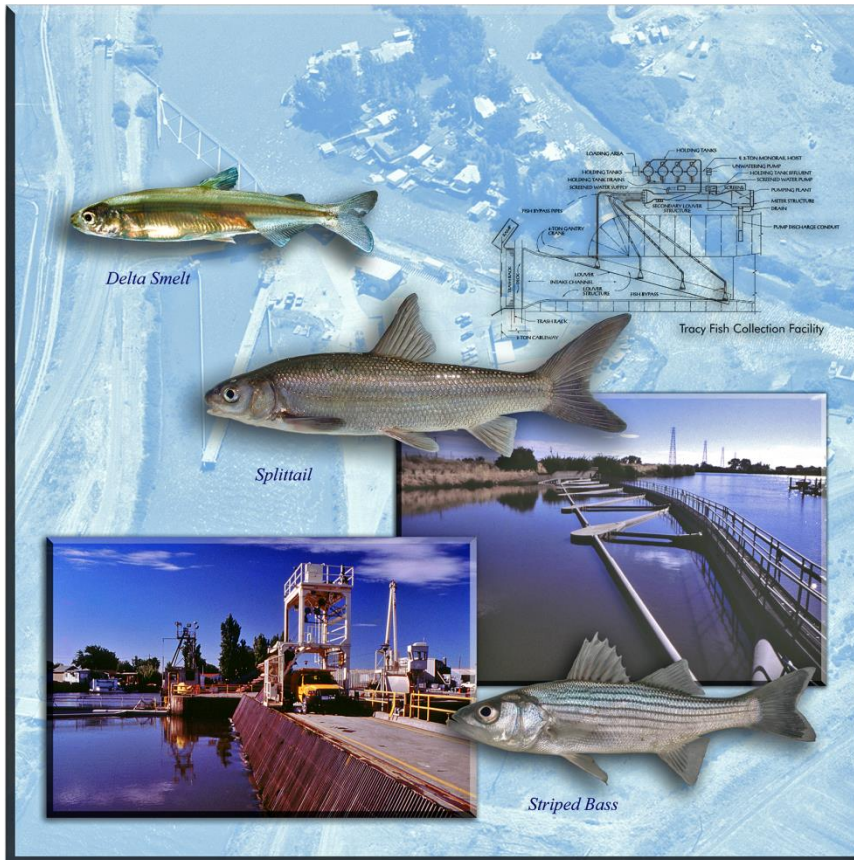


RECLAMATION

Managing Water in the West

Tracy Series Volume 49

Evaluating the Use of Carbon Dioxide as an Alternative Predator Removal Technique to Decrease Tracy Fish Collection Facility Predator Numbers and Improve Facility Operations



U.S. Department of the Interior
Bureau of Reclamation
Mid-Pacific Region and
Denver Technical Service Center

January 2014

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) January 2014		2. REPORT TYPE Final		3. DATES COVERED (From - To)	
4. TITLE AND SUBTITLE Evaluating the Use of Carbon Dioxide as an Alternative Predator Removal Technique to Decrease Tracy Fish Collection Facility Predator Numbers and Improve Facility Operations				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Brandon J. Wu and Brent B. Bridges				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Bureau of Reclamation Tracy Fish Collection Facility 16650 Kelso Road, Byron, CA 94514				8. PERFORMING ORGANIZATION REPORT NUMBER Volume 49	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Bureau of Reclamation, Tracy Fish Collection Facility 16650 Kelso Road, Byron, CA 94514				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Available from the National Technical Information Service (NTIS) Operations Division, 5285 Port Royal Road, Springfield, VA 22161					
13. SUPPLEMENTARY NOTE					
14. ABSTRACT The U.S. Department of the Interior, Bureau of Reclamation's (Reclamation) Tracy Fish Collection Facility (TFCF; Byron, California) functions to separate fish from water pumped south at the C.W. "Bill" Jones Pumping Plant (formerly Tracy Pumping Plant; JPP). Operations at the TFCF divert, collect, hold and return salvaged fish to the confluence of the Sacramento-San Joaquin Delta (Delta). To improve fish salvage, research is being completed to find ways to minimize TFCF fish loss. Many factors, including predation by piscivorous fish, contribute to fish loss at the TFCF. A new predator removal method would be beneficial to reduce time the TFCF is out of operation and increase employee safety. The new predator removal method tested in this study uses carbon dioxide (CO ₂) as an anesthetic to force fish, including predatory fish, downstream into holding tanks. This study demonstrates fish are easily removed using a CO ₂ dose (250–350 mg/L), and this method can be easily implemented at the TFCF. The CO ₂ treatment removed significantly more fish, of all species, than the control treatment. Median length of striped bass collected during CO ₂ treatment was significantly greater than that of striped bass salvaged at the TFCF during the week of testing. There was no significant difference in the median lengths of white catfish collected before and during CO ₂ treatment. In comparison to current predator removal methods, this method improves employee safety, reduces labor, and likely increases fish survival and the salvage efficiency of threatened and endangered species.					
15. SUBJECT TERMS TFCF, CO ₂ , dry ice, hypercapnia, acidosis, anesthetic, predation, salvage, salvage efficiency, striped bass, white catfish					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES 61	19a. NAME OF RESPONSIBLE PERSON Donald E. Portz, Ph.D.
a. REPORT	b. ABSTRACT	a. THIS PAGE			19b. TELEPHONE NUMBER (Include area code) 303-445-2220

Tracy Fish Facility Studies California

Evaluating the Use of Carbon Dioxide as an Alternative Predator Removal Technique to Decrease Tracy Fish Collection Facility Predator Numbers and Improve Facility Operations

Tracy Series Volume 49

by

Brandon J. Wu¹ and Brent B. Bridges²

¹ Bureau of Reclamation
Tracy Fish Collection Facility; TO-417
16650 Kelso Road
Byron, CA 94514-1909

² Bureau of Reclamation
Tracy Fish Collection Facility; TO-411
16650 Kelso Road
Byron, CA 94514-1909



**U.S. Department of the Interior
Bureau of Reclamation
Mid-Pacific Region and
Denver Technical Service Center**

January 2014

MISSION STATEMENTS

The U.S. Department of the Interior protects America's natural resources and heritage, honors our cultures and tribal communities, and supplies the energy to power our future.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

TRACY FISH FACILITY IMPROVEMENT PROGRAM

J. Carl Dealy, Manager

TRACY SERIES EDITOR

Donald E. Portz, Ph.D.
U.S. Department of the Interior – Bureau of Reclamation
Technical Service Center
Fisheries and Wildlife Resources Group, 86-68290
PO Box 25007
Denver, CO 80225-0007

COVER

Fish photography by René Reyes, Tracy Fish Collection Facility, Tracy, California.
Design by Doug Craft.

DISCLAIMER

Mention of commercial products does not constitute endorsement.

Table of Contents

	<i>Page</i>
Executive Summary	ES-1
Introduction.....	1
Methods.....	5
Phase 1: Properties of Carbon Dioxide in Water	5
Phase 2: Methods to Inject Carbon Dioxide and Estimate Dry Ice Sublimation Rate in Water.....	7
Phase 3: Dose-Response of Predators and Prey.....	8
Phase 4: Test Dose-Response in Flume	8
Phase 5: Pilot Test in Bypass Tubes and Secondary Channel at the Tracy Fish Collection Facility	11
Data Analyses	13
Results and Discussion	14
Phase 1: Properties of Carbon Dioxide in Water	14
Phase 2: Methods to Inject Carbon Dioxide and Estimate Dry Ice Sublimation Rate in Water.....	19
Phase 3: Dose-Response of Predators and Prey.....	20
Phase 4: Test Dose-Response in Flume	24
Phase 5: Pilot Test in Bypass Tubes and Secondary Channel at the Tracy Fish Collection Facility	26
Acknowledgments.....	36
References.....	36

Tables

<i>Table</i>	<i>Page</i>
1 Summary of bypass tube lengths and volumes, along with amounts of dry ice injected, hydraulics, peak carbon dioxide concentrations and times for peak concentrations to reach secondary channel during the pilot test.....	29
2 Summary of amounts of carbon dioxide sublimated, amounts of carbon dioxide measured in the water and percent of carbon dioxide dissolved into water over time (transfer efficiency) during the first 20 min of the pilot test for each bypass tube and amount of dry ice injected.....	30

Figures

<i>Figure</i>	<i>Page</i>
1	Map of the Sacramento-San Joaquin Delta showing the Tracy Fish Collection Facility, C.W. “Bill” Jones Pumping Plant and Emmaton Fish Release Site. 1
2	Current predator removal method at the TFCF which consists of seining the secondary channel (top) and flushing each of the four bypass tubes into a sieve net (bottom). 3
3	Small oval flume (track width = 0.4 m, depth = 0.5 m, length = 8.2 m) used to examine fish swimming behavior with flowing water and elevated carbon dioxide concentrations at the Tracy Fish Collection Facility (top) and clear windows on the side of the flume for observing fish (bottom). 9
4	Aluminum perforated plate installed across the flume at a 15 degree angle to mimic the secondary louvers and bypass. 10
5	Team of 2 people inserting dry ice into bypass tube opening at the Tracy Fish Collection Facility. The bypass tube leads to the secondary channel which directs fish to a holding tank. Dry ice was inserted to move fish from the bypass tubes and secondary channel into a holding tank. 12
6	Schematic of the Tracy Fish Collection Facility illustrating amount of dry ice injected (green text), injection locations (green arrows) and treatment area (green fill). 13
7	Laboratory results showing the relationship between carbon dioxide concentration and pH for water with alkalinities of 50, 75, 100, 125 and 150 mg/L calcium carbonate and mean (minimum–maximum) temperature of 21.3 (20.2–22.1) °C. 14
8	Laboratory measured carbon dioxide concentrations overlaid on trend lines published by Hargreaves and Brunson (1996) for water with alkalinities of 50, 100 and 150 mg/L calcium carbonate and temperature of 24 °C. 15
9	Laboratory results showing the differing relationships between carbon dioxide concentration and pH for distilled and Delta water with alkalinities of 50 and 100 mg/L calcium carbonate and temperatures of approximately 21 °C. 16
10	Laboratory measured carbon dioxide concentrations overlaid on trend lines published by Wurts and Durborow (1992) for water at temperatures of 10, 20 and 30 °C with a constant alkalinity of 85 mg/L calcium carbonate. 17
11	Dissolved carbon dioxide concentration in 10 L of water (100 mg/L calcium carbonate, approximately 19 °C) as a function of time when exposed to muriatic acid (14% hydrochloric acid) and carbon dioxide gas (21 L/min through a microbubble diffuser). 17

Figures (continued)

<i>Figure</i>	<i>Page</i>
12	Carbon dioxide dissipation rate in water (21.5 ± 0.5 °C) with 0, 0.1, 1, 18, 54, 102, 150 and 174 L/min air flow. 18
13	Exponential decay of dry ice blocks of various mass immersed in water at 11.7 °C and 4.9 m deep (M_o = Initial mass [g], M_t = Mass at time t [g]). A reference block was left continually in the water and weighed at the end to verify that lifting the blocks periodically for weighing did not greatly change sublimation rate..... 19
14	Times until partial and total loss of equilibrium for striped bass exposed to water with carbon dioxide (n = 52, mean [minimum–maximum] length = 341 [260–398] mm FL). 21
15	Mean 24–96 h survival of striped bass following 20-min exposure to various concentrations of carbon dioxide (n = 52, mean [minimum–maximum] length = 341 [260–398] mm FL). ... 21
16	Times until partial and total loss of equilibrium for Chinook salmon exposed to water with carbon dioxide (n = 70, mean [minimum–maximum] length = 178 [132–209] mm FL). 22
17	Times until partial and total loss of equilibrium for delta smelt exposed to water with carbon dioxide (n = 60, mean [minimum–maximum] length = 70 [58–87] mm FL). 23
18	Mean 24–96 h survival of Chinook salmon following 20-min exposure to various concentrations of carbon dioxide (n = 70, mean [minimum–maximum] length = 178 [132–209] mm FL). ... 23
19	Mean 24–96 h survival of delta smelt following 20-min exposure to various concentrations of carbon dioxide (n = 60, mean [minimum–maximum] length = 70 [58–87] mm FL). 24
20	Striped bass (mean [minimum–maximum] length = 88 [80–100] mm FL) and Chinook salmon (mean [minimum–maximum] length = 213 [180–250] mm FL) downstream passage time in a flume at elevated carbon dioxide concentrations. Control trials (n = 18 striped bass, n = 20 Chinook salmon) demonstrated fish would typically not move downstream with flow when no carbon dioxide was added. Treatment trials for striped bass (n = 4) and Chinook salmon (n = 15) demonstrated fish move downstream more rapidly when exposed to increasing levels of carbon dioxide. 25
21	Downstream passage times of striped bass in a flume (n = 22, mean [minimum–maximum] length = 88 [80–100] mm FL) set at various carbon dioxide concentrations in comparison with times until partial loss of equilibrium and total loss of equilibrium
22	Concentration of carbon dioxide in water measured from each bypass tube over time..... 26

Figures (continued)

<i>Figure</i>		<i>Page</i>
23	Fish were abundant in the secondary channel before treatment with carbon dioxide (top); few fish were observed in the holding tank at this time (bottom).....	27
24	After carbon dioxide treatment (349 mg/L peak carbon dioxide concentration), fish were forced from the secondary channel (top) into the holding tank (bottom).....	28
26	Frequency distributions of striped bass fork lengths (mm) salvaged during the week of testing (n = 33, median, mean [minimum–maximum length = 89, 100 [38–317] mm FL) and collected in the holding tank during carbon dioxide treatment (n = 492, median, mean [minimum–maximum] length = 282, 291 [89–698] mm FL; 349 mg/L peak carbon dioxide concentration).	32
27	Frequency distributions of white catfish fork lengths (mm) collected in the holding tank during control (n = 11, median, mean [minimum–maximum] length = 135, 127 [69–190] mm FL) and carbon dioxide treatment (n = 558, median, mean [minimum–maximum] length = 135, 133 [40–328] mm FL; 349 mg/L peak carbon dioxide concentration).	32
28	Total number of each species collected in the sieve net behind secondary channel louvers during carbon dioxide treatment (349 mg/L peak carbon dioxide concentration). No fish were collected in the sieve net during the control.	33
29	Frequency distribution of white catfish fork lengths (mm) collected in the sieve net during carbon dioxide treatment (n = 236, mean [minimum–maximum] length = 88 [62–138] mm FL; 349 mg/L peak carbon dioxide concentration). Only a representative sample (30 fish) was measured.....	34

Appendices

Appendix

- 1 Chemical and Physical Properties of Carbon Dioxide
- 2 Interrelationships Between Carbon Dioxide, Temperature, pH, and Total Alkalinity
- 3 Stages of Anesthesia

EXECUTIVE SUMMARY

The U.S. Department of the Interior, Bureau of Reclamation's (Reclamation) Tracy Fish Collection Facility (TFCF; Byron, California) functions to separate fish from water pumped south at the C.W. "Bill" Jones Pumping Plant (formerly Tracy Pumping Plant; JPP). Operations at the TFCF divert, collect, hold and return salvaged fish to the confluence of the Sacramento-San Joaquin Delta (Delta). To increase fish salvage, research is being completed to minimize or reduce TFCF fish loss. Many factors, including predation by piscivorous fish, contribute to fish loss at the TFCF. Predatory fish take up residence throughout the facility, including the primary channel, bypass tubes and secondary channel.

