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Proposal Package for Tracy Fish Facility Improvement Program

Tracy Fish Facility Improvement Program
California-Great Basin · Interior Region 10

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Proposal Package for Tracy Fish Facility Improvement Program

Tracy Fish Facility Improvement Program
California-Great Basin · Interior Region 10

prepared by

Bureau of Reclamation
Tracy Fish Collection Facility

Bureau of Reclamation
Technical Service Center

Cover Photograph: Tracy Fish Collection Facility, Byron, California (San Luis Delta Mendota Water Authority).

Mission Statements

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Tracy Fish Facility Improvement Program

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Disclaimer

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ExCell Plus as a Substitute for Formalin: Decomposition Rates and DNA Extraction of Larval Stage Delta Smelt (*Hypomesus Transpacificus*)

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Summary

The Bureau of Reclamation's Tracy Fish Collection Facility (TFCF), located in Byron, California, was developed to collect and salvage fish from Sacramento-San Joaquin Delta (Delta) water being pumped by the Central Valley Project's C.W. "Bill" Jones Pumping Plant. Fish that are salvaged by the TFCF are transported to designated release sites in the Delta away from the influence of the water export pumps. The salvage has historically included a wide variety of both introduced and native fish species, including listed species such as the Delta Smelt (*Hypomesus transpacificus*; Federal Register 1993). The TFCF may potentially collect and salvage Delta Smelt throughout the year but is usually limited to the non-summer months. The TFCF implements a larval sampling program targeting larval Delta Smelt, from spring to early summer of each year to meet regulatory requirements.

The Delta Smelt is the primary species of focus of larval sampling throughout the Sacramento-San Joaquin Delta. Once collected in the field, a fixative is necessary to preserve the important characteristics (fin structure, physical body condition, pigments, etc.) needed for accurate identification of Delta Smelt in a laboratory environment. Using formalin as the primary fixative has been the standard for many years.

Preparing, handling, and disposing of formalin requires significant time, expense, and effort. Any formalin spill could create a major safety hazard requiring intervention by hazardous materials specialists. Formalin poses an inhalation risk and can produce symptoms even at very low atmospheric concentrations around 0.10 ppm (Cancer.gov 2018). The EPA has classified formaldehyde, the main ingredient of formalin, as a "probable human carcinogen" (EPA 1984). The storage of formalin, particularly at its 37% concentration, requires the use of flammable storage cabinets due to its highly flammable nature.

While formalin is recommended as a fixative agent for larval fish (Kelso et al. 2012), its use does have major disadvantages. Formalin can damage specimen DNA in typical conditions (Koshiba et al. 1993) and dissolve otoliths (McMahon 1979). This is a major disadvantage for larval sampling operations. Delta Smelt can be very difficult to identify in their larval stage, even for experts (Wang 1991). Therefore, positive identification of Delta Smelt ID can be difficult without DNA testing as a QA/QC measure.

One alternative to formalin is ExCell Plus, an ethanol and glyoxal based tissue fixative. While commonly used to preserve histological samples, ExCell Plus has been used previously to preserve freshwater fish larvae (Acre 2015, Gilbert 2016). ExCell Plus is a low hazard fixative, posing relatively low risk to personnel and the environment. It is less flammable than 10% formalin (standard concentration for larval preservation), has a reduced inhalation risk, and does not require any special procedures for disposal.

Problem Statement

There have been no direct comparisons between ExCell Plus and 10% formalin for preserving freshwater fish larvae. It is unknown whether ExCell Plus preserves important larval characteristics similar to 10% formalin. The manufacturers of ExCell Plus advertise its use as leaving biological

tissue 'capable of having DNA and similar materials extracted.' ExCell Plus would hold a major advantage over formalin as a fixative for larval sampling programs if it can preserve important larval characteristics and allow for DNA testing.

Goals and Hypotheses

Goals:

1. Compare the effectiveness of ExCell Plus vs. 10% formalin for the preservation of tissue structure and pigmentation of Delta Smelt larval fish specimens, both in the short and long term.

2. Determine whether advertised benefits of ExCell Plus – particularly its preservation of specimen DNA – hold true in Delta Smelt larval fish.

Hypotheses:

1. ExCell Plus will be just as effective at preserving tissue structure and pigmentation of larval Delta Smelt specimens.

2. Usable DNA for species identification will be able to be obtained from larval Delta Smelt preserved in ExCell Plus at all study timelines.

Materials and Methods

504 larval Delta Smelt (*Hypomesus transpacificus*; 15mm – 25 mm) will be gathered alive at the University of California Davis Fish Conservation and Culture Laboratory (FCCL) and divided into 8 groups, each containing 63 fish. Four of the groups of fish (252 fish total) will be placed into separate 250 mL containers containing 10% formalin. The remaining four groups of fish (252 fish total) will be placed into separate 250 mL containers containing ExCell Plus. No live fish will be transported offsite from the FCCL. A Leica microscope and attached camera will be used to photograph all the larval Delta Smelt in the designated 250 mL containers at the following time intervals: 1 day, 30 days, 90 days, 365 days (Table 1). Each individual fish will be photographed only once at its designated time interval. This will rule out physical degradation of the specimen due to over handling. The fish in each picture will be visually assessed and four key larval fish identification characteristics; myomere integrity, fin/ray integrity, gut integrity and pigments will be recorded as present or absent (Table 2).

Table 1.—Number of larvae to be photographed at each time interval for both 10% formalin and ExCell Plus.

	Day 1	Day 30	Day 90	Day 365
Ten Percent Formalin	63 Larvae	63 Larvae	63 Larvae	63 Larvae
ExCell Plus	63 Larvae	63 Larvae	63 Larvae	63 Larvae

Table 2.—Characteristics larval Delta Smelt to be assessed by image reviewers. "P" represents a present characteristic and "A" marks that characteristic as absent.

Myomeres	P or A	P or A	P or A	P or A
Fin/Rays	P or A	P or A	P or A	P or A
Gut	P or A	P or A	P or A	P or A
Pigments	P or A	P or A	P or A	P or A

Thirty-two Delta Smelt (15mm – 25 mm) will be randomly chosen from the ExCell Plus groups after they are photographed at each time interval. To confirm accurate species identification, the fish will go through DNA bar coding testing performed by Cramer Fish Sciences at the approximate time intervals; 30 days, 90 days, 120 days, 395 days. The DNA bar coding test will provide useful information about the timeline that DNA testing is successful in confirming a larval Delta Smelt's identification when ExCell Plus was the fixative (Table 3).

Table 3.—Number of larvae to be tested via DNA barcoding at each time interval for ExCell Plus.

