

A CONTINUATION OF EVALUATING THE USE OF CARBON DIOXIDE AS AN ALTERNATIVE PREDATOR REMOVAL TECHNIQUE TO DECREASE TFCF PREDATOR NUMBERS AND IMPROVE FACILITY OPERATIONS

Investigators

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Summary

The Tracy Fish Collection Facility (TFCF) was developed in 1956 by the Department of the Interior, Bureau of Reclamation (Reclamation) as a means of salvaging fish ≥ 20 mm in length and returning them to the Sacramento-San Joaquin River Delta (SSJD) beyond the influence of C.W. “Bill” Jones Pumping Plant (JPP). To improve the overall salvage process and efficiency of the TFCF it is necessary to minimize fish loss throughout the facility. Many factors, including predation, contribute to the total fish loss at the TFCF. Predators accumulate throughout the facility, including in front of the trash rack, the primary channel, the bypass tubes, the secondary channel, and the holding tanks (HT; Liston *et al.* 1994). Over the years, Reclamation has discussed various means of moving fish through the system (Liston *et al.* 1994, Fausch 2000).

A predator removal program in the secondary channel was studied and implemented in the early 1990’s (Liston *et al.* 1994) and continued through the decade. Predators were washed into fyke nets, seined, and dip netted out during times when the secondary channel was drained. Striped bass (*Morone saxatilis*) were the main predatory species and fish up to 700 mm TL were removed. Other abundant predators at the facility include ictalurids, centrarchids and gobiids. Stomach analyses of some of these fish have yielded, among others, Chinook salmon (*Oncorhynchus tshawytscha*), delta smelt (*Hypomesus transpacificus*), and threadfin shad (*Dorosoma petenense*; Liston *et al.* 1994). In recent years, predator removal activities have slowed because of logistics and the length of time the facility is down to complete the fish removal effort. In 2004, a new

predator removal method using carbon dioxide (CO₂) was approved for study. This method would not reduce daily salvage due to secondary channel downtime and could prove to be more efficient, and safer for employees and fish than the current predator removal method. This project was divided into five phases. Phases 1–4 were completed in Sept. 2007 and the first draft document is currently in peer review. Portions of Phase 5 that have been preliminarily investigated have been summarized below.

Phase 5: Implementation of CO₂ for Predator Removal

Several portions of Phase 5 were preliminarily investigated during the 2010–2012 research period. This includes work performed to investigate CO₂ injection methods, determine predator location, and determine the effectiveness of the existing predator removal processes by performing the alternative (CO₂) predator removal process immediately after the existing predator removal process. Additional work is still necessary for all portions of Phase 5.

The investigation of CO₂ injection devices suggested that a slide or chute would be a feasible piece of equipment to quickly and efficiently inject dry ice blocks into the primary bypass tubes. The use of a slide or chute would also minimize safety hazards during CO₂ injection.

The preliminary investigation of predator location in the bypass tubes and secondary channel suggests that approximately 89% (1820/2046) of the striped bass in the secondary system (bypass tubes and secondary channel) are located in the bypass tubes and approximately 11% (226/2046) of the striped bass are located downstream of the bypass tubes in the secondary channel. This suggests that it will be necessary to inject CO₂ into the bypass tubes instead of just the secondary channel in order to remove the majority of the predators from the secondary system.

Preliminary data for the optimal dose investigation suggests that the CO₂ concentration that removes the greatest proportion of tagged striped bass while maintaining acceptable survival is different for cool (<18.0 °C) and warm (≥18.0 °C) water. During times when the water temperature was cool, the striped bass could survive treatment at a higher CO₂ concentration and the optimal dose was determined to be approximately 180 mg/L. When the water was warm, the optimal dose was only determined to be approximately 65 mg/L. This is likely due to overall reduced 96-h survival of striped bass during times of high water temperature. When data for all water temperatures are combined, it is suggested that 75 mg/L is the optimal CO₂ concentration to remove the greatest proportion of tagged striped bass while maintaining acceptable survival.

The preliminary investigation of the effectiveness of the existing predator removal method suggests that, on average, 69% (0%–100%) of striped bass are removed

by using this method. This suggests that the old predator removal method is not always 100% effective and that the CO₂ predator removal method removes fish that the existing method does not.