A predator removal program in the secondary channel is ongoing and started in the early 1990's. This program requires dewatering the secondary channel to manually remove predatory fish with sieve nets, seines and dip nets. This labor intensive process exposes employees to water engulfment dangers while in a confined space, presumably reduces daily salvage, and may be harmful to fish. To minimize these problems, a new predator removal technique is being tested that raises carbon dioxide (CO₂) levels in the water. At elevated concentrations, the anesthetic properties of CO₂ reduce fish swimming performance. Fish that are not capable of swimming at full capacity are forced downstream with the water flow into holding tanks, after which they are ultimately transported by truck to release sites within the Delta.

A pilot test in the bypass tubes and secondary channel at the TFCF was completed in November of 2006. The CO₂ treatment (349 mg/L peak CO₂ concentration) used during the pilot test removed significantly more fish, of all species (n = 1,118), than the control treatment (n = 18). The numbers of striped bass (*Morone saxatilis*; mean fork length [FL] = 292 mm) and white catfish (*Ameiurus catus*; mean FL = 134 mm) collected in the control (n = 0, n = 11, respectively) and CO₂ treatment (n = 492, n = 558, respectively) were significantly different. Median length of striped bass collected during CO₂ treatment was significantly greater than that of striped bass salvaged the week of testing (n = 33). There was no significant difference in the median lengths of white catfish collected in the control and CO₂ treatment.

INTRODUCTION

The U.S. Department of the Interior, Bureau of Reclamation's (Reclamation) Tracy Fish Collection Facility (TFCF; Byron, California; Figure 1) functions to separate fish from water pumped south at the C.W. "Bill" Jones Pumping Plant (formerly Tracy Pumping Plant; JPP). Operations at the TFCF divert, collect, hold and return salvaged fish to the confluence of the Sacramento-San Joaquin Delta (Delta). The TFCF salvages approximately 7 million fish per year, but this number varies between dry and wet years (Aasen 2012). Most of these fish are less than 15 cm long and include threatened and endangered species: delta smelt (*Hypomesus transpacificus*), longfin smelt (*Spirinchus thaleichthys*), green sturgeon (*Acipenser medirostris*), steelhead trout (*Onchorhynchus mykiss*) and Chinook salmon (*O. tshawytscha*). To maintain a whole facility efficiency of 75%, as mandated by Action Suite IV.4 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 (Biological Opinion), it is necessary to minimize fish losses throughout the facility. Many factors, including predation by piscivorous fish (Bridges *et al.* in press), contribute to fish loss at the TFCF.



Figure 1.—Map of the Sacramento-San Joaquin Delta showing the Tracy Fish Collection Facility, C.W. "Bill" Jones Pumping Plant and Emmaton Fish Release Site.

Piscivorous predatory fish, such as striped bass (*Morone saxatilis*), white catfish (*Ameiurus catus*) and channel catfish (*Ictalurus punctatus*), accumulate throughout the TFCF by taking up residence in the primary channel, bypass tubes (concrete underground pipes that deliver fish from the primary to the secondary channel) and secondary channel (Liston *et al.* 1994). Although a 56-mm spaced trash rack heads the facility, fish up to ~ 400-mm fork length (FL) can still pass through the rack (Bridges *et al.* in press) and maintain their position inside of the TFCF (Liston *et al.* 1994).

A predator removal program in the secondary channel is ongoing and started in the early 1990's (Liston *et al.* 1994). This program requires dewatering the secondary channel to provide access to manually remove predators with sieve nets, seines and dip nets (Figure 2). This labor intensive process exposes employees to unsafe working conditions, presumably reduces the number of salvaged fish while the secondary channel is dewatered and is potentially harmful to fish due to net impingement, atmospheric exposure and abrasions or physical damage caused by handling and contact with other fish.

Over the years, Reclamation personnel have reviewed various means of moving predators through the TFCF system, such as sound, light, and mechanical methods. Many of these methods are largely ineffective for removing large predatory fish, expensive to install and operate and are logistically difficult to implement at the TFCF because many areas cannot be dewatered (Fausch 2000). An alternative is to focus on a method that encourages predators to move downstream on their own by changing the physical/chemical properties of the water.

Adding CO₂ to water was selected as the most promising solution for controlling predators within the TFCF. The chemical and physical properties of CO₂ make this compound suitable for predator removals (Appendix 1, Table A1-1). Carbon dioxide is soluble in water (Bell 1987; Appendix 1, Figure A1-1) and the interrelationships between CO₂, temperature, pH and total alkalinity are well documented for aqueous solutions (Hargreaves and Brunson 1996; Appendix 2, Figure A2-1). Since CO₂ acts as an acid in water and produces a predictable drop in pH, measurements of pH and alkalinity can be used to estimate CO₂ concentration (Hargreaves and Brunson 1996; Appendix 2, Figure A2-2). Carbon dioxide is a natural component of respiration and fermentation and is listed as a Low Regulatory Priority compound by the Food and Drug Administration (Bowser 2001). This compound is currently used for anesthetic purposes in cold, cool and warm water fish (Bowser 2001), produces a consistent and reproducible decrease in blood pH (Ackerman *et al.* 2005), does not leave tissue residues that could be ingested by people (Ackerman *et al.* 2005), is generally recognized as safe by the public as people are in contact with it daily (Murphy and Willis 1996) and rarely causes direct toxicity to fish at low concentrations (Wurts and Durborow 1992). Carbon dioxide is also readily available from local merchants and can be acquired in three different forms (solid [dry ice], liquid, gas).



Figure 2.—Current predator removal method at the TFCF which consists of seining the secondary channel (top) and flushing each of the four bypass tubes into a sieve net (bottom).

The effect of CO_2 on respiration makes it an ideal compound for removing fish in flowing water. When CO_2 enters the tissues, it diffuses down the concentration gradient into the blood. Most of the CO_2 is drawn into red blood cells where it combines with water to form carbonic acid (H_2CO_3), which dissociates to

bicarbonate (HCO_3^-) and hydrogen ions (H^+ ; Appendix 2, Figure A2-3). The rapid production of H^+ from dissociating carbonic acid inside the red blood cells causes the pH to drop. This alters hemoglobin and causes a release of oxygen, which diffuses out of the red blood cells and into the tissues. Some hemoglobin molecules bind CO_2 , while others bind the excess H^+ , preventing blood pH from dropping too low (Helfman *et al.* 1997).

The anesthetic effect of CO_2 at lower concentrations arises from its ability to reduce the animal's capacity to extract oxygen from the environment (Black *et al.* 1954), which reduces its swimming performance. Elevated dissolved CO_2 in water reduces the concentration gradient between the water and the fish's blood. This reduces the rate at which CO_2 from the fish's own metabolism can be released from the blood through the gills and causes a rise in blood CO_2 (hypercapnia). A rise in blood CO_2 results in a drop in the pH of the blood (acidosis), which reduces the oxygen-carrying ability of the hemoglobin in the blood (Southgate 2005). In the short term, the physiology of the fish can counteract the effect of hypercapnia by balancing acidosis with an exchange of ions, such as increasing the uptake of bicarbonate and losing hydrogen and phosphate ions (Southgate 2005). If excess CO_2 is not removed, blood and tissue pH will drop low enough to interfere with normal metabolic processes (Helfman *et al.* 1997) and can have a profound effect on the health of fish, including nephrocalcinosis (blocked excretion of minerals), an increased susceptibility to pathogens, spinal abnormalities from bone demineralization (Southgate 2005) and mortality at high levels (Brauner and Baker 2009).

The purpose of this project is to determine the effectiveness of exposing fish to durations of elevated CO_2 concentrations for the purpose of removing them from the bypass tubes and secondary channel at the TFCF. Short-term exposure to CO_2 should prevent fish, including piscivorous predatory species, from holding in place within the bypass tubes and secondary channel, allowing increased water flow to push them downstream into holding tanks. If proven efficient, this method of predator removal will increase employee safety by not requiring entry into a confined space, reduce labor by eliminating the need for manual methods of fish removal and likely reduce damage sustained by fish by net impingement, exposure and handling. In addition, the method will likely result in an increase in salvage and salvage efficiency of fish because it does not require the secondary channel to be shut down for any duration of time.

The main objectives of this study were to:

- 1) Learn about physical and chemical properties of CO_2 in relation to Delta water.
- 2) Develop a cost effective and safe method to inject and monitor CO_2 .

- 3) Determine a CO₂ dose-response relationship for striped bass, as well as certain threatened and endangered fish species (Chinook salmon and delta smelt), in Delta water.
- 4) Test the dose-response relationship in a laboratory flume with flowing water.
- 5) Demonstrate this method can be implemented at the TFCF.

METHODS

The CO₂ predator removal project was divided into five phases. Phase 1 was a training tool as it summarized the chemical properties of CO₂ in water and provided laboratory personnel time to train with the test kits and equipment. Phases 2–5 evaluated CO₂ water injection techniques and sublimation rate in water, species-specific dose-response, fish responses to CO₂ in flowing water and fish responses to CO₂ in the bypass tubes and secondary channel at the TFCF (pilot test), respectively. If this method shows potential and is of interest to management, a follow-up study will be completed to determine an effective manner to fully implement this alternative predator removal technique at the TFCF.

Phase 1: Properties of Carbon Dioxide in Water

Small-scale laboratory experimentation was necessary to demonstrate that the predictable relationships reported in the literature between dissolved CO₂, pH, alkalinity and temperature could be duplicated and monitored adequately. It was also necessary to determine if these relationships were influenced by the use of Delta water due to the presence of compounds that may affect alkalinity but do not release CO₂. These water quality analyses were completed in a laboratory setting at the Tracy Aquaculture Facility (TAF), within the TFCF. Carbon dioxide and pH levels were measured using hand-held titration cells (K-1910 [range = 10–100 mg/L CO₂] and K-1920 [range = 100–1,000 mg/L CO₂], CHEMetrics Inc., Midland, Virginia) and a pH meter (Model pH 110, Oakton Instruments, Vernon Hills, Illinois), respectively. Temperature was measured using a handheld multiparameter meter (Model 85, YSI Inc., Yellow Springs, Ohio) calibrated to a certified mercury thermometer (Thermo Fisher Scientific, Waltham, Massachusetts). Alkalinity was measured with hand-held titration cells (K-9815 [range = 50–500 mg/L calcium carbonate (CaCO₃)], CHEMetrics Inc., Midland, Virginia). If alkalinity was under 50 mg/L CaCO₃ (under titration cell detection range), total alkalinity water quality test strips (Hach Co., Loveland, Colorado) were used.

The relationship between CO₂ concentration, pH and alkalinity was examined with both distilled and Delta water by intermittently injecting CO₂ (0.25 L/min) from a pressurized CO₂ cylinder (8 m³), using a MBD-75 microbubble diffuser (15.2 x 0.3 cm [l x w] diffusing area; Point Four Systems Inc., Richmond, BC Canada), into 18.9-L buckets filled with 3.8 L of water at approximately 21 °C (20.2–22.1 °C). Five different alkalinities (50, 75, 100, 125 and 150 mg/L CaCO₃) were used with distilled water. These alkalinities were achieved by adding appropriate amounts of a 25 g/L CaCO₃ alkalinity standard solution (Hach Co., Loveland, Colorado) to the distilled water. Delta water used was at ambient alkalinities of 50, 75 and 100 mg/L CaCO₃. Carbon dioxide concentrations and pH levels were recorded from each bucket until a CO₂ concentration of 300 mg/L was reached.

Gas solubility usually decreases with increased temperature (Senese 2010). In order to demonstrate this property in the laboratory, three water temperatures were chosen that span the temperatures normally seen at the TFCF (10–30 °C). Carbon dioxide was injected at 0.25 L/min from a pressurized CO₂ cylinder, using a MBD-75 microbubble diffuser, into four 18.9-L buckets filled with 15 L of 85 mg/L CaCO₃ distilled water at steady temperatures (10, 20, 30 ± 0.5 °C). Carbon dioxide concentrations and pH levels were recorded through time until CO₂ concentrations of 300 mg/L were reached.

Promoting a higher rate of CO₂ solubility in water would potentially make the technique more efficient to administer at the TFCF. Adding acid to water just prior to the addition of CO₂ gas was one potential technique evaluated to increase the rate of rise in dissolved CO₂. Five 18.9-L buckets filled with 10 L of 100 mg/L CaCO₃ distilled water at approximately 19 °C (18.7–19.3 °C) were acidified with muriatic acid (14% HCl) until the desired pH was attained (pH 3, 4, 5, 6, and 7). Carbon dioxide gas was injected from a pressurized CO₂ cylinder into each of the buckets, at 21 L/min, using a MBD-75 microbubble diffuser. Carbon dioxide concentrations were recorded from each bucket every 5 min for the duration of the test.

