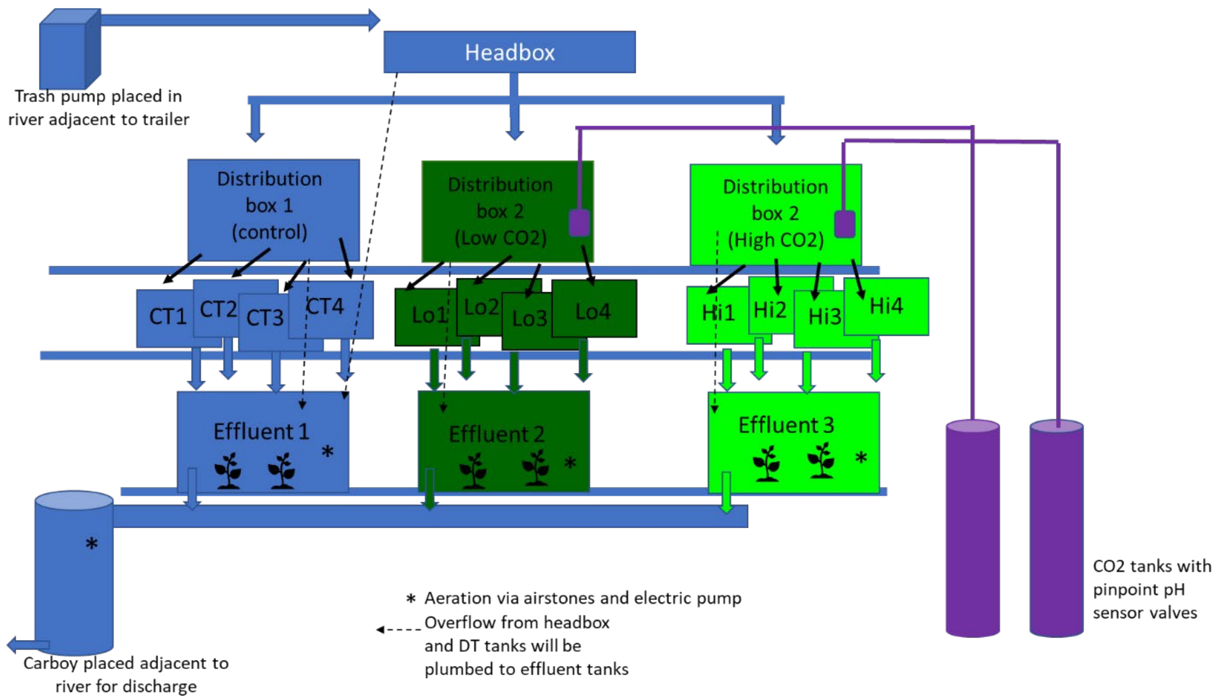

Figure 6 Continuous flow-through test system with raw water supply from Davis Dam forebay. Credit: UMESC.

2.2.2 Water Chemistry

Measurements of DO (AEH 394 or equivalent), pH (AEH 307 or equivalent), and temperature will be taken daily in each test tank. Daily P_{CO_2} (µatm) measurements will be calculated with the USGS CO_2 calculator http://coastal.er.usgs.gov/co2calc/CO2calcNet.html using tank pH, tank temperature, and average alkalinity; P_{CO_2} will be verified at least once a week with the CO_2 probe (Vaisala,) and by titration with concentration NaOH to a pH endpoint of 8.3 (AEH 726). Alkalinity, hardness, (UMESC SOP 706.4, UMESC SOP 712.3), conductivity (AEH 188) and total ammonia (UMESC SOP 306) will be measured once a week. Unionized ammonia concentrations will be determined by calculation. Chlorophyll concentration will be measured in test tanks biweekly by spectrophotometric methods (APHA 1998).

2.2.3 Water Discharge

Carbon dioxide infusion in the test system will decrease the pH to 6.7 – 6.8 in the lowest treatment tanks. Water from the test system will be discharged into an effluent tank located outside of the trailer. The maximum volume of water discharged will be 65 gallons per minute (GPM) and the minimum will be ~20 GPM. About 12 GPM will be infused with CO_2 which will be mixed with untreated water at a minimum ratio of 1:1 treated to untreated, but more likely at a ratio of 1:5 treated to untreated. Effluent water will be vigorously aerated and diluted with overflow water from the intake line. The pH of water in the carboy will be continuously monitored with a HOBO sonde unit to ensure that pH in the discharged water is within 0.1 units of the incoming water (ambient level). The sonde unit will be programmed to provide a cell phone alert to study personnel if pH is more than 0.10 units below ambient (indicating CO_2 concentration is above ambient levels). Study personnel will shut off water discharge until CO_2 has been off gassed from the carboy water and returned to ambient levels.