Problem Statement

Predation may be significant within the primary bypass tubes and secondary channel because striped bass continue to reside within them. Removing these fish with the current methods is dangerous for employees, decreases daily salvage, and causes damage to the fish and/or fish mortality. An alternate method to remove predators is needed for the facility. Phases 1–4 demonstrated that CO₂ is effective at removing predatory fish from the bypass tubes and secondary channel at the TFCF. Phase 5 will focus on developing methods and guidelines on how to implement CO₂ predator removals at the TFCF.

Goals and Hypotheses

Goals:

1. Determine if an injection device, such as a slide or chute, should be used to inject dry ice into bypass tube openings relative to time and safety.
2. Determine if the injection of dry ice into the bypass tubes reduces the velocity and total volume of water allowed to flow down the transition boxes and through the bypass tubes.
3. If injection of dry ice is found to reduce the total volume of water allowed to flow through the bypass tubes, determine if gradual injection of small amounts (pellets or broken pieces) of dry ice throughout the injection period will stabilize flows and CO₂ concentrations in the bypass tubes.
4. Determine if use of gaseous CO₂ would be effective, feasible, and result in more stable CO₂ concentrations in the bypass tubes.
5. Produce a calculation to predict the peak CO₂ concentration in the bypass tubes depending on the amount of dry ice injected and flow through each bypass tube.
6. Determine if more striped bass hold within the bypass tubes or secondary channel.
7. Determine Optimal CO₂ concentration for a 10-minute exposure in relative to removal efficiency and survival.

8. Determine removal efficiency and survival for the current and alternative predator removal methods.

Hypotheses:

1. There will be no difference in the amount of time required and safety hazards present during injections performed with and without an injection device.
2. Injection of dry ice will not affect the velocity and total volume of water allowed to flow through the bypass tubes.
3. The injection of small amounts (pellets or broken pieces) of dry ice throughout the injection period will result in the same flows and CO₂ concentrations observed when all dry ice was injected at once.
4. The use of gaseous CO₂ will not result in more stable CO₂ concentrations in the bypass tubes than the use of dry ice.
5. The bypass tubes and secondary channel hold equal amounts of striped bass.
6. All CO₂ concentrations will result in equal removal efficiency and survival over a 10-minute exposure.
7. Removal efficiency will be equal for the current and alternative predator removal methods.
8. The proportion of fish that die or show signs of damage (*i.e.*, fungus, hemorrhaging) after 96 h will be equal for the current and alternative predator removal methods.
9. The amount of time and cost to complete the old and new predator removal methods will be equal.

Materials and Methods

Phase 5: Implementation of CO₂ for Predator Removal

In the final phase of this project (Phase 5) we intend to apply the knowledge gained through our initial studies (Phases 1–4) in order to implement the combined use of CO₂ and pulsed flows as a predator removal technique at the TFCF. Information learned during the different components of Phase 5 will direct the next step in the research process. Phase 5 consists of two primary components: hydraulics and fisheries.

The examination of CO₂ injection on TFCF hydraulics will be investigated to determine a suitable location for CO₂ injection, develop a device for dry ice injection, determine whether dry ice is causing flow rate changes, determine how to stabilize flows and CO₂ concentration when using dry ice, determine if another method of CO₂ injection would provide more stable CO₂ concentrations and flows, and produce a calculation that predicts peak CO₂ concentration from the amount of dry ice injected and the flow through each bypass tube in the secondary channel.

In order to investigate the response of the TFCF fishery to the combined use of CO₂ and pulsed flows, we must first determine where the majority of predators are congregating and the dose at which predators are most efficiently removed from the bypass tubes and secondary channel. Once predator location and an optimal dose are established, we will compare the alternative predator removal efficiency and survival to that of the current predator removal method.

A. Hydraulics

1) CO₂ Injection Method

In order to determine if an injection device, such as a slide or chute, should be used we will need to compare injection time and safety hazards for the use of the device to that for the injection where no device is used. The dry ice will be separated into four equal portions and kept in a cooler near the bypass tube into which it will be injected. The same amount of dry ice will be injected into each bypass tube regardless of whether or not a device is used. The time it takes two workers to introduce dry ice into all of the four bypass tubes, with and without an injection device, will be determined and compared. A safety evaluation will also be completed to identify all hazards that are encountered when injecting with and without a device. The safety evaluation for dry ice injections, with and without a device, will then be compared with the safety evaluation for the existing predator removal technique.