In order to demonstrate that CO₂ could be removed from water, it was necessary to examine the dissipation rate of CO₂ as a function of tank aeration. Long-term effects of elevated CO₂ levels in water needed to be taken into consideration prior to performing the pilot test in the bypass tubes and secondary channel at the TFCF. A Sweetwater® regenerative air blower (Model S51-230, Aquatic Eco-Systems, Inc., Apopka, Florida) was used to supply low pressure air (0.136 atm [2 psi]) to all experimental tanks. Brass threaded pipe valves (Foster and Smith, Inc., Rhinelander, Wisconsin) were used to adjust air flows. Eight 1.2-m diameter (757-L) tanks were set up with different amounts of air flowing through Sweetwater® 76.2 mm x 38.1 mm diffusers (Aquatic Eco-Systems, Inc., Apopka, Florida). One tank was set up as a control with no air flow, while 7 tanks were aerated at 0.1, 1, 18, 54, 102, 150 and 174 L/min. The amount of air flowing to each tank was measured by recording the time for air to displace the water in an

inverted 18.9-L graduated cylinder (diameter = 26.7 cm, height = 35.6 cm) held over the diffusers. Tanks receiving more air had a greater number of diffusers to help keep the bubble size uniform between treatments. After the flows were verified, the air blower was shut off and the tanks were drained. A 2.4-m diameter (4270-L) tank was filled with raw Delta water and a CO₂ concentration of 230 mg/L (the approximate concentration expected to be used during fish removal efforts in the bypass tubes and secondary channel at the TFCF) was achieved using a pressurized CO₂ cylinder and a MBD-300 microbubble diffuser (30.8 x 6.0 cm [l x w] diffusing area; Point Four Systems Inc., Richmond, BC Canada). Water in the 2.4-m diameter tank was mixed with a boat paddle during CO₂ injection to ensure CO₂ gas was evenly distributed. Approximately 568 L of 230 mg/L CO₂ water was pumped into each of the 8 empty experimental tanks using a 373-W (0.5-hp) sump pump (Model CSU, Ebara International Corporation, Rock Hill, South Carolina) and a flexible 38-mm diameter pool drain hose. After all experimental tanks were filled, the CO₂ concentration was verified to be 230 mg/L in each of the tanks (at time 0 min). Water temperatures were also measured and verified to be 21.5 ± 0.5 °C in each tank. The air blower was turned on to initiate low pressure air flow to tanks to dissipate CO₂. Carbon dioxide measurements were taken at known time intervals, over 1 d, from each of the tanks.

Phase 2: Methods to Inject Carbon Dioxide and Estimate Dry Ice Sublimation Rate in Water

Carbon dioxide gas was supplied from a pressurized cylinder in the laboratory dose-response and flume studies because cylinders and microbubble diffusers provide a reliable, rapid method for adding CO₂ to small volumes of water. However, large amounts of CO₂ were needed for anesthetizing fish in the secondary channel as flows exceed 2 m³/s most of the time and cylinders do not hold enough mass to be used for dosing large volumes of water. Additionally, both the liquid and gas phases of CO₂ require extensive equipment to hold and deliver the chemical compound into water; therefore, the solid phase (dry ice) was selected during the pilot test in the bypass tubes and secondary channel as it was the simplest and least costly in terms of injection devices, storage containers and employee training. Blocks of dry ice (4.5 kg, 25 x 25 x 5 cm [l x w x h]) were provided by Innovative Federal Operations Group, LLC (Vista, California) at a cost of approximately \$1.20/kg and stored in large, outdoor coolers (0.85 m³; Polar Tech Industries, Inc., Genoa, Illinois).

To estimate the transfer efficiency of dissolving solid CO₂ into water, it was necessary to determine how the size and dimension of a dry ice block affects the CO₂ sublimation rate under water. This was examined by placing different sized dry ice blocks, with similar shape, individually into a porous net bag and placing the bag on the bottom of the canal (4.9-m deep, 11.7 °C; n = 1). The bag was lifted out of the water every 4 min and each block was weighed individually using a digital bench scale (Model BW-30, CAS-USA Corp., East Rutherford,

New Jersey). A reference block was left continually in the water and weighed at the end to verify lifting the treatment blocks periodically did not greatly change the sublimation rate. These measurements were used to estimate the amount of dry ice outgassed/min in water and the transfer efficiency of CO₂ gas into water in Phase 5 (pilot test). Warmer water temperatures accelerate the sublimation rate of dry ice (Goodman *et al.* 2008), although this effect was not evaluated in this study.

Phase 3: Dose-Response of Predators and Prey

The objective of Phase 3 was to determine CO₂ concentrations resulting in partial loss of equilibrium (PLE; Stage 3 anesthesia, Bowser 2001) and total loss of equilibrium (TLE; Stage 4 anesthesia, Bowser 2001; Appendix 3, Table A3-1). Fish selected for this study were representative of commonly salvaged species at the TFCF and included striped bass (260–398 mm FL), Chinook salmon (132–209 mm FL) and delta smelt (58–87 mm FL). Striped bass were collected at the TFCF, Chinook salmon were obtained from Nimbus Fish Hatchery (Gold River, California) and delta smelt were obtained from UC Davis' Fish Conservation and Culture Laboratory (Byron, California). All fish were held in ambient, sand filtered, ozonated Delta water. The number of replicates (individual fish) used in each test group depended on the variability of the physiological response, with more replicates tested at doses with higher response variability.

Observation tests were completed in 757-L tanks containing static Delta water. As recommended by Bowser (2001), the desired CO₂ concentrations (<10, 50, 100, 150, 200, 250 and 300 mg/L) were preset before fish were added. The pressurized CO₂ cylinder and MBD-300 microbubble diffusers described in Phase 1 were used to inject CO₂. Delta smelt were not tested at the 150 mg/L CO₂ concentration. Treatment time, for PLE and TLE, was monitored immediately after fish were added to the hypercapnic water. All experimental fish were exposed to elevated CO₂ concentrations for 20 min, which was the anticipated CO₂ exposure time for the pilot test in the bypass tubes and secondary channel at the TFCF. Fish were then transferred to a recovery tank (757-L), at ambient gas concentration and Delta water temperature (9.6–11.4 °C), for 96-h survival monitoring. Measurements of all fish were taken upon mortality or after completion of 96-h survival monitoring.

Phase 4: Test Dose-Response in Flume

In Phase 4, results from laboratory studies were applied to lotic conditions in a test flume. The purpose of this phase was to determine if elevated CO₂ concentrations influence downstream swimming behavior of fish in a flowing environment. An oval shaped flume (track width = 0.4 m, depth = 0.5 m, length =

8.2 m; Frigid Units Inc., Toledo, Ohio) was selected for monitoring fish behavior as it had several advantages. The desired temperature, water velocity and CO₂ concentration could be easily achieved and maintained for long periods, while the clear windows on the side of the flume made it easy to observe fish (Figure 3).



Figure 3.—Small oval flume (track width = 0.4 m, depth = 0.5 m, length = 8.2 m) used to examine fish swimming behavior with flowing water and elevated carbon dioxide concentrations at the Tracy Fish Collection Facility (top) and clear windows on the side of the flume for observing fish (bottom).

Striped bass ($n = 22$, mean [minimum–maximum] length = 88 [80–100] mm FL) and Chinook salmon ($n = 35$, mean [minimum–maximum] length = 213 [180–250] mm FL) were tested individually in the flume. The size of tested striped bass and Chinook salmon was determined by availability. Delta smelt were not tested in the flume because they were determined to be extremely sensitive to elevated CO_2 during Phase 3.

A 1491-W (2-hp) axial-flow submersible water pump (Model 316, Carry Manufacturing, Inc., Munger, Michigan) was used to maintain a flume velocity of 0.22–0.26 m/s. Water velocity was measured with a flow meter (Model 2000, Marsh McBirney, Frederick, Maryland), while temperature and oxygen were measured with a handheld multiparameter meter. A 746-W (1-hp) chiller (Model D1-100, Frigid Units, Inc., Toledo, Ohio) was used to maintain water temperature (± 0.5 °C) in the flume. An aluminum perforated plate (6.3-mm diameter holes, 48% open area) was installed across the flume (15 degrees to the direction of water flow) at the end of a straight section to mimic the secondary louvers and bypass (Figure 4). A dip net was attached downstream to catch fish that succumbed to CO_2 and passed through the bypass. A square wooden board (61 x 61 x 1.3 cm [l x w x h]) was placed across the flume, upstream of the screen where fish were inserted, to provide a shaded refuge. This board was intended to keep fish at a stationary location until the effects of elevated CO_2 concentrations caused downstream movement.



Figure 4.—Aluminum perforated plate installed across the flume at a 15 degree angle to mimic the secondary louvers and bypass.

Testing was completed by exposing fish to one CO₂ dose at a time (0, 100, 120, and 140 mg/L CO₂). Treatment CO₂ concentrations were selected based on results of previous phases of the project. Prior to the addition of fish, CO₂ was injected into the flume from a pressurized cylinder using an MBD-300 microbubble diffuser until the target dose was reached according to hand-held titration cells. Individual fish were then transferred by dip net to the flume and released under the shade board. Travel time from the shaded area to the bypass was recorded (min) for each species and condition, but was stopped after 10 min if fish did not move.

Phase 5: Pilot Test in Bypass Tubes and Secondary Channel at the Tracy Fish Collection Facility

The Phase 5 pilot test was completed to determine if predators could be forced downstream from the bypass tubes and secondary channel into the holding tank using CO₂. Target CO₂ levels were > 200 mg/L because this dose was reported to cause salmonids to lose equilibrium in less than 3 min by Ackerman *et al.* 2005 and was found to cause striped bass to reach TLE in less than 10 min during Phase 3 of this study. Based on results from Phase 2 of this study, a 30-min exposure was selected to allow for complete dry ice sublimation. Fish health was not a concern during the pilot test and an excessive CO₂ concentration and exposure time were intentionally selected to demonstrate the technique would force fish downstream. A control sample (no CO₂ injected) was obtained 2 h earlier in the day to demonstrate that the pulsed flow was not the main mechanism driving fish downstream into the holding tank.

Control and treatment trials were performed in a nearly identical manner (n = 1). For both the control and treatment, the secondary channel was dewatered, cleaned, back-filled, then underwent 30 min of reduced flow (0.17–0.33 m³/s) to increase contact time between CO₂ gas and water and reduce the cost of dry ice during treatment trials, followed by 30 min of elevated flow (> 3.4 m³/s) to flush lethargic predatory fish downstream into the holding tank. A 3-mm mesh sieve net was lowered into the secondary channel, behind the louvers, for the duration of each replicate to capture fish not successfully louvered into the holding tank and determine if certain species or sizes of fish are more readily lost through the secondary louvers during CO₂ treatment. The only differences for the treatment group were that dry ice was inserted at the entrances of the bypass tubes during the low velocity period and 149-W (0.2-hp) submersible pumps (Alita Co., Ltd, Baldwin Park, California) were installed in the exit of each bypass tube to provide water samples for monitoring CO₂, pH and alkalinity over time.

Dry ice was removed from storage coolers, weighed and stored in smaller coolers (0.14 m³; Igloo Products Corp., Katy, Texas) near primary bypass tube entrances 1 h before injection. Two teams of 2 people injected dry ice into bypass tubes (Figure 5). Employees working at the injection site were required to wear



Figure 5.—Team of 2 people inserting dry ice into bypass tube opening at the Tracy Fish Collection Facility. The bypass tube leads to the secondary channel which directs fish to a holding tank. Dry ice was inserted to move fish from the bypass tubes and secondary channel into a holding tank.

personal protective equipment (life jacket, harness, gloves, safety glasses and hardhat). Bypass tubes 1–4 received 180, 135, 90 and 45 kg of dry ice, respectively, due to differences in bypass flows (Figure 6). Total time to insert dry ice was < 5 min. The goal was to achieve the same peak dose in each bypass. Air quality in the secondary channel was monitored with a hand held multi-gas detector (Model M40, Industrial Scientific Corp., Oakdale, Pennsylvania) after dry ice injection to determine if oxygen (O_2) levels were compromised. Water flow and pH in each bypass tube were recorded every 2 min. Carbon dioxide concentrations were measured from bypass 1 water samples every 2 min to generate a formula for the pH versus CO_2 relationship. This formula was used to estimate the CO_2 concentration in each of the remaining bypasses; hence, an alkalinity measurement was not needed to predict CO_2 dose. Transfer efficiency of CO_2 into the water was determined from the rate of gas sublimation (estimated in Phase 2) and the amount of CO_2 dissolved in the water.

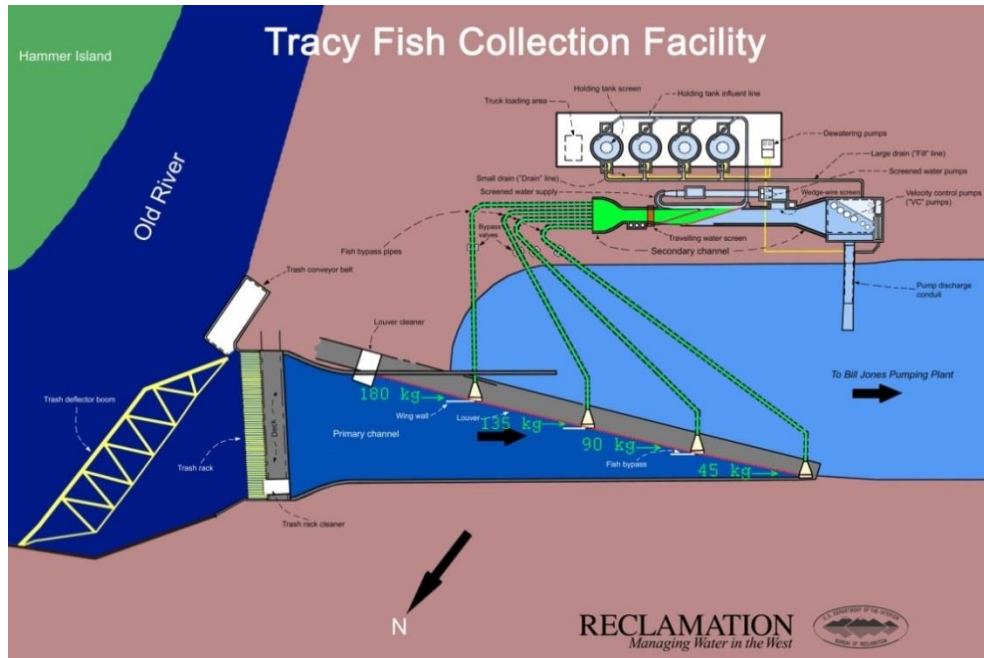


Figure 6.—Schematic of the Tracy Fish Collection Facility illustrating amount of dry ice injected (green text), injection locations (green arrows) and treatment area (green fill).

Following each trial, control and treatment fish were collected from the holding tank and sieve net. The holding tank sample was transferred into a 356 x 74 x 76-cm (l x w x h) trough equipped with oxygen and flow-through raw Delta water. Fish not successfully louvered into holding tanks were removed from sieve nets and kept in a 133-L plastic container with raw Delta water. Fish collected in the holding tank and sieve net were identified, measured (FL) and released back into the holding tank for truck transport to the Delta.

Data Analyses

Scatter plots were generated showing species-specific and CO₂ dose-dependent PLE and TLE. Carbon dioxide dose-dependent group survival was graphed with bar charts; however, no statistical comparison was attempted as survival was generally high for Chinook salmon and striped bass.