	Day 30	**Day 90**	**Day 120**	**Day 365**
ExCell Plus	32 Larvae	32 Larvae	32 Larvae	32 Larvae

The preservative comparison sample size calculations are for a power analysis comparing two proportions with two-tailed tests (Figure 1). Sample sizes are given as the number of fish for each treatment for each time point. The total number of fish necessary for each time point is two times the number given because there are two treatments (Table 4).

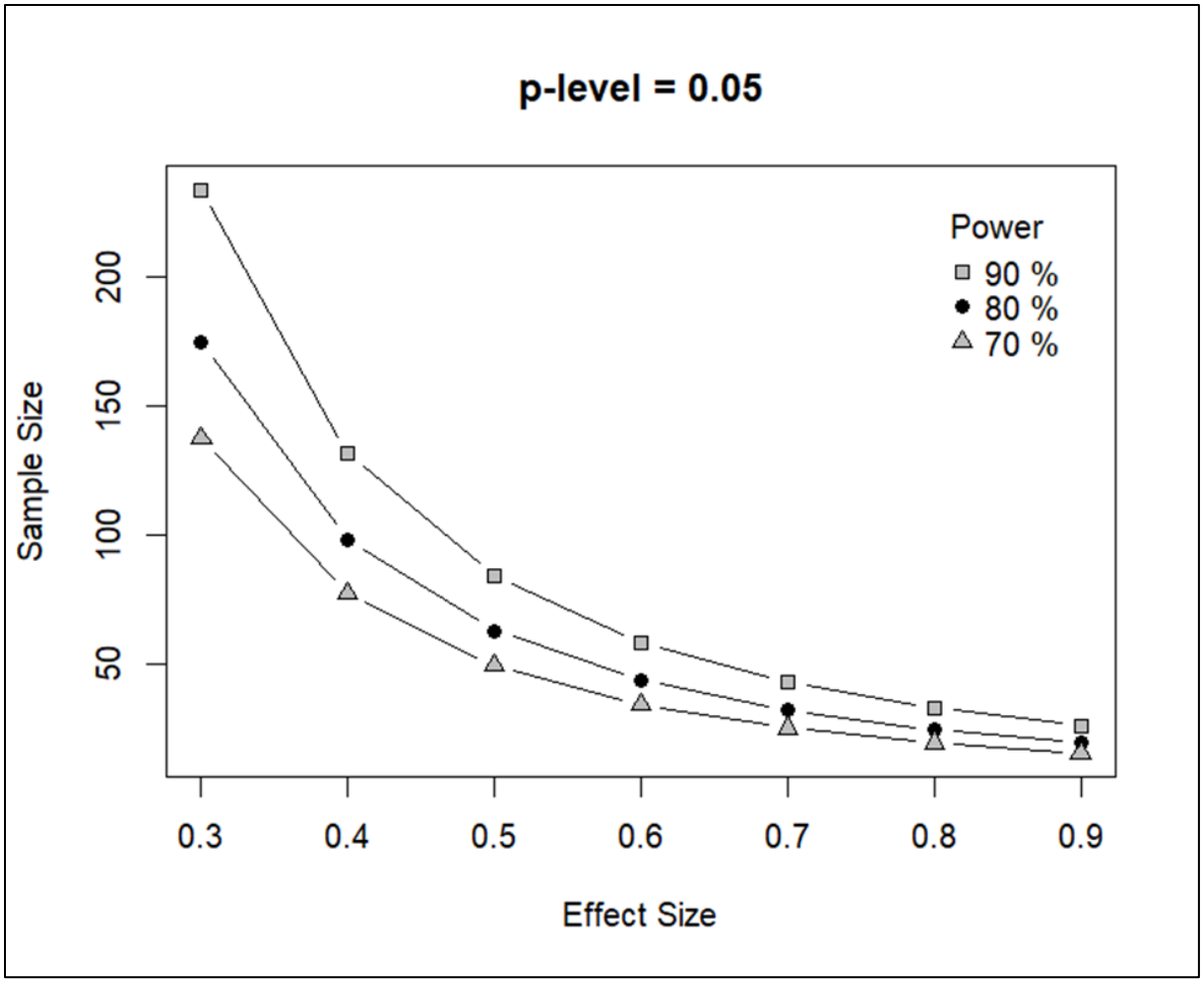

Figure 1.—Pairwise sample sizes necessary to achieve various levels of power (70, 80, and 90%), given various effects sizes for a study comparing proportions of samples that have certain fish identification markers. For reference, 0.3 is a small difference between the two treatments (i.e., the proportions are similar between the two treatments) and 0.9 is a large difference. Estimates of sample sizes are also given in Table 4.

Table 4.—**Estimated Sample Size for Power Levels**: Pairwise sample sizes necessary to achieve various levels of power (70, 80, and 90%), given various effects sizes for a study comparing proportions of samples that have certain fish identification markers. For reference, 0.3 is a small difference between the two treatments (i.e., the proportions are similar between the two treatments) and 0.9 is a large difference.

Effects Size	70 Percent	80 Percent	90 Percent
0.3	138	175	234
0.4	78	99	132
0.5	50	63	85
0.6	35	44	59

The DNA testing sample size calculations are for a single proportion power analysis with two-sided test (Figure 2). Sample sizes are given as the number of fish for each treatment for each time point (Table 5).