2) Hydraulic Changes When CO₂ is Injected into the Bypasses

It is possible that the injection of dry ice into the bypass tubes, and subsequent rising of CO₂ up the transition boxes, reduces the velocity and total volume of water allowed to flow down the transition box and through the bypass tube. The build-up of gas in the actual bypass tubes could also prove to reduce the volume and increase water velocity inside the tubes. These changes to volume and water velocity possibly caused by injecting dry ice in the bypass tubes could have a direct effect on the concentration of CO₂. In order to determine that CO₂ injection is causing flow rate

changes and to develop a method to stabilize flows we must obtain flow measurements through each bypass tube after varying amounts of dry ice have been injected.

The control trial will be completed first by examining flow through each bypass tube without any dry ice injection. All velocity control (VC) pumps will be turned off and the secondary channel will be allowed to flow at about 0.57 cubic meters per second (cms) for 20 min. Flow measurements will be recorded for each of the four bypass tubes every 2 min. This same procedure will be completed after injecting 11.4, 22.7, 34.1, 45.5, 68.2, and 90.0 kg of dry ice into each bypass tube. After each treatment the secondary channel will be flushed for 5 min to remove the remaining dry ice inside the bypasses. A flow vs. time graph will be plotted for each bypass and amount of dry ice tested in an effort to illustrate the flow rate changes caused by CO₂ injection.

3) Stabilizing CO₂ Levels During 10 Min Fish Dose

To determine if CO₂ concentration can be stabilized within the bypass tubes and secondary channel we are proposing to inject small amounts of dry ice (pellets or broken blocks) throughout the 20 min dose time. In order to do this we must first drain the secondary channel so 1/5 hp pumps can be installed at the mouth of each bypass tube in order to obtain water for pH and CO₂ measurements. The secondary channel will then be back-filled and all VC pumps will be shut off in order to achieve a flow of 0.57 cms for 20 min. The same known amount of dry ice, for each bypass tube, will be broken into small pieces (or dry ice pellets will be used) and injected into each bypass opening throughout the 20 min dose period. Carbon dioxide and pH measurements will be performed for each bypass every 5 min. This procedure will be repeated, with the same amount of dry ice per bypass tube, except that the ice will not be broken and will be injected, all at once, into each of the bypass tubes. A CO₂ concentration vs. time graph will be made for each treatment and bypass tube in order to determine if CO₂ levels can be stabilized by injecting small amounts of dry ice throughout the dose time rather than all at once.

4) Alternate Forms of CO₂

It is possible that more stable CO₂ concentrations and water flows could be achieved by using gaseous CO₂ instead of dry ice. To investigate this, we would follow the same procedures as described above, except that a pressurized CO₂ cylinder will be used to continuously inject CO₂ gas, with a stable flow (LPM), into the mouth of each bypass tube throughout the 20 min dose period. Flow through each bypass tube will be recorded every 2 min while CO₂ concentration and pH measurements will be taken every 5 min. This data will be used to construct a CO₂ concentration vs.

time graph and a flow vs. time graph for the use of gaseous CO₂. These graphs will be compared to those developed for the dry ice injections in order to determine if using gaseous CO₂ allows for more stable CO₂ concentrations and flows than using dry ice as a CO₂ source.

5) Predicting CO₂ Dose Fish are Exposed to

A calculation will be produced to predict the peak [CO₂] in the bypass tubes depending on the amount of dry ice injected and the flow through each bypass tube. It will also be necessary for us to make a calculation that determines the amount of dry ice to add to the water in order to get to a target CO₂ concentration with a known bypass tube flow. When constructing these calculations we will need to take into consideration the percent of each dry ice block that is gassed off each minute and the efficiency of the gas dissolving into the water as a function of water temperature.