Carbon dioxide concentration, as function of bypass location and time, was plotted to show CO₂ transport through the TFCF bypass tubes and secondary channel. The effect of using CO₂ to move fish through the bypass tubes and secondary channel was analyzed using a 2 proportions test (MiniTab version 15, State College, Pennsylvania). It is important to note the use of a 2 proportions test assumes all fish that could potentially be caught were the combination of the control and treatment group. Mann-Whitney tests (MiniTab version 15) were used to compare median lengths of striped bass and white catfish between groups.

This non-parametric test was used because fish lengths were not normally distributed. Due to lack of striped bass in the control trial, lengths of striped bass salvaged at the TFCF during the week of testing (November 1–7, 2006) were used for this analysis.

RESULTS AND DISCUSSION

Phase 1: Properties of Carbon Dioxide in Water

The relationship between dissolved CO₂ concentration, pH and alkalinity of water developed in the laboratory (mean [minimum–maximum] water temperature = 21.3 [20.2–22.1] °C; Figure 7) was very similar to that provided by Hargreaves and Brunson (1996) for water near 24 °C. However, a step occurred in the data around the 100 mg/L CO₂ level and was likely caused by using test kits that covered two different ranges of CO₂ (10–100 and 100–1000 mg/L). Even with the small shift around the 100 mg/L level, laboratory measured CO₂ concentrations generally matched trend lines published by Hargreaves and Brunson (1996) when overlaid on top (Figure 8).

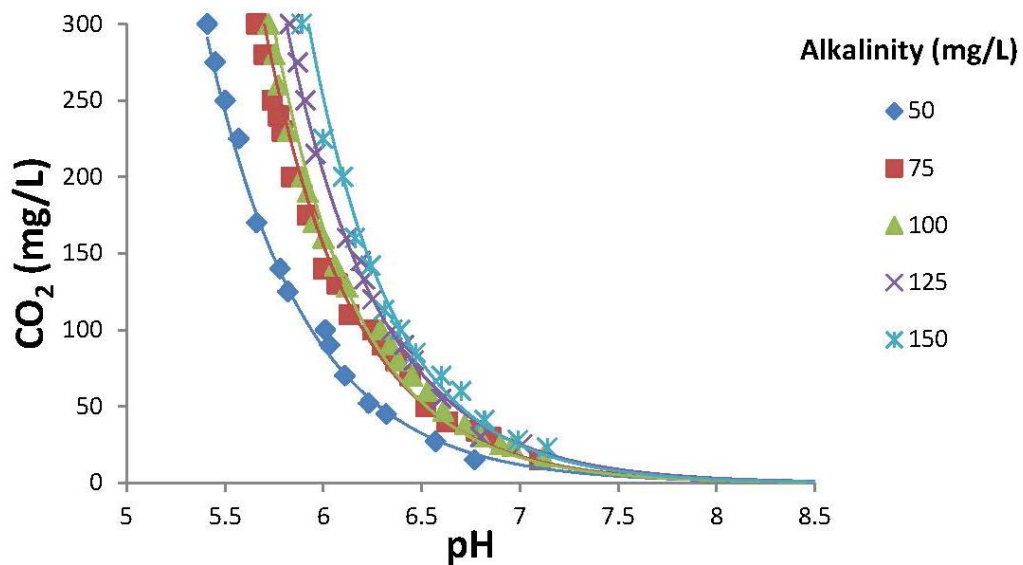


Figure 7.—Laboratory results showing the relationship between carbon dioxide concentration and pH for water with alkalinities of 50, 75, 100, 125 and 150 mg/L calcium carbonate and mean (minimum–maximum) temperature of 21.3 (20.2–22.1) °C.

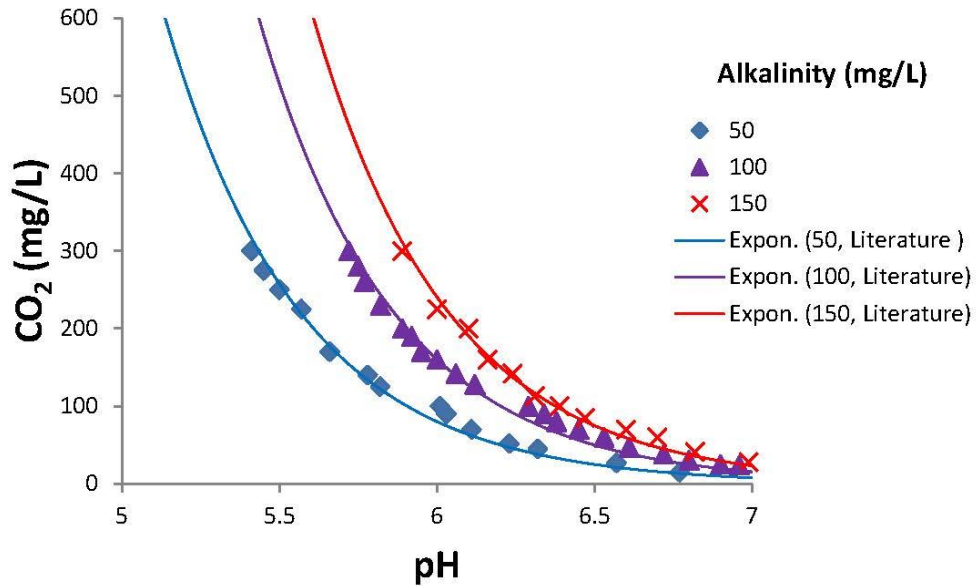


Figure 8.—Laboratory measured carbon dioxide concentrations overlaid on trend lines published by Hargreaves and Brunson (1996) for water with alkalinities of 50, 100, and 150 mg/L calcium carbonate and temperature of 24 °C.

Data tables in Hargreaves and Brunson (1996) were used to generate Equation 1, which was a good starting point for estimating the amount of CO₂ at a known water alkalinity and pH.

$$CO_{2(Dis)} = ((1.91E + 06)e^{-2.32pH})Alk \tag{Eq. 1}$$

Where:

$CO_{2(Dis)}$ = Dissolved CO₂ in water (mg/L)

Alk = Alkalinity (mg/L CaCO₃/1 mg/L CaCO₃, unitless)

Laboratory results with Delta water (approximately 21 °C) did not show the relationship predicted by Equation 1; less CO₂ was being released at a given pH than predicted (Figure 9). This was to be expected because part of the Delta water alkalinity comes from other bases in solution such as hydroxide, borate, phosphate, silicate, nitrate and berates (Wurts and Durborow 1992).

Compounds other than carbonate and bicarbonate increased alkalinity but did not necessarily release CO₂; therefore, it was difficult to predict the amount of CO₂ in water based on pH and alkalinity alone and the presence of these compounds made it problematic to use Equation 1 exclusively for predicting dissolved CO₂ accurately. This equation was acceptable for estimating dissolved CO₂ to ± 50 mg/L in Delta water.

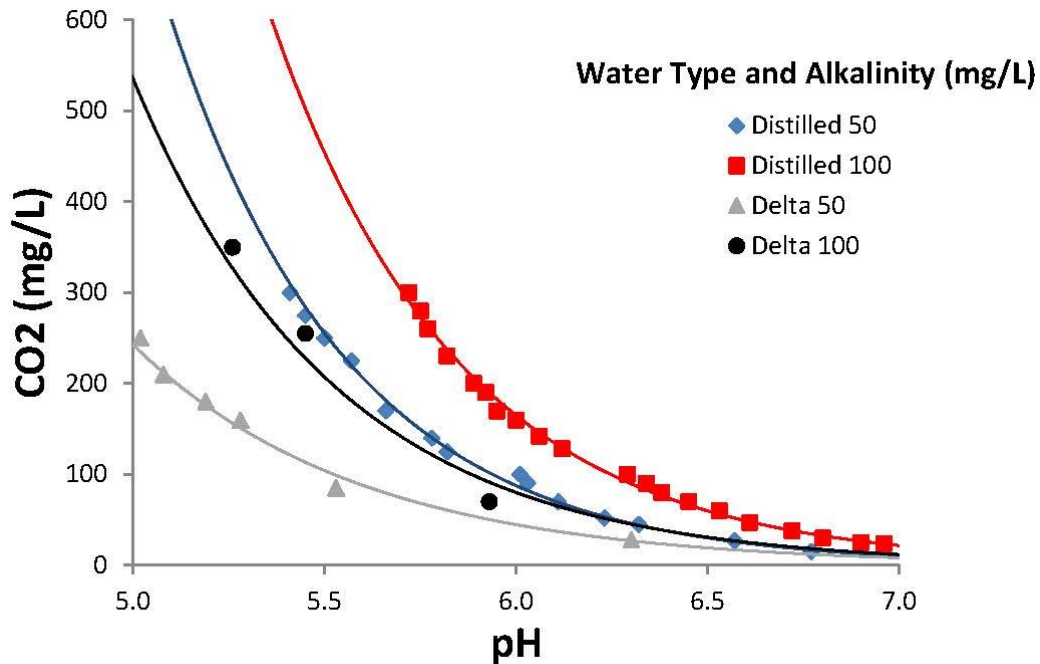


Figure 9.—Laboratory results showing the differing relationships between carbon dioxide concentration and pH for distilled and Delta water with alkalinities of 50 and 100 mg/L calcium carbonate and temperatures of approximately 21 °C.

Temperature influenced the amount of dissolved CO₂ in water (approximately 35 mg/L from 10–25 °C at a pH of 6) but its influence was not as great as the fluctuation in CO₂ (approximately 70 mg/L) caused by seasonal changes in alkalinity at the TFCF (range = 50–100 mg/L CaCO₃). Laboratory tests with distilled water, with an alkalinity of 85 mg/L at 10, 20 and 30 °C, demonstrated that CO₂ concentration shifted in a similar manner to that published by Wurts and Durborow 1992 (Figure 10). These results were in agreement with Hargreaves and Brunson's (1996) statement that, "in general, water can hold more CO₂ as temperature declines, although differences in temperature are less important than differences in total alkalinity and thus, for practical purposes, application of some kind of temperature correction is not necessary for estimation of CO₂." Since the bulk of the predator removals at the TFCF will be completed between 15 and 25 °C, it may not be necessary to closely monitor temperature to predict CO₂ concentration in the water. However, small changes in temperature may have a large impact on the health and survival of certain fish species exposed to low dissolved oxygen environments (Downing and Merckens 1957).

The CO₂ absorption rate was greatly influenced by the pH of the water (Figure 11). This result was a combination of the carbonate in the water being converted to CO₂ with the addition of muriatic acid and the injected CO₂ more readily dissolving in water. This suggests that dosing large volumes of water would be more effective using a combination of muriatic acid and CO₂.

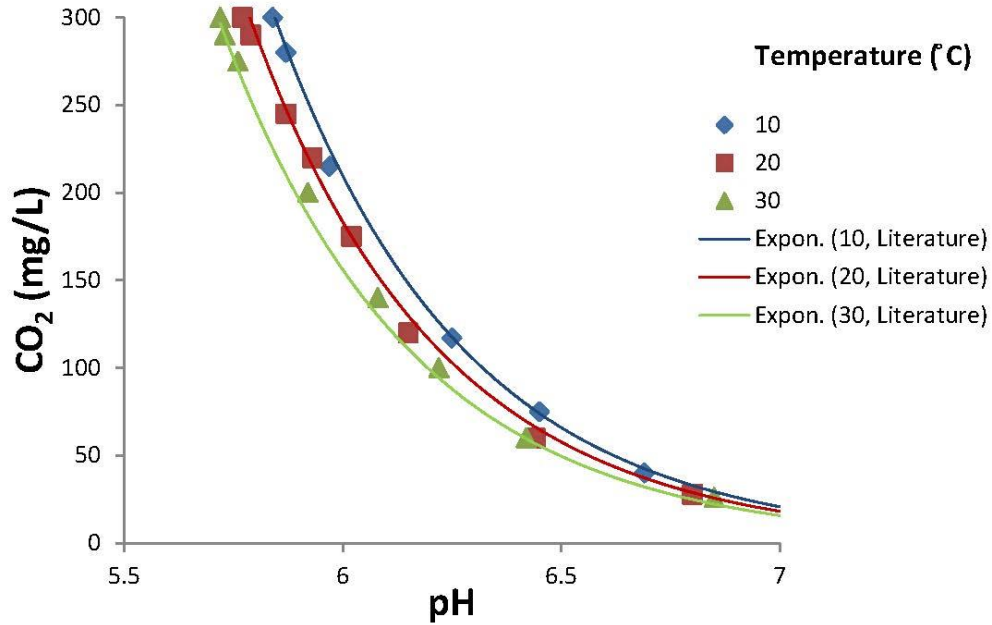


Figure 10.—Laboratory measured carbon dioxide concentrations overlaid on trend lines published by Wurts and Durborow (1992) for water at temperatures of 10, 20, and 30 °C with a constant alkalinity of 85 mg/L calcium carbonate.

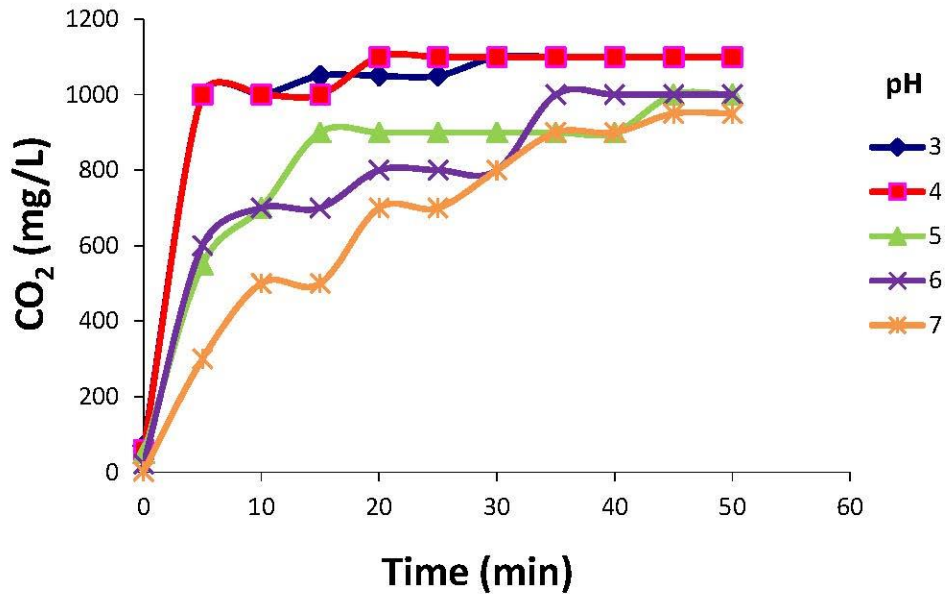


Figure 11.—Dissolved carbon dioxide concentration in 10 L of water (100 mg/L calcium carbonate, approximately 19 °C) as a function of time when exposed to muriatic acid (14% hydrochloric acid) and carbon dioxide gas (21 L/min through a microbubble diffuser).