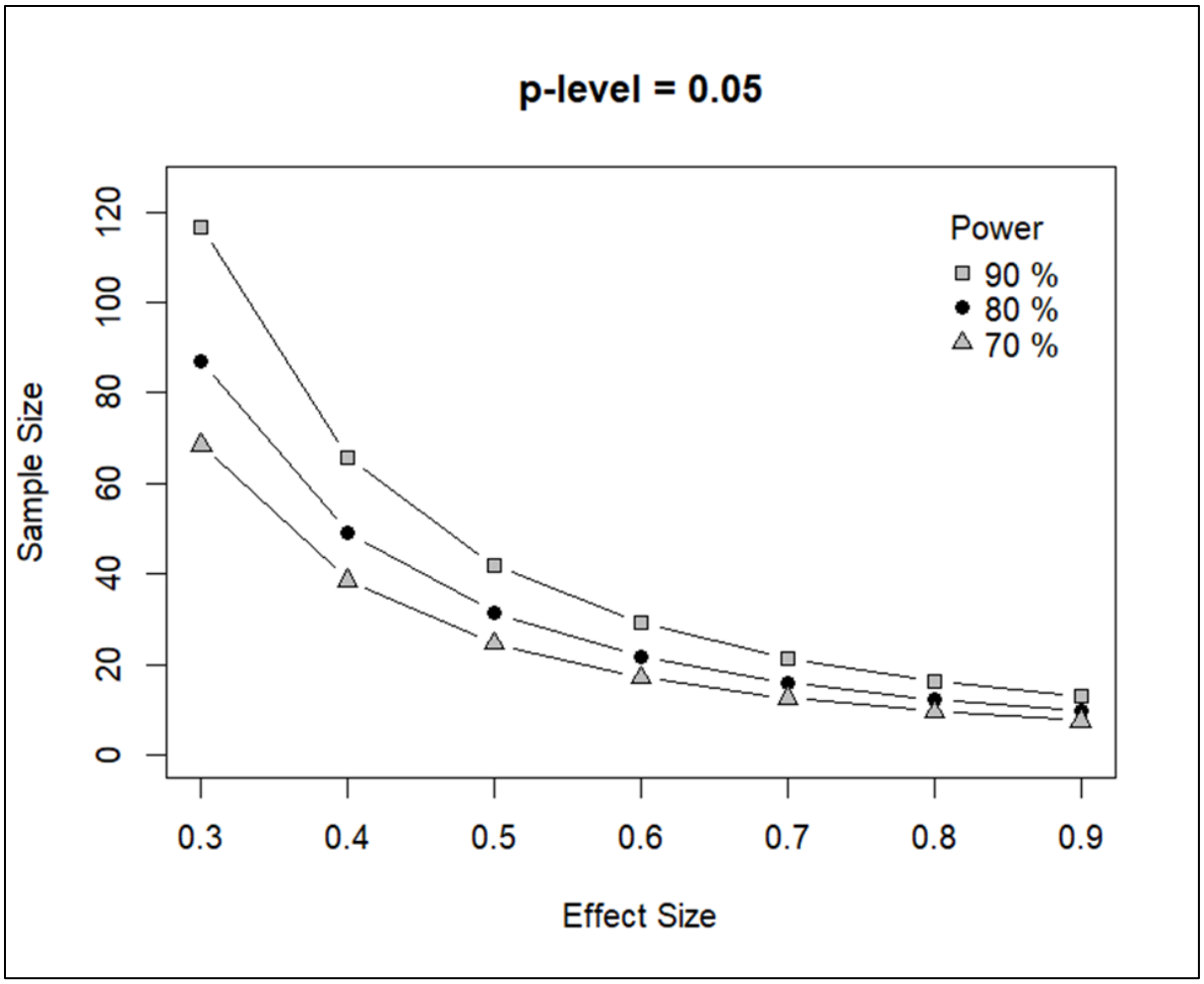

Figure 2.—DNA testing sample size estimates for a one-sample proportion test.

Table 5.—**Estimated Sample Size for Power Levels:** DNA testing sample size estimates for a one-sample proportion test.

Effects Size	70 Percent	80 Percent	90 Percent
0.3	69	88	117
0.4	39	50	66
0.5	25	32	43
0.6	18	22	30
0.7	13	17	22
0.8	10	13	17
0.9	8	10	13

Assumptions and Limitations

It is assumed that the TFCF laboratory and its equipment, specifically the microscope and attached camera, will be fully functional for the entire course of the project. It is also assumed that all staff will remain present and available to work.

Coordination and Collaboration

This research will be coordinated and conducted by the TFCF Biological Resources Section in collaboration with U.S. Fish and Wildlife Service and Cramer Fish Science's Genidaqs Lab. USFWS has a vested interest in this project because they conduct a very similar larval sampling program and protocols. Cramer Fish Science's Genidaqs Lab has previous agreements with USBR and conducts genetic work with the TFCF Biological Resources Section regularly. The USFWS biologists and Cramer Fish Sciences genetics research associate will serve as co-PIs, and assist with all aspects of the study, including study design, data analysis and report writing. UC Davis' participation is essential to the project's success by providing cultured Delta Smelt and fish husbandry.

The project development, equipment, and data collection portions of this project have already been completed. The data analysis portion of this project is in progress and will be finalized in FY22.

Endangered Species Issues, "Take" Considerations

The use of cultured Delta Smelt for this project is covered under the University of California-Davis, Fish Conservation and Culture Lab (FCCL) Federal Fish and Wildlife Permit TE-027742-5 which expires June 25, 2022.

Dissemination of Results (Deliverables and Outcomes)

Data analysis and results will be shared at a Tracy Technical Advisory Team (TTAT) meeting and a Tracy Series Report will be completed in tandem with publication in an appropriate scientific journal. The information gained will be utilized by operations and other larval sampling programs throughout the system.

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Evaluation of the Traveling Water Screen at the Secondary Channel Using Larval and Juvenile Delta Smelt (*Hypomesus Transpacificus*)

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Summary

The Tracy Fish Collection Facility (TFCF) is located at the head of the Delta-Mendota Canal in the southern region of Sacramento-San Joaquin Delta (Delta) near Tracy, California. The facility was constructed in the 1950s to salvage fish that would otherwise be entrained by the Central Valley Project's C.W. "Bill" Jones Pumping Plant (JPP). Since inception, the TFCF used behavioral louver arrays in the primary and secondary channels that were angled 15° to the flow of water with 2.5 cm (1 in) spaced vertical slats angled 90° to the direction of flow that create a disturbance in the water and guide fish into one of four recessed holding tanks (6.1 m wide, 5.0 m deep). Reclamation replaced the secondary louvers (2.5 cm opening) in June 2014 with traveling water screens (Hydrolox™, Intralox LLC, Harahan, Louisiana) that have smaller screen opening (1.5 mm width x 50 mm length).

Delta Smelt (*Hypomesus transpacificus*) is a federally listed threatened species native to the Delta (Federal Register 1993) and is salvaged at the TFCF (CDFW, ftp salvage records website). The larval, juvenile, and adult life stages are reported when they are observed during fish counts and when they are detected during larval fish sampling. Delta Smelt larvae and juveniles were expected to be guided successfully (salvaged) to the holding tanks by the new screens. Data collected from this study will determine how velocity affect larval and juvenile Delta Smelt secondary channel efficiency. The field data collection portion of the study was completed in 2016. Data analysis is planned for FY21; funds are being requested for the development of the report for FY22.

Problem Statement

The traveling water screen's efficiency in guiding Delta Smelt larvae and juveniles to the holding tanks is unknown. Furthermore, the State Water Resources Control Board Decision 1485 (i.e., D-1485) states the secondary channel be operated at salmon criteria, or 3.0–3.5 fps, between February and May, months when larval and juvenile Delta Smelt are observed at the TFCF. It is uncertain how this range of velocities and the traveling screen interact and affect the diversion of larval and juvenile Delta Smelt to the holding tanks.

Goals and Hypotheses

Goals:

1. Determine if secondary channel water velocity (0.3–0.9 mps) affect the salvage of Delta Smelt larvae and juvenile to the holding tank.