B. Fisheries

1) Predator Location

In order to determine the best location and method for CO₂ injection we must first figure out where striped bass are holding up within the secondary system. This will be done by injecting high doses of CO₂ (>200 mg/L) into two different areas of the secondary system (head of the secondary channel and entrance of bypass tubes) and comparing the number of predators removed. The secondary channel will be drained in order to install 1/5 hp submersible pumps at the mouth of each bypass tube which will be used to obtain water samples for CO₂ and pH measurements. The secondary channel will then be back-filled and all VC pumps will be shut off to achieve a flow of about 0.57 cms. The SN downstream of the secondary channel will be lowered before dry ice injection and will be used to evaluate the proportion and spp. of fish that are not successfully louvered into the HT (lost).

Dry ice (contained in a mesh bag) will first be lowered into the head of the secondary channel using a rope-pulley system to deliver the CO₂ to the mouth of the bypass tubes. Once a high [CO₂] is reached the flow in the secondary channel will be maintained at 0.57 cms for 20 min and then an empty HT will be opened and VC pumps will be turned on, for 10 min, in order to achieve a flow of 0.46–0.61 m/sec and flush predators from the secondary channel into the HT. This HT sample will contain all of the predators that resided in the secondary channel but will not include any that were holding up in the bypass tubes.

After collection of the first sample, all VC pumps will be shut off again and a flow of 0.57 cms will be achieved. Dry ice will then be injected into the opening of each bypass tube until a high [CO₂] is reached in the secondary channel. A secondary channel flow of 0.57 cms will be maintained for 20 min after which an empty, HT will be opened and VC pumps will be turned on in order to flush the bypass tubes for 10 min at 0.46–0.61 m/sec. This HT sample should contain all predators that resided within the bypass tubes with the assumption that fish holding up in the secondary channel were previously removed.

If most of the predators are present in the secondary channel (1st HT sample), after the bypass tube mouths, then CO₂ injection in this area should be sufficient. If the majority of predators hold up in the bypass tubes (2nd HT sample) then CO₂ injection should take place at the mouth of these tubes in order to effectively remove these predators. If both locations hold predators then the CO₂ injection should take place at the bypass tube opening in order to collect predators from both locations.

2) Determining Optimal CO₂ Concentration for a 10 Min Exposure

To determine the CO₂ concentration that is optimal for the removal of TFCF predators it is necessary to inject unique groups of ten striped bass for each of five consecutive predator removals exposing fish to five different CO₂ concentrations (0, 75, 150, 200, and 300 mg/L). The order of the concentration tested will be randomized each day.

Groups consisting of ten striped bass each will be given a distinct color/fin tag using a phototonic marking gun and BMX1000 phototonic marking formulation (NEWEST Technologies, Santa Rosa, CA). The secondary channel will be drained in order to install a 1/5 hp pump at the mouth of each bypass tube which will be used to obtain water samples for CO₂ and pH measurements. The secondary channel will then be back-filled, one group of ten striped bass will be released, dry ice will be injected to obtain the target CO₂ concentration and a secondary and holding tank flow of 0.57 cms and 0.23 cms, respectively, will be achieved for 10 min. The SN downstream of the secondary channel will be lowered before fish injection and will be used to evaluate the proportion and spp. of fish that are not successfully louvered into the HT (lost).

After 10 min, VC pumps will be turned on in order to achieve a secondary flow of 0.46–0.61 m/sec and flush the bypass tubes. Flushing time will be limited to 5 min as all CO₂ will have cleared the secondary channel by this time. The fish collected in the HT will be placed in a 3.6 m x 0.74 m x 0.76 m trough, equipped with O₂ and flow through Delta water, while the

fish collected in the SN will be put into a 132.5-L garbage can containing Delta water. All fish will be identified and measured and the proportion of tagged fish recovered in each sample will be determined.

These methods will be repeated for CO₂ concentrations of 0, 50, 75, 100, 125, 150, 200, 250, and 300 mg/L. Ninety-six h survival will be recorded for all recovered tagged striped bass. In order to detect the true probability of capture within 25%, it will be necessary to complete 30 replicates for each treatment (3 releases of 10 striped bass). The CO₂ concentration that is found to remove the greatest proportion of tagged striped bass (>90%), while maintaining acceptable survival (>90%) and least loss of fish (<10%), will be considered the optimal dose and will be used to compare the current predator removal technique to the proposed alternative predator removal method.