Carbon dioxide had a strong affinity with water but could be removed with aeration (Figure 12). These results were in agreement with Hargreaves and Brunson’s (1996) statement that dissipation of CO₂ gas from water could be accelerated with aeration and/or mixing. The dissipation rate was described with an exponential decay function (Equations 2 and 3). As agitation increased, the dissipation rate increased.

$$CO_{2(t)} = CO_{2(o)}e^{-t/\tau} \tag{Eq. 2}$$

$$\tau = (1.7E - 03)Air^2 + 0.52Air + 50 \tag{Eq. 3}$$

Where:

$CO_{2(t)}$ = Dissolved CO₂ (mg/L) at time t (min)

$CO_{2(o)}$ = Dissolved CO₂ (mg/L) at time 0 (min)

Air = Volume of atmosphere air injected through diffusers (lpm/1 lpm, unitless)

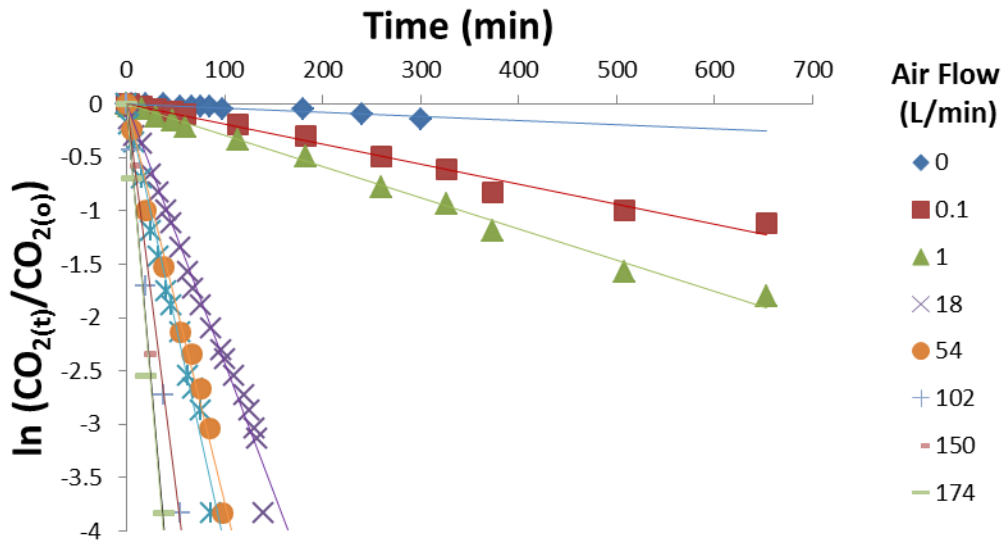


Figure 12.—Carbon dioxide dissipation rate in water (21.5 ± 0.5 °C) with 0, 0.1, 1, 18, 54, 102, 150, and 174 L/min air flow.

Interestingly, this simple experiment revealed that without agitation the CO₂ remained largely in solution for the entire 1440 min (1 d) test. This has important implications for the use of CO₂ within the TFCF because treated water may not undergo an extensive amount of agitation; therefore, to reduce dissolved CO₂ the water must be diluted. This method was appropriate for use in the bypass tubes and secondary channel at the TFCF where the CO₂ was rapidly diluted back to < 20 mg/L when the secondary channel flow entered the canal downstream of the

facility. Carbon dioxide may also be chemically removed by treating water with liming agents such as quicklime, hydrated lime or sodium carbonate, which chemically react directly with CO₂, resulting in reduced CO₂ concentration, increased alkalinity and increased pH (Hargreaves and Brunson 1996). While this technology exists, it would likely be expensive to perform due to the amount of liming agent that would be necessary to use.

Phase 2: Methods to Inject Carbon Dioxide and Estimate Dry Ice Sublimation Rate in Water

Surface to volume ratio is inversely proportional to block size; consequently, smaller blocks should see a faster decline in mass with time when immersed in water. Results using four sizes of blocks, of roughly the same shape, immersed 4.9-m deep in 11.7 °C water, supported our prediction based on this physical property (Figure 13). Smaller blocks lost mass faster than larger blocks; however, once large blocks were reduced in size due to sublimation, they did not decline in size as fast as a newly submerged small block. This was likely due to the gradual buildup of an ice layer around the dry ice block that was observed during the test. The largest block size tested was the standard size delivered by the dry ice supplier; consequently it was used in the pilot test.

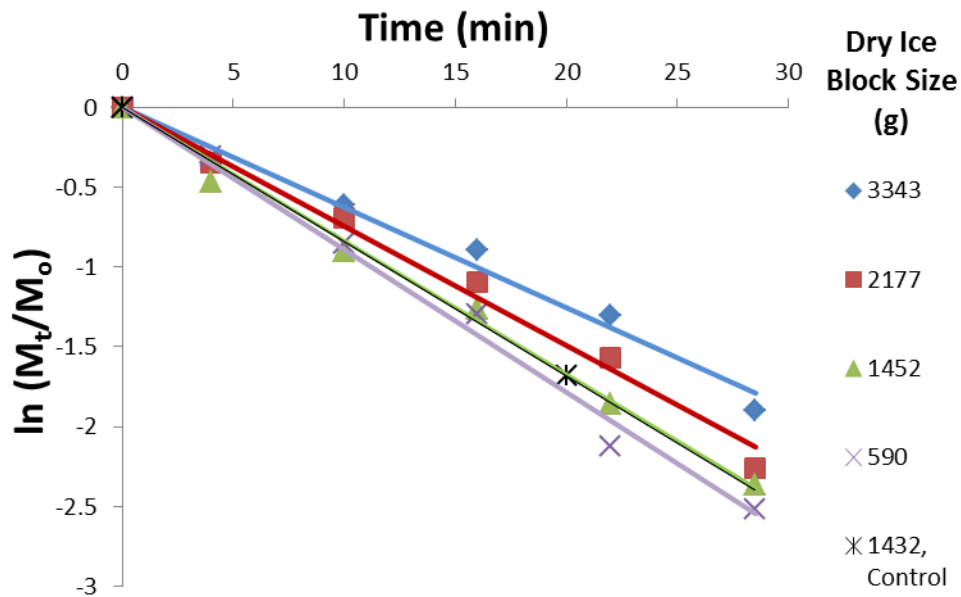


Figure 13.—Exponential decay of dry ice blocks of various mass immersed in water at 11.7 °C and 4.9 m deep (M_o = Initial mass [g], M_t = Mass at time t [g]). A reference block was left continually in the water and weighed at the end to verify that lifting the blocks periodically for weighing did not greatly change sublimation rate.

When dry ice entered water it vigorously sublimated; however, mass loss followed an exponential decay function and was predicted based on initial block size (Equation 4). The time constant declined as block size was reduced (Equation 5). These equations were used to estimate amount of gas entering the water, which was subsequently used to determine how efficiently CO₂ gas dissolved into water flowing down each bypass tube during Phase 5 (pilot test). The decay constant for a standard block used for field work was 15.9, meaning approximately 63.3% of the dry ice mass sublimated after 15.9 min. The reference dry ice block, which was only removed from water once after 20 min, lost mass at the same rate as the same size block lifted several times. Therefore, it was assumed that the durations of atmospheric exposure necessary to weigh blocks did not affect results.

$$M_t = M_o e^{-t/\tau} \quad \text{Eq. 4}$$

$$\tau = (4E - 07)Ms^2 + (3E-04)Ms + 10.8 \quad \text{Eq. 5}$$

Where:

- M_t = Block mass (g) at time t (min)
 M_o = Initial block mass (g)
 Ms = Starting block weight ($M_o/1$ g, unitless)

Dry ice was delivered in blocks, which were seldom the same size or fully intact. Over the size range of dry ice blocks delivered, sublimation rate differed by 10–15% over 10–20 min (see Figure 13). For all sizes tested, the rate of sublimation per min was between 4–6% in the first 10 min. For short term CO₂ exposure (~10 min) in the bypass tubes and secondary channel, it will be more cost effective to use smaller blocks (pellets) because the whole pellet will be gassed off, reducing waste. However, water flow may push small dry ice pellets downstream if they are too light or buoyant. Therefore, it will likely be more efficient to use full sized dry ice blocks for longer dose periods, as they last for at least 20 min.

Phase 3: Dose-Response of Predators and Prey

Striped bass (n = 52, mean [minimum–maximum] length = 341 [260–398] mm FL) generally reached PLE and TLE in < 10 min when the CO₂ concentration was ≥ 50 and ≥ 150 mg/L, respectively (Figure 14). Times to PLE and TLE were less variable at the higher doses and were nearly indistinguishable at CO₂ concentrations > 250 mg/L. An optimal 10-min dose for striped bass appears to be in the range of 50–150 mg/L CO₂.

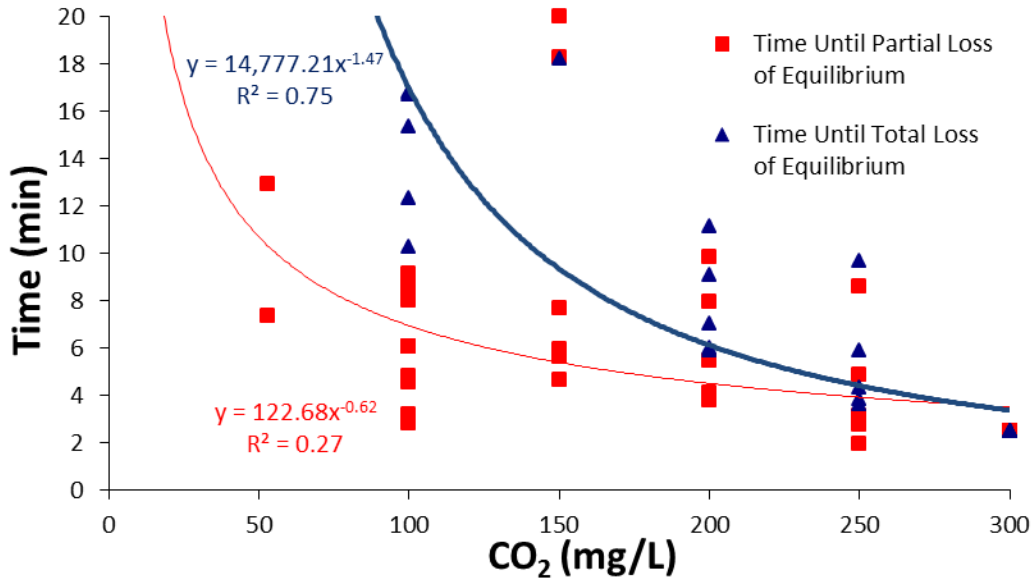


Figure 14.—Times until partial and total loss of equilibrium for striped bass exposed to water with carbon dioxide (n = 52, mean [minimum–maximum] length = 341 [260–398] mm FL).

Survival was 100% for striped bass exposed to CO₂ concentrations < 250 mg/L for 20 min (Figure 15). Striped bass exposed to the 2 highest concentrations (250 mg/L and 300 mg/L) for 20 min displayed 80% survival over the 96-h observation period.

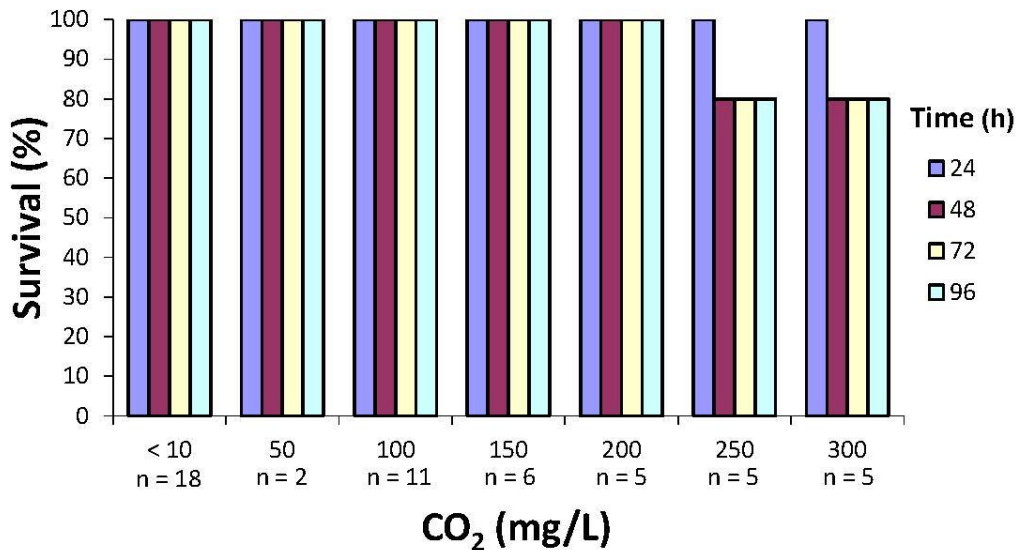


Figure 15.—Mean 24–96 h survival of striped bass following 20-min exposure to various concentrations of carbon dioxide (n = 52, mean [minimum–maximum] length = 341 [260–398] mm FL).

Chinook salmon (n = 70, mean [minimum–maximum] length = 178 [132–209] mm FL) and delta smelt (n = 60, mean [minimum–maximum] length = 70 [58–87] mm FL) generally exhibited PLE responses in less than 10 min at the lowest treatment dose (50 mg/L CO₂), although times to PLE were not consistent until CO₂ concentrations of ≥ 100 mg/L. In addition, TLE was highly variable (Figures 16 and 17); anecdotal evidence suggests this is likely due to fish balancing on the tank bottom rather than rolling over. Future testing should incorporate gentle prodding or firmly squeezing the base of the tail, as described by Gourdon (2003), to determine response to stimuli and verify if test fish can maintain equilibrium. Chinook salmon and delta smelt TLE generally occurred within 10 min at CO₂ concentrations of ≥ 100 mg/L and ≥ 50 mg/L, respectively, although times to TLE were not absolute until CO₂ concentrations of ≥ 250 mg/L for Chinook salmon and ≥ 200 mg/L for delta smelt. Optimal 10-min doses for Chinook salmon and delta smelt appear to be in the range of 30–100 and 30–50 mg/L CO₂, respectively.