Hypotheses:

1. Secondary channel velocity and fish size have no effect on traveling screen efficiency.

Materials and Methods

Because Delta Smelt is a protected species and wild Delta Smelt cannot be used, cultured Delta Smelt were obtained from the UC Davis Fish Conservation and Culture Laboratory (FCCL) and these fishes were used as surrogates for wild Delta Smelt. A memorandum of understanding (MOU) was prepared with CDFW allowing the use of cultured Delta Smelt within the compounds of the TFCF for this study. In 2015, 3,000 juveniles measuring 20–30 mm fork length (FL) and in 2016, 10,000 individuals measuring 15–40 mm FL were used.

Five secondary channel velocities were tested to cover the full range of typical operations: 1.0, 1.5, 2.0, 2.5, 3.0 fps (or 0.3–0.9 mps). All test trials were conducted during the daytime. Predator removal using carbon dioxide following protocols published by Wu and Bridges (2014) was completed before each trial. Traveling water screen efficiency and participation will be calculated using the following equations:

$$\text{Efficiency} = HT/(HT + SN)100$$
$$\text{Participation} = [(HT + SN)/200)]100 \quad \text{(Eq.1)}$$

where:
HT = number of Delta Smelt recovered in the holding tank,
SN = number of Delta Smelt recovered in the sieve net behind the screen.

Coordination and Collaboration

This study was coordinated with the UC Davis Fish Culture and Conservation Laboratory. Participation and inclusion of research-related updates will be provided at regularly scheduled Tracy Technical Advisory Team (TTAT) and Central Valley Fish Facilities Review Team (CVFFRT) meetings. Statistical analysis will be provided by the Technical Service Center and a final Tracy Series Report will be prepared by the TFCF Biological Resources Section with input from the Technical Service Center.

Endangered Species Issues, "Take" Considerations

Field data collection has been completed for this study and no take is anticipated.

Dissemination of Results (Deliverables and Outcomes)

Field data collection and separation of samples were completed in winter 2016; laboratory data collection which includes measuring specimens, identification, and data entry is 99% complete. The remaining 1% is expected to be completed by August 2021. Data analysis is expected for FY21. A written report is expected for FY22. The venue for dissemination of results will be through the Tracy Series Reports. Data and metadata will be made available for digital archive. Results will be provided at TTAT and CVFFRT meetings.

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Determining Optimal Carbon Dioxide Concentration for Implementation of Carbon Dioxide Predator Removals in the Bypass Pipes and Secondary Channel at the Tracy Fish Collection Facility

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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 (Delta) beyond the influence of Central Valley Project's 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 (Liston et al. 1994, Fausch 2000).

Predators accumulate throughout the facility, including in front of the trashrack, the primary channel, the bypass pipes, the secondary channel, and the holding tanks (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 flushed 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 included catfish, sunfish and gobies. 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, an alternative predator removal method using carbon dioxide (CO_2) was approved for study. This method does not reduce daily salvage due to secondary channel downtime and is likely more efficient and safer for employees and fish than the historic predator removal method (Wu and Bridges 2014). An initial evaluation of the use of CO_2 as an alternative predator removal technique in the TFCF bypass pipes and secondary channel was completed in September 2007 and demonstrated that elevated CO_2 concentrations are effective for removing predatory fish from the bypass pipes and secondary channel at the TFCF. Results from this initial evaluation have been published as a Tracy Series Report (Wu and Bridges 2014), although the authors did not recommend a CO_2 concentration that should be used upon implementation of this method at the TFCF.

Fourteen replicates have been completed for this project during which Striped Bass removal efficiency and 96-h post-treatment survival were determined. In addition, 5 replicates have been completed during which only Striped Bass 96-h post-treatment survival was investigated. Minimal data collection occurred for this project during the FY2020 and FY2021 research period due to the COVID-19 pandemic. It will be necessary to complete at least 6 more replicates (at approximately 25, 125, 150, 200, 250 and 300 mg/L) to thoroughly investigate Striped Bass removal efficiency and 96-h post-treatment survival for CO_2 concentrations up to 300 mg/L.

Preliminary results suggest the optimal CO_2 concentration for use during predator removals in the bypass pipes and secondary channel at the TFCF (i.e., the CO_2 concentration that resulted in the highest combination of Striped Bass removal efficiency and 96-h post-treatment survival throughout the range of water temperature and water quality conditions observed at the TFCF) is approximately 150 mg/L. To obtain this CO_2 concentration within the bypass pipes and secondary channel at the TFCF using current procedures, it is recommended that approximately 68.0 kg of dry ice be injected into each bypass tube for each predator removal effort.

Problem Statement

Predation may be significant within the primary bypass pipes and secondary channel because Striped Bass continue to reside within them. Removing these fish with the historic method is dangerous for employees, likely decreases daily salvage, and likely causes damage to the fish and/or fish mortality. An initial evaluation of the use of CO_2 as an alternative predator removal technique in the TFCF bypass pipes and secondary channel has been completed and published (Wu and Bridges 2014), although authors did not recommend a CO_2 concentration that should be used upon

implementation of this method. The goal of this project is to determine the optimal CO_2 concentration for the implementation of CO_2 predator removals in the bypass pipes and secondary channel at the TFCF considering removal efficiency and 96-h post-treatment survival.

Goals and Hypotheses

Goals:

1. Determine the optimal CO_2 concentration for a 15-minute exposure relative to removal efficiency and survival throughout the range of temperature and water quality conditions observed at the TFCF.

2. Estimate a single, set amount of dry ice (kg) that should be inserted per bypass pipe to approximately obtain the optimal CO_2 concentration within the bypass pipes and secondary channel at the TFCF.

Hypotheses:

1. All CO_2 concentrations will result in equal removal efficiency and survival over a 15-minute exposure period.

Materials and Methods

The optimal CO_2 concentration for the removal and survival of wild Striped Bass will be investigated by performing consecutive CO_2 injections with increasing CO_2 concentration in the TFCF bypass pipes and secondary channel. Replicates for this study will be performed in concurrence with monthly facility CO_2 predator removals at the TFCF; therefore, labor and materials costs will be split 50/50 with TFCF operations.

To obtain water samples for monitoring of pH and CO_2 concentration, it will be necessary to install a 1/5 hp pump in the secondary channel prior to the initiation of consecutive CO_2 predator removal replicates. The secondary channel Velocity Control (VC) pumps will be operated to achieve a secondary flow of approximately 0.57 m3/s and water flow will be initiated into an empty holding tank. Carbon dioxide (in the form of dry ice) will then be injected into the bypass pipes to obtain an initial target CO_2 concentration (approximately 25, 125, 150, 200, 250 and 300 mg/L for FY2022) and minimal water flow in the bypass pipes and secondary channel will be maintained for 15 minutes to increase contact time between CO_2 gas and water. Using the submersible pump, hose, and pH meter previously described, pH will be continuously monitored throughout the 15-minute minimal flow treatment period. Carbon dioxide concentration will be measured from a water sample taken at the lowest observed pH to estimate the maximum CO_2 concentration that was achieved during each initial CO_2 treatment. After the 15-minute minimal water flow period, the number of secondary channel VC pumps in operation will be adjusted to maximize (> 3.4 m3/s) water flow in the bypass pipes and secondary channel for 15 minutes to flush lethargic fish downstream into a holding tank.