The optimal CO₂ concentration for the removal and survival will also be investigated by removing wild striped bass from the bypass tubes and secondary channel with consecutive CO₂ injections of increasing concentration. The same procedure as described above will be performed except that no striped bass will be injected into the secondary channel before treatment. After the predator removal effort is completed with a certain CO₂ concentration (0, 50, 75, 100, 125, 150, 200, 250, or 300 mg/L) the secondary channel will be flushed until the CO₂ concentration returns to an ambient level and another predator removal effort with a 300 mg/L CO₂ concentration will be performed. Preliminary data suggests that a 300 mg/L concentration is well over the concentration that is 100% effective (150 mg/L) at removing striped bass from the bypass tubes and secondary channel, therefore, any fish remaining after the first predator removal should be collected at the 300 mg/L concentration. This allows us to determine the effectiveness of each CO₂ concentration tested. Ninety-six h survival will be determined for all striped bass recovered from the initial predator removal efforts at concentrations of 50, 75, 100, 125, 150, 200, 250, and 300 mg/L. Survival of striped bass collected during the 300 mg/L predator removal efforts that followed each tested CO₂ concentration will not be determined due to the fact that fish collected in this sample will be exposed to numerous CO₂ concentrations.

3) Current vs. Alternative Predator Removal Method

To evaluate the current and alternative predator removal techniques we will compare removal efficiency, survival, salvage loss time, cost, and safety. This will be done by performing five repetitions of each predator removal in which groups of 30 comparable sized striped bass (300-800 mm FL) will be given a distinct color/fin tag and released into the secondary system prior to each trial.

In order to perform the current predator removal technique we would first inject a distinctly marked group of 30 striped bass into the secondary channel. The secondary channel will then be drained, by closing all bypass tubes, in order to remove any readily available tagged predators using a dip or seine net. The SN downstream of the secondary channel will be lowered in order to collect any fish that are lost (not successfully louvered) during this predator removal process. The order that each bypass tube (1–4) will be flushed will be randomly determined and each tube will be individually opened for about 30 s while two biologists, equipped with waders and safety harnesses, hold a 6.35 mm mesh fyke net at the bypass mouth in order to collect flushed fish. After each of the bypass tubes has been flushed all bypasses will be opened and the secondary channel will be filled. The sieve net will be raised and any fish will be removed, identified, and measured. The proportion of tagged striped bass successfully recovered will then be determined. All tagged striped bass will be held for 96 h to determine survival. The time it takes to perform each trial will be determined in order to evaluate salvage loss due to secondary downtime. Time will be started the moment that HT flow is stopped until HT flow is resumed. The cost to perform each trial will also be estimated and will include labor, waders, harnesses, and price of the fyke, dip and seine nets. This process will be repeated until five repetitions of the current predator removal method are completed. Five replicates were chosen due to the fact that we are only interested in seeing differences greater than 25% between capture efficiencies of the two methods.

The evaluation of the alternative predator removal technique involves using the CO₂ concentration that was previously determined to be optimal for the removal of striped bass from the secondary channel. The secondary channel will be drained in order to install 1/5 hp pumps at the mouth of each bypass tube to provide water samples for CO₂ and pH measurements. The secondary channel will then be back-filled and all VC pumps will be turned off in order to achieve a secondary flow of 0.57 cm/s. The SN will be lowered and a distinctly marked group consisting of 30 striped bass will be injected into the secondary channel. Dry ice will then be introduced (location to be determined) until the optimal [CO₂] is reached. A secondary flow of 0.57 cm/s will be maintained for 10 min. After this time period an empty HT will be opened and VC pumps will be turned on in order to flush the bypass tubes at 0.46–0.61 m/sec. The proportion of tagged striped bass successfully louvered into the HT while using the optimal CO₂ concentration will be determined. All successfully recovered tagged striped bass will be held to determine 96 h survival. The time it takes to perform the alternative predator removal method will be determined by starting the timer when flow into the HT ceases and stopping the timer when HT flow is resumed. This will allow us to determine salvage loss due to secondary downtime.