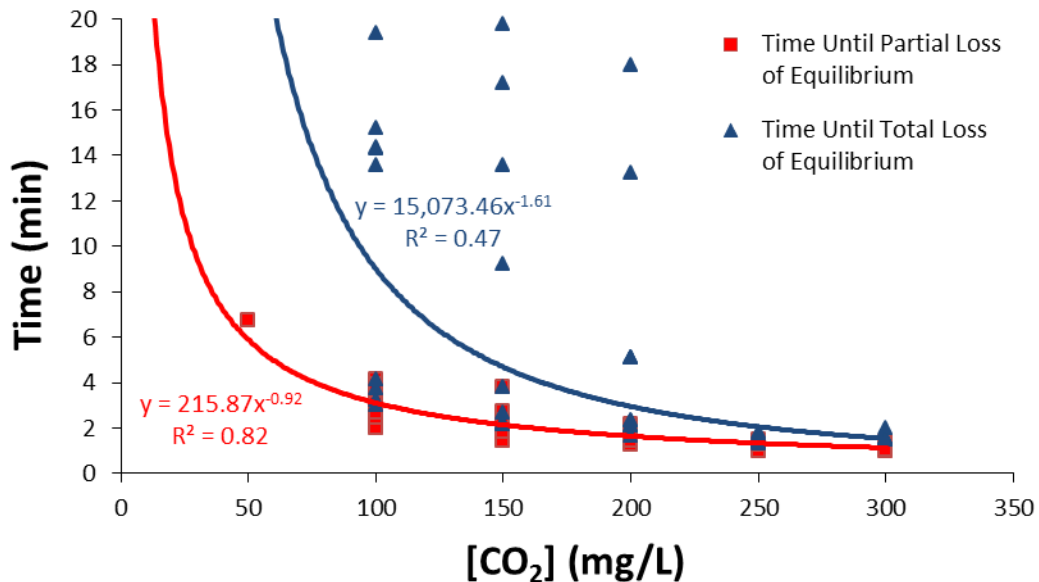


Figure 16.—Times until partial and total loss of equilibrium for Chinook salmon exposed to water with carbon dioxide (n = 70, mean [minimum–maximum] length = 178 [132–209] mm FL).

All Chinook salmon exposed to CO₂ concentrations ≤ 250 mg/L for 20 min survived 96 h (Figure 18). Ninety-six hour survival dropped to 20% for Chinook salmon exposed to 300 mg/L for 20 min.

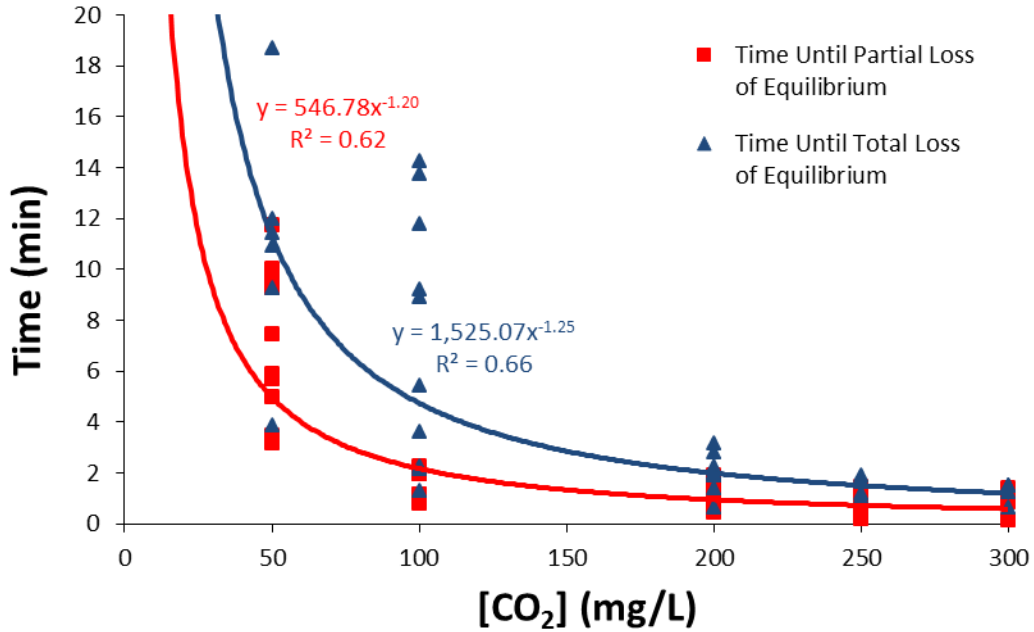


Figure 17.—Times until partial and total loss of equilibrium for delta smelt exposed to water with carbon dioxide (n = 60, mean [minimum–maximum] length = 70 [58–87] mm FL).

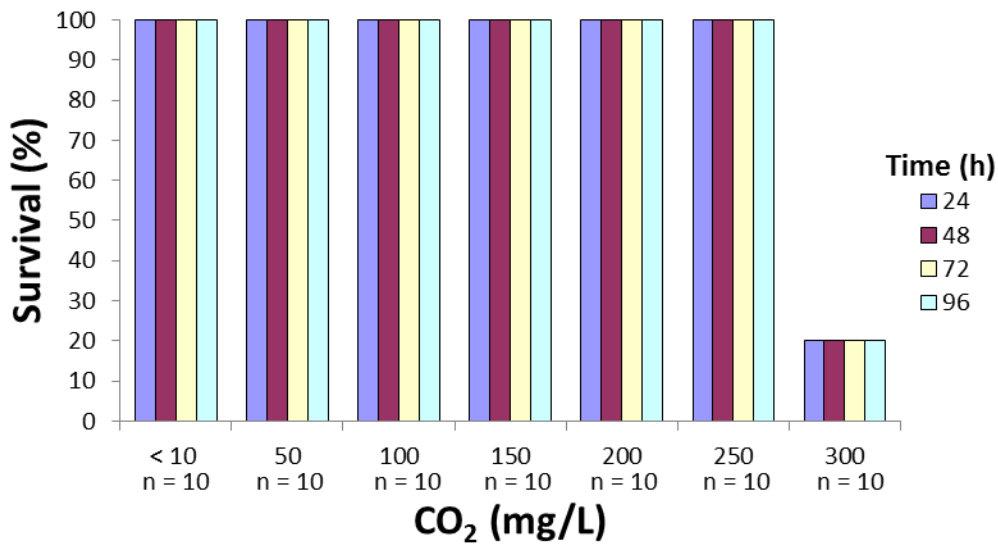


Figure 18.—Mean 24–96 h survival of Chinook salmon following 20-min exposure to various concentrations of carbon dioxide (n = 70, mean [minimum–maximum] length = 178 [132–209] mm FL).

All delta smelt survived when exposed to CO₂ concentrations < 10 mg/L (Figure 19). Ninety-six hour survival of delta smelt exposed to CO₂ concentrations of 50, 100, 200, 250 and 300 mg/L were 40, 30, 10, 0 and 0%, respectively. While delta smelt did not tolerate elevated CO₂ concentrations, this

should not preclude using this method at the TFCF. As a result of predation, fewer delta smelt will likely enter TFCF holding tanks if piscivorous predatory fish are not removed regularly from the bypass tubes and secondary channel. The CO₂ predator removal process only takes a small portion of a salvage day (1 h) to complete; therefore, only a small percentage of delta smelt salvaged throughout the day will potentially be affected. To prevent incidental mortality, the CO₂ predator removal process could be performed when delta smelt, or other sensitive species, are typically not present at the TFCF.

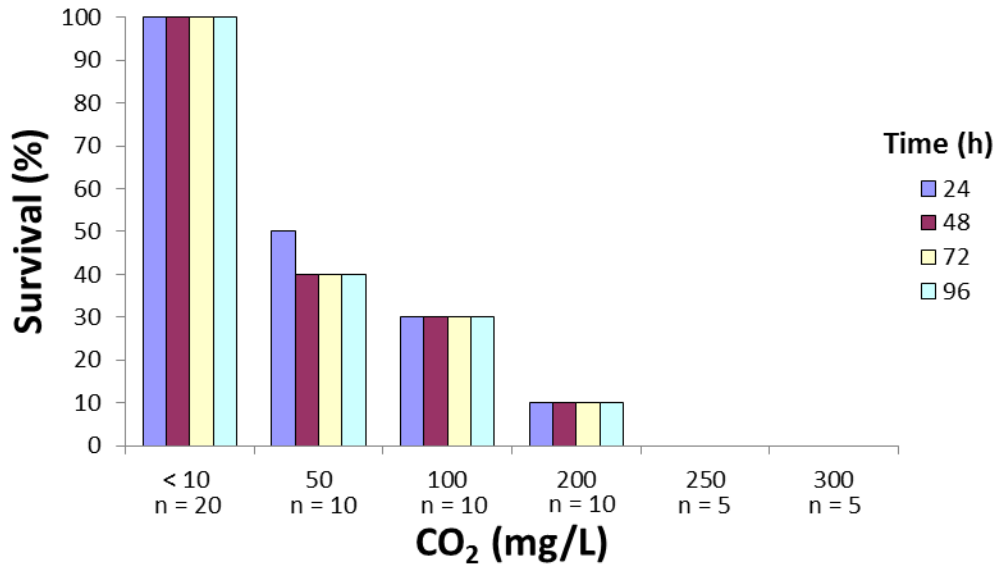


Figure 19.—Mean 24–96 h survival of delta smelt following 20-min exposure to various concentrations of carbon dioxide (n = 60, mean [minimum–maximum] length = 70 [58–87] mm FL).

Although adult striped bass appeared less sensitive to hypercapnic water than juvenile Chinook salmon and adult delta smelt, this may have been a result of differences in the efficacy of the anesthetic due to variation in body size as well as differences between species (Ackerman *et al.* 2005). Data collected in this study did not provide information on effects of fish size or temperature on species specific PLE, TLE or survival.

Phase 4: Test Dose-Response in Flume

Flume test results were promising, as experimental doses (100–140 mg/L CO₂) promoted downstream fish movement within 10 min (Figure 20). Striped bass downstream movement occurred before PLE; therefore, they were not impinged on the screen angled across the flow (Figure 21). This suggests fish do not need to be rendered completely insensible with anesthetic to promote downstream movement and that fish will not be impinged on the secondary channel louvers if exposed to comparable CO₂ concentrations. Based on these findings, it is likely

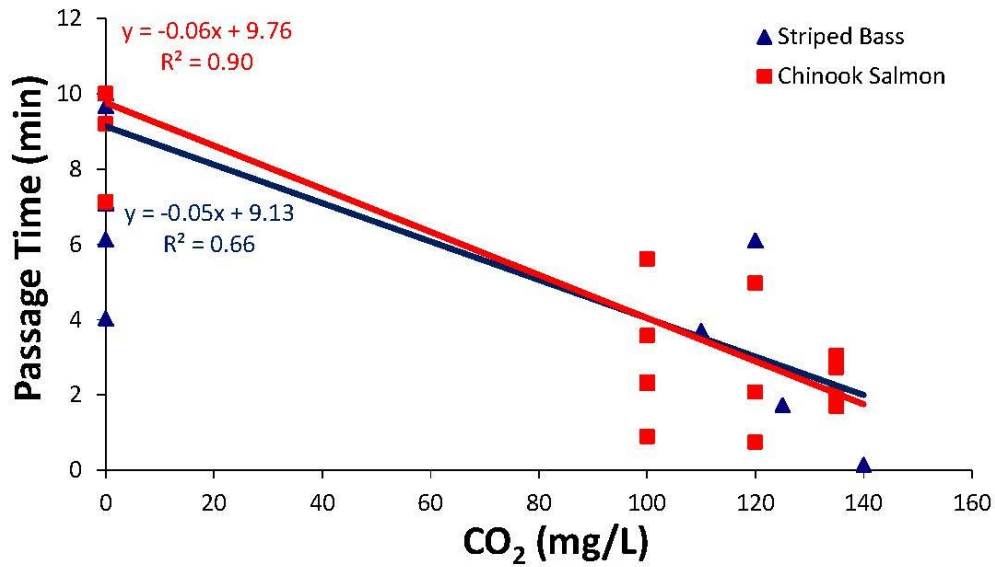


Figure 20.—Striped bass (mean [minimum–maximum] length = 88 [80–100] mm FL) and Chinook salmon (mean [minimum–maximum] length = 213 [180–250] mm FL) downstream passage time in a flume at elevated carbon dioxide concentrations. Control trials (n = 18 striped bass, n = 20 Chinook salmon) demonstrated fish would typically not move downstream with flow when no carbon dioxide was added. Treatment trials for striped bass (n = 4) and Chinook salmon (n = 15) demonstrated fish move downstream more rapidly when exposed to increasing levels of carbon dioxide.

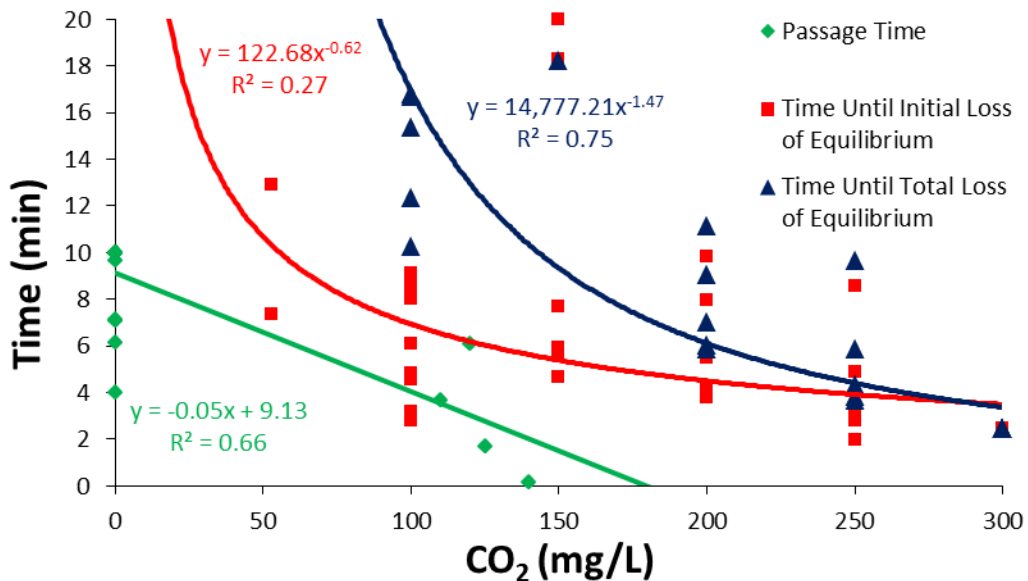


Figure 21.—Downstream passage times of striped bass in a flume (n = 22, mean [minimum–maximum] length = 88 [80–100] mm FL) set at various carbon dioxide concentrations in comparison with times until partial loss of equilibrium and total loss of equilibrium.

that the optimal dose ranges estimated in Phase 3 from times to ILE and PLE are higher than necessary to achieve downstream fish movement. This experimental setup was not ideal because the CO₂ concentration needed to be preset before fish were added rather than having fish acclimated and then administering CO₂. This tradeoff was acceptable as the CO₂ concentration could be tightly controlled and the cumulative effects over time with a gradual increase in concentration did not need to be taken into consideration.