After conclusion of the 15-minute maximum flow period, water flow will be switched to an empty holding tank, minimized in the bypass pipes and secondary channel, and the process will be repeated with the insertion of approximately 136.1 kg of dry ice per bypass pipe with the intention of obtaining a CO_2 concentration of approximately 300 mg/L in the bypass tubes and secondary channel to remove any fish that may have remained after the initial CO_2 treatment. Preliminary data suggests that a 300 mg/L concentration is well over the concentration that is 100 percent effective (150 mg/L) at removing Striped Bass from the bypass pipes and secondary channel, therefore, any fish remaining after the first predator removal should be collected at the 300 mg/L concentration. This will allow us to determine the effectiveness of each CO_2 concentration tested.

Fish collected in holding tanks will be transferred to a 356 x 74 x 76-cm (l x w x h) rectangular tank (equipped with aerated, flow-through, raw Delta water) using the 1544.5-L haul-out bucket at the TFCF. Ninety-six h survival will be determined for all wild Striped Bass recovered from initial CO_2 treatments. Ninety-six h survival will not be investigated for non-target species. Survival and efficiency of removal for wild Striped Bass collected during the 300 mg/L predator removal efforts that follow each tested CO_2 concentration will not be determined due to the fact that fish collected in this sample will be exposed to numerous CO_2 concentrations. Striped Bass saved for 96-h survival will be removed from the rectangular tank using a dipnet with clear rubber mesh and placed in an ice chest containing oxygenated raw Delta water. These Striped Bass will then be transported and transferred to 757.1-L circular tanks (at a density of up to 5 fish/tank) containing only aerated raw Delta water (no salt will be added). Feed will be withheld for the first 24 h of the 96-h survival monitoring period, after which fish will be fed live feed daily.

The CO_2 concentration that is determined to exhibit the highest combination of removal efficiency and 96-h post-treatment survival throughout the range of temperature and water quality conditions observed at the TFCF will be considered the optimal dose for implementation of CO_2 predator removals in the bypass pipes and secondary channel at the TFCF.

Data Analyses

Logistic regression will be used to determine if a significant capture-dose response exists within the range of 0–300 mg/L for all sizes of Striped Bass combined. A scatterplot will be used to illustrate the relationships between CO_2 concentration, removal efficiency, and 96-h post-treatment survival. The CO_2 concentration at which best-fit trend lines for removal efficiency and 96-h post-treatment survival intercept (the CO_2 concentration at which there is the highest combination of removal efficiency and 96-h post-treatment survival) will be considered the optimal dose for implementation of CO_2 predator removals in the bypass pipes and secondary channel at the TFCF. A scatterplot illustrating the relationship between the amount of dry ice injected (kg) and the maximum CO_2 concentration obtained (mg/L) during historical CO_2 treatments performed at the TFCF will be used to recommend a single, set amount of dry ice (kg) that should be inserted into each bypass pipe at the TFCF for each predator removal effort.

Assumptions and Limitations

It is assumed wild Striped Bass will be available and the Tracy Aquaculture Facility will be operational and able to adequately hold this species. In addition, it is assumed that an appropriate

number of personnel (4–5 individuals) will be available to perform consecutive CO_2 injections to determine optimal CO_2 concentration for the removal and survival of Striped Bass. Access to appropriate safety equipment (dry ice gloves, eye protection, etc.) will be necessary to perform dry ice injections. The Biological Resources group at the TFCF will also need the ability to adjust secondary channel flow as needed for this study. It is assumed that the contract for dry ice delivery will remain active and that no other projects or studies will take priority or precedence during the FY2022 research period.

For data analyses, it is assumed that Striped Bass size does not affect removal efficiency or survival; therefore, all sizes of Striped Bass collected during each treatment will be combined for data analyses. It is also assumed that CO_2 concentration and water temperature are the only variables that affects Striped Bass removal efficiency and survival. In addition, it is assumed that removal efficiency is 0% and 96-h survival is 100% without CO_2 treatment.

Coordination and Collaboration

This study will be coordinated with the TFCF staff, Tracy Technical Advisory Team (TTAT), and California Department of Fish and Wildlife (CDFW). 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

Winter-run Chinook Salmon (*Oncorhynchus tshawytscha*), Steelhead Trout (*O. mykiss*), and Delta Smelt (*Hypomesus transpacificus*) may be encountered during these experiments. Based on results from Wu and Bridges (2014), it is possible that mortality of listed species could occur if predator removals using CO_2 as an anesthetic are completed during the normal entrainment season of these species. This is because certain species, such as Delta Smelt, exhibited a lower tolerance to elevated CO_2 levels than Striped Bass and displayed up to 70% mortality over 96 h after being exposed to 100 mg/L CO_2 for 20 min. If listed species are encountered, they will be immediately documented, returned to the Sacramento-San Joaquin Delta (if alive), 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. All fish take for this project is covered under the most recent National Marine Fisheries Service Biological Opinion as well as current CDFW Scientific Collecting Permits held by the biology staff at the TFCF. Although the procedures during experimentation may lead to mortality of listed species, the cumulative lethal take of listed species for the facility is likely much higher in the absence of predator removal activities.

Dissemination of Results (Deliverables and Outcomes)

Data collection and analyses will likely be completed during the FY2022 research period. Updates will be provided at TTAT and CVFFRT meetings. The primary deliverable will be an article published as a Tracy Series Report. A draft report for peer review is anticipated to be completed by

the end of FY2022. The final published report is expected to be posted to the Tracy Fish Facility Improvement Program website in FY2023. Information will be gained on the successes and limitations of this alternate predator removal technique at the TFCF. This knowledge will help guide future development and implementation of predator removal procedures at the TFCF and other fish facilities.