2.2.4 Veliger Density and Settlement

Veliger density will be monitored three days/week in the headbox beginning when water temperature is ≥ 12°C. A plankton net or filter cup (mesh size 35 μm) will be placed under the inflow to the headbox to filter a minimum of 1000 L. The plankton sample will be immediately examined or poured into a leak-proof Nalgene bottle and preserved with 70% ethanol buffered to a pH of 7.0. Samples will be examined for veliger presence by transferring ~10 mL to a glass petri dish and scanning the contents of the dish with a stereomicroscope using cross-polarized light (CPL) at 6× to 90× magnification. The number of veligers in the sample will be enumerated. Infusion of CO_2 will begin in the test system after veligers are observed in the incoming water. Veliger density will be monitored at the inflow to the headbox, from outflow of each distribution tank, and from outflow of each test tank, up to 3 times per week. Settlement of the pediveliger is expected 3 –5 weeks after veligers appear in the water column.

Settlement samplers (slides) will be placed into each distribution tank, test tank, and effluent tank, at least one week before the onset of CO_2 infusion to allow formation of a biofilm. Settlement samplers will consist of a microscope slide rack with acrylic microscope slides (n = 12). Six settlement samplers will be placed into each of the aforementioned tanks. Up to four slides per tank will be removed once weekly, starting 4 weeks after the first detection of veligers in the water column, and examined with a stereomicroscope using CPL at 6× to 90× magnification. The number of settled mussels on both sides of the slide will be counted. Shell length of mussels from one slide per tank will be measured with digital calipers or from a digital image with NBS software. At the conclusion of the trial, the contents of all tanks will be removed and rinsed through a screen (~0.5 mm mesh) to retain zebra mussels. The number of zebra mussels (live and dead) in each tank will be counted. A subsample (~10%) will be measured (shell length) to estimate mean size.

3. Discussion

The project described in this report covers the initial efforts by Reclamation and UMESC to investigate the use of CO_2 to control settlements of zebra and quagga mussels in Reclamation facilities. Further investigation is required before CO_2 may be implemented on a permanent basis inside of Reclamation facilities.

3.1 Pandemic Related Field Work Stoppage

Reclamation and UMESC entered into an IAA agreement in FY19 and initiated plans to start the field study at Davis Dam in March 2020. This field study was expected to take between 6 to 8 weeks to complete. However, the COVID-19 pandemic interrupted plans and all non-mission critical travel were not approved. DOI employees were required to maximize teleworking. Since then, it has not been possible to travel to Davis Dam. Current plans are to start the Davis Dam field study in the spring of 2022, pending the lifting of COVID-19 travel restrictions. The current IAA between

Reclamation and USGS has been extended twice with a new completion date of September 30, 2022.

After Reclamation and UMESC employees were allowed to return to work in their laboratories with some restrictions, UMESC continue to perform laboratory scale CO_2 studies in their laboratories. Reclamation also returned to their laboratories and focused on completing the Speece Cone apparatus and monitoring water quality during carbonation using laboratory deionized water with adjusted alkalinity and pH like those found in the reservoir at Davis Dam. Because these laboratory studies do not involve actual lower Colorado River water or quagga mussels from the lower Colorado River, there is still a need to perform the field study at Davis Dam to obtain relevant data in preparation for a pilot-scale study at Davis Dam.

The original S&T proposal for this project was accepted for funding for FY19-21. After discussion with S&T administration, a new S&T proposal was submitted for FY22-24 to avoid extending this project for additional years without a placeholder report documenting the milestones achieved to date and as described here in this report. Modifications in tasks and intermediate completion dates were documented using change orders. The major modification was for Reclamation to shift its focus from obtaining mussel-related data to optimizing the Speece Cone apparatus and understanding better how a Speece Cone may best be implemented at Davis Dam in the future.

4. Conclusion

The project described in this report covers the initial efforts by Reclamation and UMESC to investigate the use of CO_2 to control settlements of zebra and quagga mussels in Reclamation facilities. Due to delays caused by the COVID-19 pandemic to the Davis Dam field study, project split into a two-prong research approach. The first prong of the project continues to be a collaboration with UMESC. The Davis Dam field study will commence when travel restrictions are lifted. The second prong is ongoing with a focus on completion of the Speece Cone apparatus and an understanding of how key water parameters are affected by the addition of CO_2 and how to best manage these effects to prevent the settlement of zebra and quagga mussels inside of Reclamation's dam and powerplant facilities.

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