Phase 5: Pilot Test in Bypass Tubes and Secondary Channel at the Tracy Fish Collection Facility

Dry ice inserted directly into primary bypass entrances sank to the bottom of the bypasses and stayed in place. Water flow entering the bypasses carried the CO₂-rich water along the 0.9-m diameter concrete bypass tube towards the secondary channel. Peak CO₂ concentrations in bypasses 1, 2, 3 and 4 were 267, 349, 280, and 238 mg/L, respectively, and the time for peak CO₂ concentrations to reach the secondary channel ranged from 6–22 min (Figure 22). The effects of elevated CO₂ levels, combined with pulsed water flow, successfully moved fish from the bypass tubes and secondary channel to the holding tank (Figures 23 and 24). A total of 1,136 fish, of various species, were collected in the holding tank during the pilot test. Eighteen (1.6%) of these fish were collected during the 10-min control flush, while 1,118 fish (98.4%) were collected after treatment with CO₂. The large difference between the numbers of fish collected in the holding tank during the control and CO₂ treatment indicates elevated CO₂ was responsible for moving fish downstream (2 proportions test, $n = 1,136$, $Z = -184.8$, $P < 0.001$).

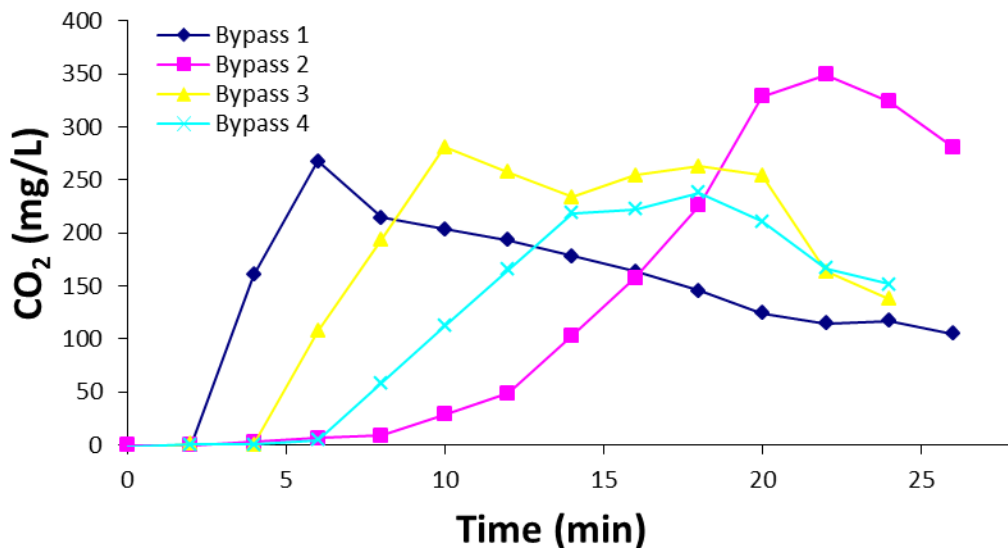


Figure 22.—Concentration of carbon dioxide in water measured from each bypass tube over time.

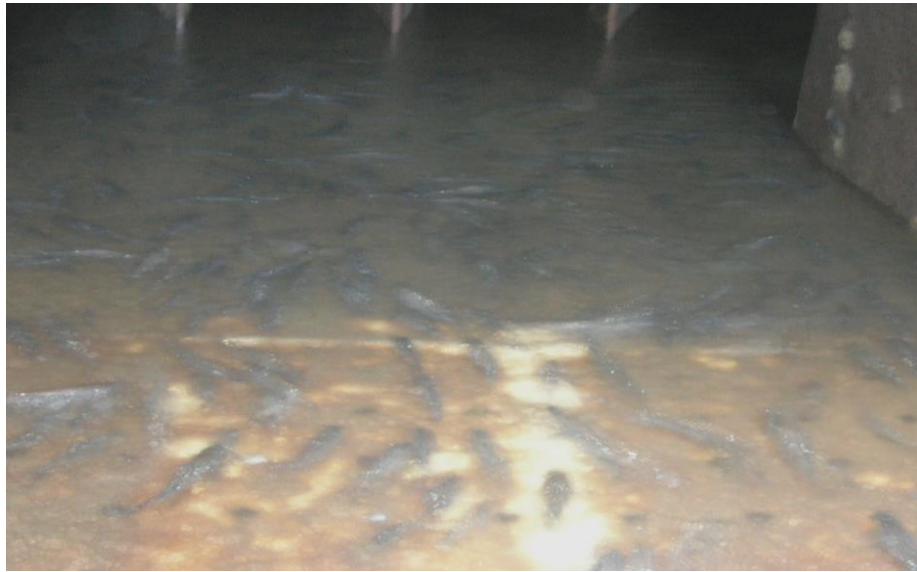


Figure 23.—Fish were abundant in the secondary channel before treatment with carbon dioxide (top); few fish were observed in the holding tank at this time (bottom).

While CO₂ concentrations in the bypass tubes increased as predicted, peak level and the time for the CO₂-rich water to reach the secondary channel was not as expected. The range of CO₂ concentrations observed and times necessary for peak concentrations to reach the secondary channel were most likely due to fluctuations in bypass tube water flow (Table 1). Bypass flows, under normal conditions, decline from bypass 1 to 4 because of differences in water elevation at the bypass entrances and bypass tube length. In addition to the natural flow variation between bypass tubes, there is also evidence of an upwelling effect after dry ice is injected. If the amount of dry ice added to a bypass tube was too great, the rise of sublimated gas generated an upwelling, which slowed water flow in the bypass tube.



Figure 24.—After carbon dioxide treatment (349 mg/L peak carbon dioxide concentration), fish were forced from the secondary channel (top) into the holding tank (bottom).

Bypass 1 received the most dry ice but did not generate the highest peak CO_2 concentration because of the large volume of water that traveled down the bypass in the first 5 min. Flow rate in this bypass decreased once dry ice was inserted into the other bypasses. Peak CO_2 concentration in bypass 1 reached the secondary channel quickly compared to other bypasses. While the peak CO_2 concentration was lower than expected, the transfer efficiency (percent of CO_2 dissolved over time) into water during the first 20 min, estimated from the sublimation rate and CO_2 concentration, was the best of all four bypasses (Table 2).

Table 1.—Summary of bypass tube lengths and volumes, along with amounts of dry ice injected, hydraulics, peak carbon dioxide concentrations and times for peak concentrations to reach secondary channel during the pilot test.

Bypass Tube	Length (m)	Volume (L)	Mean Velocity \pm SD (m/s)	Mean Flow \pm SD (m ³ /s)	Amount of Dry Ice Injected (kg)	Peak Carbon Dioxide Dose (mg/L)	Time for Peak Dose to Reach Secondary Channel (min)
1	55	36,118	0.08 \pm 0.01	0.37 \pm 0.05	180	267	6
2	61	40,058	0.03 \pm 0.03	0.13 \pm 0.12	135	349	22
3	72	47,282	0.04 \pm 0.02	0.18 \pm 0.06	90	280	10
4	92	60,416	0.02 \pm 0.00	0.09 \pm 0.01	45	238	18

Bypass 2 received a large amount of dry ice; however, bypass flow nearly stopped because gas rising inside the bypass created an upwelling flow that nearly countered the natural flow down the bypass. This was apparent from the bubbles coming out the entrance to bypass 2 (but not bypasses 1, 3 or 4) during the pilot test. The reduction in flow, caused by upwelling, increased the contact time between the CO₂ and water and resulted in the highest peak CO₂ concentration of all four bypasses. Despite this, the transfer efficiency into water was the lowest of all bypasses during the first 20 min and the peak CO₂ concentration took the longest to reach the secondary channel.

The percent of dry ice that dissolved into water varied from 7–70% and depended on the amount of dry ice inserted and the flow rate through the bypass tube. This data set was too limited for generating a regression equation that predicts CO₂ concentration based on the amount of dry ice inserted and water flow. Although high concentrations of CO₂ (349 mg/L) were achieved, they returned to ambient levels within 5 min after normal water flow was resumed in the secondary channel.

While 12 species of fish were collected in the holding tank during the pilot test, only striped bass and white catfish were abundant (Figure 25). A greater number of striped bass were collected in the holding tank during CO₂ treatment (n = 492) than during the control (n = 0). Similarly, white catfish collected in the holding tank during CO₂ treatment (n = 558) were significantly more abundant than during the control (n = 11; 2 proportions test, n = 569, Z = -117.76, P < 0.001).

Table 2.—Summary of amounts of carbon dioxide sublimated, amounts of carbon dioxide measured in the water and percent of carbon dioxide dissolved into water over time (transfer efficiency) during the first 20 min of the pilot test for each bypass tube and amount of dry ice injected.

Amount of Dry Ice Added (kg)	180	135	90	45
Accumulative Time (min)	Bypass 1	Bypass 2	Bypass 3	Bypass 4
Total Carbon Dioxide Sublimated at Given Time (kg)				
10	79	60	40	20
14	100	75	50	25
16	108	81	54	27
20	122	91	61	30
Total Dissolved Carbon Dioxide Measured in Water (kg)				
10	50	4	17	7
14	58	6	21	9
16	73	13	29	15
20	85	19	34	18
Percent of Carbon Dioxide Dissolved in Water Over Time (%)				
10	63	7	43	35
14	58	8	42	36
16	68	16	54	56
20	70	21	56	60

Most striped bass collected in the holding tank during the CO₂ treatment were 250–350 mm FL (median, mean [minimum–maximum] length = 282, 291 [89–698] mm FL; Figure 26). No striped bass were collected in the holding tank during the control, although those that were salvaged at the TFCF during the week of testing (n = 33) were predominantly between 100–150 mm FL (median, mean [minimum–maximum] length = 89, 100 [38–317] mm FL; Figure 26). The majority of white catfish collected in the holding tank during CO₂ treatment were 120–160 mm FL (median, mean [minimum–maximum] length = 135, 133 [40–328] mm FL), while most white catfish collected in the control group were 100–140 mm FL (median, mean [minimum–maximum] length = 135, 127 [69–190] mm FL; Figure 27). A comparison between the median length of striped bass collected in the holding tank during CO₂ treatment and the median length of striped bass salvaged at the TFCF during the week of testing suggests that significantly larger fish are removed during CO₂ treatment than during

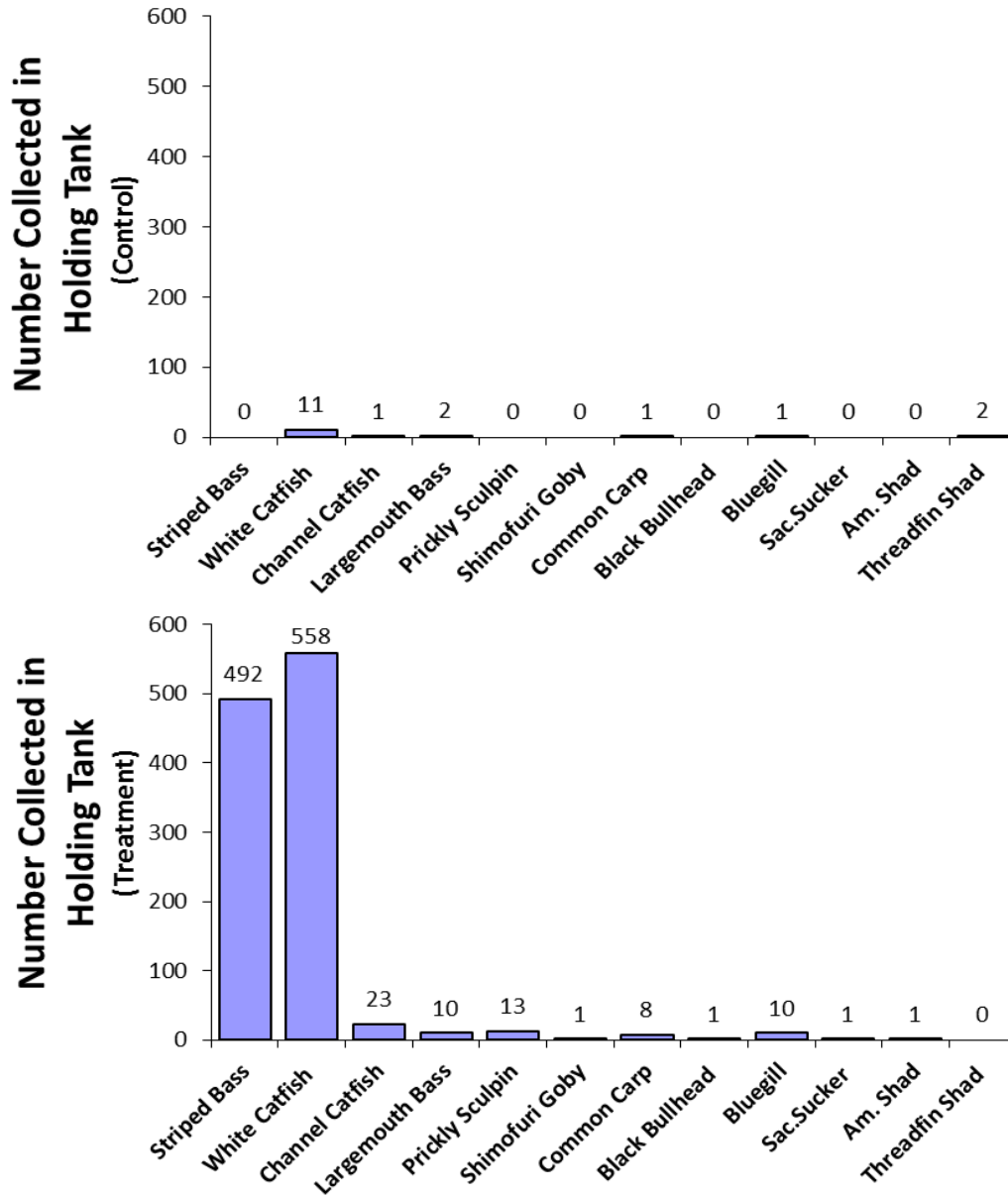


Figure 25.—Total number of each species collected in the holding tank during control (top graph) and carbon dioxide treatment (bottom graph; 349 mg/L peak carbon dioxide concentration).

regular salvage activity (Mann-Whitney test, $W = 1291$, $P < 0.001$). There was no significant difference in median lengths of white catfish collected in the holding tank during the control and CO₂ treatment (Mann-Whitney test, $W = 159063.5$, $P = 0.9513$).