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Feasibility of Using Carbon Dioxide to Remove Resident Piscivorous Fish From the Tracy Fish Collection Facility Primary Channel

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Summary

Action IV.4.1(1)(a) of the 2009 National Marine Fisheries Service (NMFS) Biological Opinion and Conference Opinion on the Long-Term Operations of the Central Valley Project and State Water Project (BiOp) mandates that the U.S. Bureau of Reclamation (Reclamation) complete studies to determine methods for removal of predators in the primary channel at the Tracy Fish Collection Facility (TFCF) with the goal of implementing measures to reduce pre-screen predation in the primary channel to ten percent or less (NMFS 2009). While a predator removal program in the secondary channel at the TFCF has been ongoing since the early 1990s, there are few options for addressing predator loads in the primary channel. Reclamation personnel have reviewed various means of moving predators through the TFCF system such as electricity, sound, light, and mechanical methods. Many of these techniques are largely ineffective for removing large piscivorous fish, expensive to install and operate, and are logistically difficult to implement

(Fausch 2000). The use of carbon dioxide (CO_2), in the form of dry ice, was recently evaluated as a predator removal technique in the bypass pipes and secondary channel at the TFCF and was found to effectively remove fish, including piscivores, from this area (Wu and Bridges 2014). This suggests the periodic use of CO_2 may also be efficacious for the removal of piscivorous fish from the primary channel at the TFCF. If so, the use of CO_2 in the primary channel could be implemented at the TFCF to meet Action IV.4.1(1)(a) of the NMFS BiOp instead of investing funds for extensive research, design, development, installation and maintenance of more complicated predator removal systems or processes.

Data collection for this evaluation was completed in FY2019. A total of four CO_2 treatments in the primary channel were completed. The four treatments that were completed include an initial investigation to determine if acoustically tagged Striped Bass (*Morone saxatilis*) could be influenced or moved to a desired location within the primary channel by injecting dry ice, as well as separate investigations to determine if acoustically tagged Striped Bass could be guided into TFCF holding tanks or pushed downstream of the TFCF (where they do not have an impact on TFCF fish salvage) through an open primary channel louver panel with CO_2 treatment of the entire primary channel. Three of the CO_2 treatments in the TFCF primary channel were completed during 1 pump operation at the C.W. "Bill" Jones Pumping Plant (JPP; approximately 22.7–28.3 m3/s [800–1000 ft3/s] water flow, approximately 0.2 m/s [0.5 ft/s] water velocity) to minimize the volume of water that needed to be treated, although 1 treatment was completed during 2 pump operation at the JPP (approximately 45.3–56.6 m3/s [1600–2000 ft3/s] water flow, approximately 0.3 m/s [1.0 ft/s] water velocity) to determine if the method was feasible with increased water flows.

Preliminary results suggest that CO_2 treatment of the primary channel is a feasible technique to remove resident Striped Bass from the TFCF during 1 pump operation at the JPP, although the process is not 100% effective, is extremely labor intensive, and must be scheduled around certain uncontrollable factors (i.e., the number of pumps in operation at the JPP, tidal height, tidal direction, timing of tides, etc.). Acoustically tagged Striped Bass appeared to exhibit an avoidance response to elevated CO_2 concentrations in the TFCF primary channel and separate treatments of the entire primary channel during 1 pump operation at the JPP removed 41.7% of acoustically tagged Striped Bass by guiding them into a holding tank and 45.4% of acoustically tagged Striped Bass by guiding them downstream of the facility through an open TFCF primary channel louver panel. No fish were collected in a holding tank during CO_2 treatment of the entire TFCF primary channel with an open primary channel louver panel (all fish that were removed were pushed through the open louver panel). It appears that CO_2 treatment of the TFCF primary channel during 1 pump operation at the JPP also results in treatment of the bypass pipes and secondary channel and likely effectively removes fish from these areas as well. Treatment of the entire TFCF primary channel with CO_2 during 2 pump operation at the JPP did not yield the sustained elevated CO_2 concentrations necessary to effectively guide acoustically tagged Striped Bass from the primary channel into a holding tank and there was 0% removal during this operational condition. This suggests that the use of CO_2 for the removal of piscivorous fish from the primary channel at the TFCF may not be feasible when the JPP is operating at more than 1 pump.

Problem Statement

Action IV.4.1(1)(a) of the 2009 NMFS BiOp mandates that Reclamation complete studies to determine methods for removal of predators in the primary channel at the TFCF with the goal of implementing measures to reduce pre-screen predation in the primary channel to ten percent or less (NMFS 2009). The use of CO_2 was recently found to effectively remove fish, including piscivorous predators, from the bypass pipes and secondary channel at the TFCF (Wu and Bridges 2014). In addition, preliminary data from Wu et al. (In Progress) suggests that CO_2 concentrations of approximately 150 mg/L are optimal for the removal of Striped Bass from the bypass pipes and secondary channel at the TFCF, considering removal efficiency and survival. This suggests that the periodic use of CO_2 at a concentration of approximately 150 mg/L may also be efficacious for the removal of piscivorous fish from the primary channel at the TFCF. Due to this, the feasibility of using CO_2 at a concentration of approximately 150 mg/L to remove piscivorous fish from the primary channel will be investigated.

Goals and Hypotheses

Primary Goals:

1. Determine if a CO_2 concentration of approximately 150 mg/L can be reasonably obtained in the primary channel at the TFCF, within 30 min, considering the volume of water that needs to be treated and the amount of dry ice necessary.

2. Determine if a CO_2 concentration of approximately 150 mg/L increases the number of piscivorous fish removed from the primary channel during a 30-min treatment period.

3. Estimate the efficiency of removal for acoustically tagged Striped Bass in the primary channel at the TFCF using a CO_2 concentration of approximately 150 mg/L over a 30-min period.

Secondary Goals:

1. Provide a population estimate of the number of piscivorous fish in the TFCF system (primary channel, bypass pipes, and secondary channel) on the day of experimentation based on the proportion of acoustically tagged striped bass recovered, as well as numbers of wild piscivorous fish collected, during CO_2 treatment in the primary channel.

Hypotheses:

1. The injection of CO_2 in the primary channel will have no effect on the CO_2 concentration in the water due to large water volume and water flow within this component of the TFCF.

2. A CO_2 concentration of approximately 150 mg/L will not increase the number of piscivorous fish species removed from the primary channel at the TFCF.

3. A CO_2 concentration of approximately 150 mg/L in the primary channel at the TFCF will have no effect on the efficiency of removal for acoustic tagged Striped Bass.

Materials and Methods

In order to investigate the feasibility of using CO_2 to remove piscivorous fish species from the primary channel at the TFCF, it was necessary to adopt procedures described by Wu and Bridges (2014) for the secondary channel and modify them for use in an area of the facility with a larger volume of water and greater flow.

Since water flow and velocity in the TFCF primary channel are largely determined by the number of pumping units (1–5) being used for water export operations at the JPP, CO_2 treatment occurred when there was 1 or 2 pump operation at the JPP, which reduced the volume of water in the primary channel that needed to be treated. If possible, CO_2 treatments were performed when there was a slack low tide to further reduce the volume of water that was necessary to treat. Secondary channel velocity and flow rate were maximized to achieve increased water velocity and flow in the primary channel bypass entrances. The maximization of secondary channel water velocity and flow also maximized primary channel bypass ratios (velocity of water going into each bypass versus the velocity of water in the channel), which promoted entrance into the bypass pipes and, ultimately, collection of fish in holding tanks during both the control (30-min facility fish-count performed immediately prior to CO_2 treatment) and CO_2 treatment.