A cost of performing the alternative method will be estimated and will include dry ice costs, titration cells, pH meter, pumps, hoses, extension cords, and labor. This procedure will be repeated until five repetitions of the new predator removal technique are completed. Five replicates were chosen due to the fact that we are only interested in seeing differences greater than 25% between capture efficiencies of the two methods.

The alternative predator removal efficiency will also be investigated by performing the two methods consecutively to remove wild striped bass from the bypass tubes and secondary channel. The same procedure a described above will be performed except that striped bass will not be injected into the secondary channel before each predator removal method is completed. The current predator removal method will be performed first and will be immediately followed by a CO₂ predator removal using at least the concentration that was previously determined to be optimal for the removal of striped bass from the secondary channel. Fish that are recovered during the CO₂ treatment will be assumed to have been missed by the current predator removal method.

After completing the necessary replicates for both the current and alternative predator removal methods we will be able to make the appropriate comparisons between predator removal efficiency, predator survival, salvage loss time, cost and safety. This will allow us to determine which method is most effective and should be implemented as a TFCF predator removal technique.

Data Analyses

Carbon dioxide concentration in the secondary channel vs. time will be graphed for each of the 3 dosing techniques (large blocks, small blocks, gas). This graph will provide information on how stable the concentration of CO₂ stays with time. Logistic regression will be used to see if a significant capture-dose response exists within the range of 0–300 mg/L and if this is influenced by water temperature. A probability-capture curve will be used to determine the probability of capture within 25% for each CO₂ concentration being tested (*i.e.*, 0, 50, 75, 100, 125, 150, 200, 250, and 300 mg/L) using Probit analysis with a logit link function. A probability-survival curve will be used to determine the probability of 96 h post survival within 25%. Contingency tables will be used to compare the proportion of injected fish removed using the current and alternative predator removal methods. Contingency tables will be used to compare the proportion of wild striped bass collected in the bypass tubes and secondary channel using the current and alternative methods. Contingency tables will also be used to compare the proportion of fish that die or show signs of damage after 96-h for each treatment. The average time needed to complete the old and new predator removal methods will be compared using a t-test.

Coordination and Collaboration

This study will be coordinated with the TFCF staff, Tracy Technical Advisory Team (TTAT), and California Department of Fish and Game (CDFG). Participation and inclusion of research-related updates will be provided at regularly scheduled TTAT and Central Valley Fish Facilities Review Team (CVFFRT) meetings.

Endangered Species Issues, “Take” Considerations

Based on results from the examination of 96 h survival of Chinook salmon and delta smelt after being exposed to varying CO₂ concentrations, it is possible that mortality of listed species could occur if predator removals using CO₂ as an anesthetic are completed during the normal entrainment season of these species. This is due to the fact that Chinook salmon and delta smelt exhibited a lower tolerance to elevated CO₂ levels than striped bass. The dose necessary in order to move adult striped bass through the TFCF bypass tubes and secondary channel may be over the concentration in which Chinook salmon and delta smelt exhibited 100% survival. Winter-run Chinook salmon, steelhead trout (*O. mykiss*), and delta smelt may also be collected in holding tanks and encountered during these experiments. If this occurs, these fish will be immediately documented, returned to the Delta, and reported to all appropriate agencies. In order to minimize the risk of mortality to listed species, all attempts will be made to complete research activity during seasonal periods in which salvage of listed species is not likely to occur.

Although the procedures during experimentation may lead to mortality of listed species, the cumulative lethal take of listed species for the facility is surely much higher in the absence of predator removal activities.

Dissemination of Results (Deliverables and Outcomes)

A draft report for peer review and for TTAT covering Phases 1–4 was completed on March 2013. Progress on the final phase (Phase 5) was minimal during the 2010-2013 research periods due to the fact that other projects took priority and a contract for dry ice was not in place for a portion of this period. This contract has since been finalized and Phase 5 will be worked on during the next two years and a draft report for peer review and for TTAT will be completed by Jan. 2015. The primary deliverable will be an article published in the Tracy Volume Series. Updates will also be provided at TTAT and CVFFRT meetings. Additionally, information will be gained on the successes and limitations of alternate predator removal techniques at the TFCF. This knowledge will help guide future development and implementation of predator removal procedures at the TFCF and other fish facilities.

Literature Cited

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