Approximately 2,721.6–3,628.7 kg (approximately 6,000–8,000 lbs.) of dry ice was requested to be delivered to the TFCF by the supplier (Innovative Federal Operations Group, LLC, Vista, California) on the day before experimentation. Upon delivery, dry ice was stored in large, outdoor dry ice coolers (0.85 m3; Polar Tech Industries, Inc., Genoa, Illinois) until preparation for injection. These coolers were conveniently located near the head of the primary channel at the TFCF, where injection of dry ice occurred.

To determine the reaction of piscivorous fish to elevated CO_2 treatment in the primary channel, as well as estimate the efficiency of removal when using a CO_2 concentration of approximately 150 mg/L during a 30-min treatment period, acoustic tags (Model 795-LY; HTI-Vemco USA, Inc., Seattle, Washington) were used, along with an acoustic system consisting of acoustic tag receivers (Model 290; HTI-Vemco USA, Inc., Seattle, Washington), hydrophones (Model 590; HTI-Vemco USA, Inc., Seattle, Washington), and hydrophone cables (Model 690; HTI-Vemco USA, Inc., Seattle, Washington) installed at the TFCF. The use of this technology allowed for the production of 2-dimensional tracks of acoustically tagged fish before, during, and after CO_2 treatment of the TFCF primary channel. In addition, the use of acoustic tags and 2-dimensional tracks allowed for estimation of removal efficiency when attempting to determine if acoustically tagged Striped Bass could be pushed downstream of the TFCF through an open primary channel louver panel.

Acoustic tags were surgically implanted in at least 10 Striped Bass (number chosen to allow for at least 10% precision) that were collected from the TFCF primary channel by angling. Striped Bass were chosen due to the fact that they were the most prevalent piscivorous fish species encountered during previous predator removal studies performed in the secondary channel at the TFCF (Liston et al. 1994; Wu and Bridges 2014; Sutphin et al. 2014) and are likely the main piscivorous fish species in the primary channel as well. Surgical implantation of acoustic tags in Striped Bass occurred up to 30 days prior to release and Tricaine Methanesulfonate (MS-222) was used as an anesthetic.

After surgical implantation of acoustic tags, Striped Bass were hand-carried to a wheeled recovery tub (228.6-L, 78.7-cm long x 50.8-cm wide x 57.1-cm deep) containing oxygenated 16 oC well water and transported to outside 1.2-m diameter (757-L) black tanks containing aerated, 16 oC well water where they were held at a density of up to two fish per tank. At least 1 week prior to release, tanks were gradually switched from well water to treated Delta water to appropriately acclimate fish. Two hours prior to release, Striped Bass were netted, transferred to perforated garbage cans containing approximately 37.9 L of treated Delta water, and transported to the head of the TFCF primary channel for release. Release of Striped Bass into the primary channel occurred 1 day prior to treatment with CO_2. This was done to demonstrate and verify that Striped Bass in the TFCF primary channel would not willingly move downstream through the facility and into a holding tank within 24 h. To prevent experimental Striped Bass from moving upstream through the 56-mm spaced trashrack at the upstream end of the facility, it was necessary to use only fish greater than 375 mm fork length (FL), which is the minimum size estimated by Sutphin et al. (2014) at which passage through the trashrack is restricted based on data collected at the TFCF. If possible, Striped Bass greater than 485 mm FL were used because Striped Bass up to this length have been found to move upstream through the 56-mm spaced trashrack at the TFCF (Karp et al. 2017). To prevent experimental Striped Bass from moving into the canal downstream of the primary louvers after being released in the primary channel, it was important to refrain from cleaning the primary louvers until after the predator removal in the primary channel was completed.

Prior to the start of CO_2 treatment, 149-W (0.2-hp) submersible pumps (Model 316; Carry Manufacturing, Inc., Munger, Michigan) were installed (at mid-water depth) throughout and downstream of the TFCF (i.e., in the primary channel, secondary channel, and intake canal to the JPP) to provide water samples for monitoring CO_2 and pH over time. The location, number, and configuration of submersible pumps varied based on the objective of each CO_2 treatment. Flow was maximized in the secondary channel to increase velocity at the primary channel bypass entrances and maximize primary channel bypass ratios to effectively guide fish from the primary channel into a bypass pipe and, ultimately, into a holding tank. When attempting to determine if acoustically tagged Striped Bass could be pushed downstream of the TFCF into the intake canal to the JPP, the louver panel immediately upstream of bypass 4 was lifted prior to CO_2 treatment of the primary channel.

To treat the TFCF primary channel, approximately 2,721.6–3,628.7 kg (approximately 6,000–8000 lbs.) of dry ice was distributed and inserted at multiple locations upstream of the trashrack at the head of the primary channel. Dry ice insertion was completed using 1–2 front-end loaders, 1–2 forklifts with tipping bins, 1–2 trashrack cleaning devices, a backhoe, and manual insertion. During insertion of dry ice, all personnel were required to wear appropriate personal protective equipment including, but not limited to, life jackets, harnesses, gloves, safety glasses, and hardhats.

Hydraulic measurements, including primary channel flow, primary channel velocity, primary channel depth, secondary channel flow, secondary channel velocity, secondary channel depth, holding tank flow and holding tank velocity, were recorded from facility meters during each trial. During the initial CO_2 treatment of the TFCF primary channel to determine if acoustically tagged Striped Bass could be influenced or moved to a desired location within the primary channel, CO_2 and pH measurements were taken every 2 min from the TFCF sampling stations using hand-held titration cells (K-1910 [range = 10–100 mg/L CO_2] and K-1920 [range = 100–1000 mg/L CO_2], CHEMetrics Inc., Midland, Virginia) and a pH meter (Model pH 110, Oakton Instruments, Vernon Hills, Illinois), respectively. During the other 3 CO_2 treatments of the TFCF primary

channel, pH loggers (Model SDL100; Extech Instruments, Nashua, New Hampshire) were used to obtain pH measurements every 10 seconds. A CO_2 vs. pH curve was then developed in a laboratory setting by bubbling gaseous CO_2 (using a compressed gas CO_2 cylinder and a microbubble diffuser [MBD100; Pentair, Apopka, Florida]) into a sample of raw Delta water collected prior to each CO_2 treatment in the primary channel. The formula from the CO_2 vs. pH curve was applied to the pH measurements taken by the pH loggers to estimate CO_2 concentration.

When determining if acoustically tagged Striped Bass could be influenced or moved to a desired location within the TFCF primary channel by injecting dry ice, treatment only occurred in the north side of the TFCF primary channel. Acoustic tag detections and/or 2-dimensional tracks were used to investigate Striped Bass behavior in the primary channel during CO_2 treatment. The information obtained during this evaluation was used to guide following research efforts.

When determining if acoustically tagged Striped Bass could be guided into TFCF holding tanks during CO_2 treatment of the primary channel, the number of piscivorous fish collected in a holding tank during the 30-min CO_2 treatment was compared to the number of piscivorous fish collected in a holding tank during the 30-min fish count performed immediately prior to CO_2 treatment (control) to determine if the use of CO_2 in the primary channel increases the total number of piscivorous fish removed from the primary channel. A chi-square test (Minitab version 15) was used to determine if there was a significant difference between the proportions of piscivorous fish collected in holding tanks during the 30-min fish-count (control) and CO_2 treatment. The percentage of acoustically tagged Striped Bass removed from the TFCF primary channel (collected in holding tanks) was used to estimate the efficiency of removal when using a CO_2 concentration of approximately 150 mg/L. The proportion of acoustically tagged Striped Bass recovered in holding tanks during CO_2 treatment in the primary channel was used along with the numbers of wild Striped Bass collected to estimate the Striped Bass population in the TFCF system (primary channel, bypass tubes, and secondary channel) on the day of experimentation, which was a secondary objective of this study. To obtain a Striped Bass population estimate using this method, it will be necessary to collect at least one acoustically tagged Striped Bass and one wild Striped Bass in a TFCF holding tank during CO_2 treatment of the primary channel.

When determining if acoustically tagged Striped Bass could be guided out of the TFCF primary channel through an open louver panel with CO_2 treatment, the most downstream primary channel louver panel was lifted while water continued to be collected in a holding tank. The continued collection of water in a holding tank was necessary since the TFCF must salvage fish whenever pumping is occurring at the JPP. Acoustic tag detections and/or 2-dimensional tracks were used, along with the number of acoustically tagged Striped Bass collected in a holding tank, to estimate removal efficiency. The use of acoustic tag detections and/or 2-dimensional tracks was necessary to determine the number of acoustically tagged Striped Bass removed from the TFCF primary channel through an open louver panel during CO_2 treatment. The number of acoustically tagged Striped Bass guided out of the TFCF through an open primary channel louver panel and the number of acoustically tagged Striped Bass collected in a holding tank were summed to estimate total removal efficiency from the TFCF primary channel when a louver panel is lifted during CO_2 treatment with a concentration of approximately 150 mg/L. The number of acoustically tagged Striped Bass guided out of the TFCF through an open primary channel louver panel and/or collected in a holding tank during the 30-min CO_2 treatment was compared to the number of acoustically tagged Striped Bass that left the TFCF through an open primary channel louver panel or were collected in a holding tank during the 30-min period immediately prior to CO_2 treatment (control) to determine if the use of

CO_2 in the primary channel increases the total number of piscivorous fish removed. A chi-square test (Minitab version 15) was used to determine if there was a significant difference between the proportions of acoustically tagged Striped Bass removed between the control and CO_2 treatment. It was not possible to estimate the Striped Bass population in the TFCF system (primary channel, bypass pipes, and secondary channel) on the day of experimentation because no Striped Bass (acoustically tagged or wild) were collected in a TFCF holding tank. In order to obtain a Striped Bass population estimate using this method, it was necessary to collect at least one acoustically tagged and one wild Striped Bass in a TFCF holding tank during CO_2 treatment of the primary channel with an open primary channel louver panel.

Assumptions and Limitations

This evaluation could only be completed during 1 or 2 pump operation at the JPP and 1-week notice of these pumping conditions was needed to order dry ice and have it delivered to the TFCF. One or two pump operation at the JPP was necessary for a minimum duration of 2 days to complete each replicate. Tidal conditions were considered to reduce the volume of water that needed to be treated. It was necessary to collect wild Striped Bass for use during this evaluation and hold them in the Tracy Aquaculture Facility (TAF); therefore, it was necessary to maintain the TAF so that it was operational. For each replicate, it was necessary to verify the HTI acoustic telemetry systems at the TFCF were fully operational and an appropriate number of personnel (10–12 individuals) were available to perform injection of dry ice into the primary channel at the TFCF. Appropriate safety equipment (dry ice gloves, eye protection, etc.) was used when performing dry ice injections. It was necessary for the Biological Resources group at the TFCF to adjust secondary channel flow during each replicate. In addition, it was necessary to prepare and maintain a contract for dry ice supply and delivery. It is assumed no other projects or studies will take priority or precedence during the FY2022 research period and that there will be ample opportunity to prepare and finalize a Tracy Technical Bulletin for this evaluation.

Coordination and Collaboration

This study was coordinated with the TFCF biological and operations staff, Tracy Technical Advisory Team (TTAT), California Department of Fish and Wildlife (CDFW), and HTI-Vemco USA, Inc. Participation and inclusion of research-related updates were provided at regularly scheduled TTAT meetings.

Endangered Species Issues, "Take" Considerations

To minimize the risk of mortality of listed species, all attempts were made to complete research activity during seasonal periods in which listed species were not typically present at the TFCF. Despite this, two Chinook Salmon (*Oncorhynchus tshawytscha*; one fall-run and one spring-run according to length-at-date) and three Delta Smelt (*Hypomesus transpacificus*) were collected in a holding tank throughout the course of this evaluation. The number and species of fish guided out of the TFCF primary channel through an open louver panel with CO_2 treatment is unknown and could have included winter-run and spring-run Chinook Salmon, Steelhead Trout (*O. mykiss*), Delta

Smelt, and other species. Based on results from Wu and Bridges (2014), it is possible that mortality of these species may have occurred because certain species, such as Delta Smelt, do not tolerate elevated CO_2 levels as well as other fish (Delta Smelt exhibited 70% mortality over 96 h after being exposed to 100 mg/L CO_2 for 20 min). All listed species encountered were immediately documented, processed according to current protocol, returned to the Sacramento-San Joaquin Delta (if alive), and reported to all appropriate agencies. All fish take for this evaluation was covered under the most recent National Marine Fisheries Service (NMFS) BiOp, as well as current CDFW Scientific Collecting Permits held by the Biological Resources staff at the TFCF. Although the procedures during experimentation may have led to mortality of listed species, the cumulative lethal take of listed species for the facility would likely be much higher in the absence of predator removal activities in the primary channel at the TFCF.

Dissemination of Results (Deliverables and Outcomes)

A Tracy Technical Bulletin will be prepared and published upon completion of the study. Updates and presentations of progress will be provided internally and upon request by TTAT and other interagency technical forums. A draft report is expected to be produced by the end of September 2021 and a final publication is anticipated by September 2022.

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