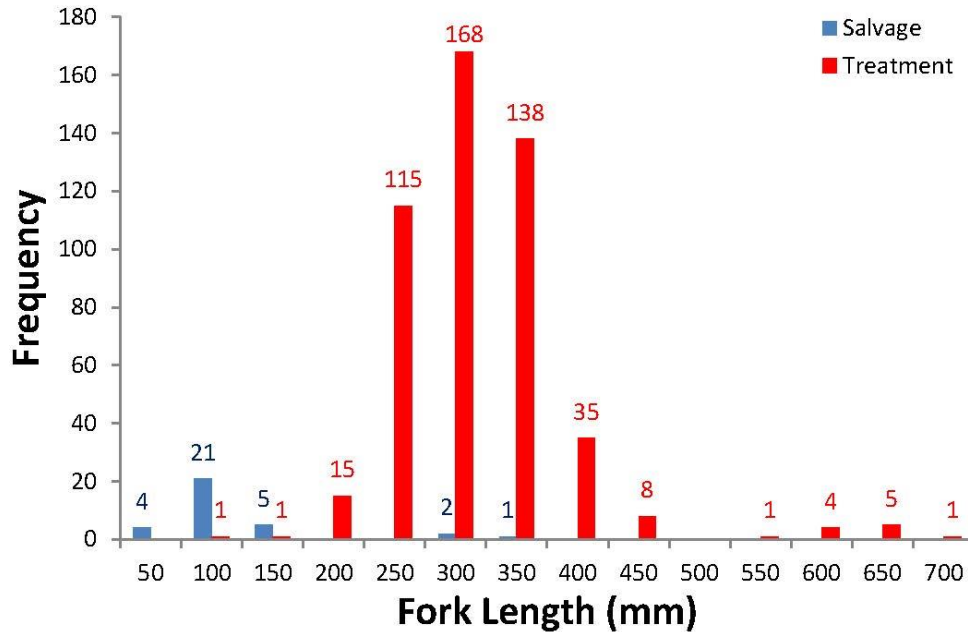


Figure 26.—Frequency distributions of striped bass fork lengths (mm) salvaged during the week of testing (n = 33, median, mean [minimum–maximum length = 89, 100 [38–317] mm FL) and collected in the holding tank during carbon dioxide treatment (n = 492, median, mean [minimum–maximum] length = 282, 291 [89–698] mm FL; 349 mg/L peak carbon dioxide concentration).

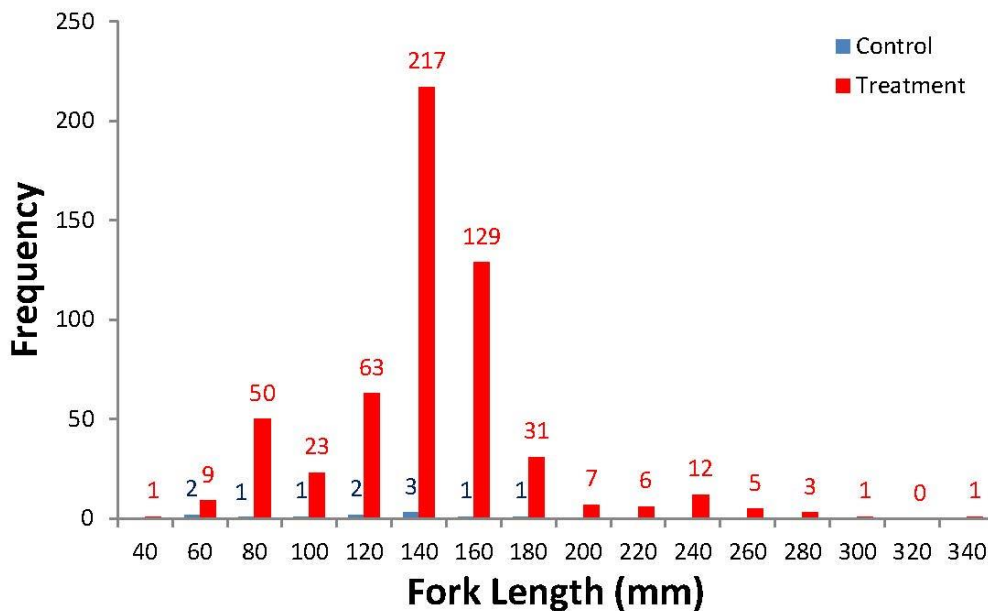


Figure 27.—Frequency distributions of white catfish fork lengths (mm) collected in the holding tank during control (n = 11, median, mean [minimum–maximum] length = 135, 127 [69–190] mm FL) and carbon dioxide treatment (n = 558, median, mean [minimum–maximum] length = 135, 133 [40–328] mm FL; 349 mg/L peak carbon dioxide concentration).

No fish were lost through the secondary channel louvers and collected in the sieve net during the control. A total of 335 fish were collected in the sieve net during CO₂ treatment. The large difference between the number of fish collected in the sieve net during the control and CO₂ treatment further support that elevated CO₂ was responsible for moving fish downstream during the pilot test.

White catfish (n = 236) comprised 70% of the fish collected in the sieve net during the CO₂ treatment (Figure 28). The median (mean [minimum–maximum]) length of a representative sample of 30 white catfish from the sieve net was 78.5 (88 [62–138]) mm FL, with the majority of fish being 60–100 mm FL (Figure 29). Striped bass (n = 2; median, mean [minimum–maximum] length = 151.5, 151.5 [145–158] mm FL) comprised 0.6% of all fish collected in the sieve net during CO₂ treatment. A comparison between the median lengths of white catfish collected in the holding tank and sieve net during CO₂ treatment suggests that significantly smaller white catfish are collected in the sieve net than in the holding tank during treatment with CO₂ (Mann-Whitney test, W = 188295.5, P < 0.001). Striped bass collected in the sieve net during CO₂ treatment were also significantly smaller than those collected in the holding tank (Mann-Whitney test, W = 122258, P = 0.0155). These results suggest that, although the majority of fish still louvered properly, certain fish species and sizes may not be as effectively guided to the holding tanks during treatment with elevated CO₂ as others and a certain degree of fish loss through the louvers in the secondary channel should be expected.

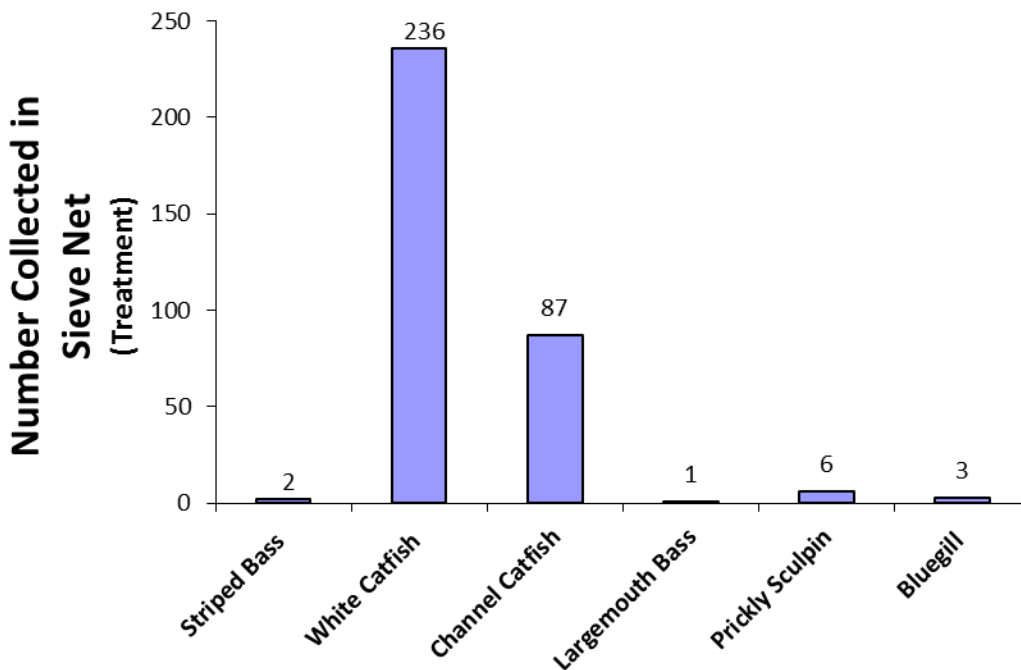


Figure 28.—Total number of each species collected in the sieve net behind secondary channel louvers during carbon dioxide treatment (349 mg/L peak carbon dioxide concentration). No fish were collected in the sieve net during the control.

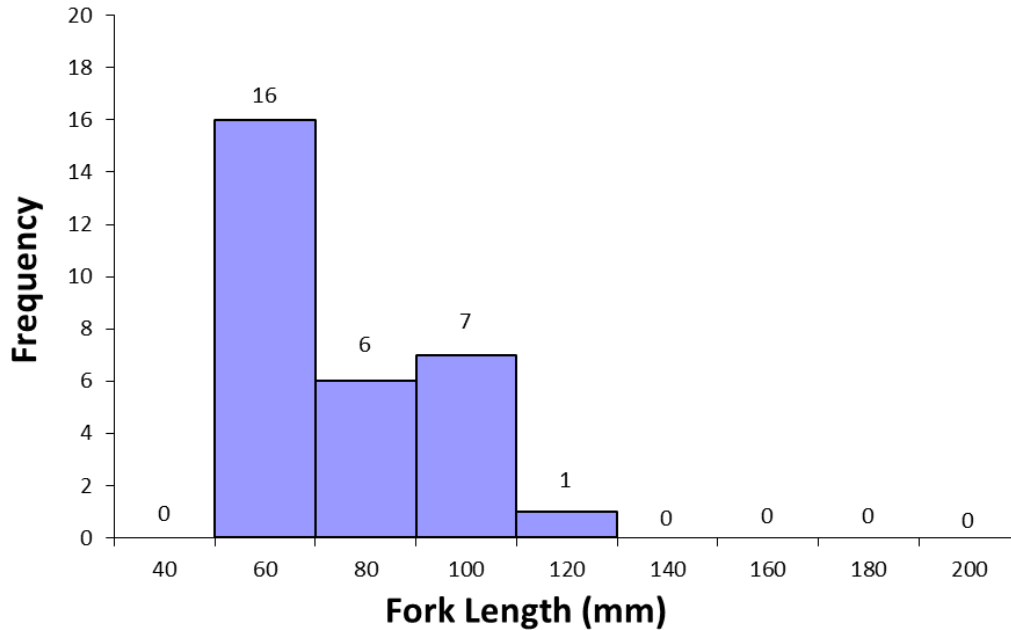


Figure 29.—Frequency distribution of white catfish fork lengths (mm) collected in the sieve net during carbon dioxide treatment ($n = 236$, mean [minimum–maximum] length = 88 [62–138] mm FL; 349 mg/L peak carbon dioxide concentration). Only a representative sample (30 fish) was measured.

CONCLUSION

Completing a predator removal with dry ice was quick, technically easy and safe. Installation of equipment and dewatering of the secondary channel were not necessarily required in order to complete this predator removal method. Only two staff members were needed to insert dry ice into a bypass tube. While dry ice was injected into two bypasses at a time using four employees, it could be possible for two employees to inject into all four bypasses in approximately 8 min, which is less than the time required to dewater the secondary channel and manually remove predators with sieve nets, seines and dip nets (≥ 45 min; Reyes 2013, personal communication). Normal atmospheric O_2 levels (20.7–20.9%) in the secondary channel were observed following the 30-min dry ice treatment. This was likely due to the secondary channel being open to the atmosphere, allowing for continuous air replenishment. Measurements of atmospheric CO_2 levels were not possible with the hand held meter used.

The laboratory and pilot tests provided evidence that CO_2 has a predictable impact on fish, including striped bass, white catfish, Chinook salmon and delta smelt. Adding CO_2 to the bypass tubes and secondary channel effectively removed fish, including piscivorous predators, from this area of the TFCF. High doses of CO_2 were attained at a reasonable cost in terms of supplies, equipment and manpower needed. There was evidence the CO_2 predator removal method will increase

employee safety, decrease labor, increase fish survival and increase daily salvage. While the efficacy of using CO₂ for removing fish was supported, the optimal technique for operators to use still needs to be developed.

Using elevated CO₂ concentrations in the bypass tubes and secondary channel at the TFCF should be further investigated for removing fish, including piscivorous predatory species. Optimal CO₂ concentrations and water flows in the bypass tubes that minimize cost, maximize fish removal and optimize survival, over all temperatures at the TFCF, should be determined. The optimal dose for striped bass removal may be deleterious for some threatened and endangered species; consequently, a minimum dose and exposure time must also be found for forcing these predators into the holding tank. While predator removal frequency could be reduced when threatened and endangered species are present, this may prove to be counterproductive as more fish may be consumed by predators than killed during, or after, treatment with elevated CO₂ concentrations. Treatment with CO₂ in the range of 50–200 mg/L, should be further evaluated in terms of survival based on fish size and water temperature. In addition, an evaluation of the old and new predator removal techniques should be completed by comparing removal efficiency, survival/injury, salvage loss time, cost and safety.

A mathematical model must be developed to predict the CO₂ dose in each bypass tube based on the dry ice block size, total amount of dry ice injected and water flow. Data for the model should be collected using a wide range of dry ice mass and bypass water flows. Currently, it appears that an excessive amount of dry ice has the potential to reduce the amount of CO₂ delivered into the secondary channel by disrupting the pattern of water flow. The conditions and mechanism behind this problem should be further evaluated.

Delta water alkalinity was not completely made of components that liberate CO₂ when acidified; consequently, a CO₂ concentration versus pH curve should be generated with each predator removal to see how accurate the equations provided in this study predict dissolved CO₂. Ideally, a technique should be developed that allows for rapid CO₂ estimation without the use of handheld titration cells.

If predator removals are needed on a regular basis (every few days when piscivorous predatory fish are present), then a permanent injection device, or methods with liquid CO₂, should be considered for future development. Injection devices and methods with liquid CO₂ will likely be more convenient for operators to use but will cost more than manually injecting dry ice.

The best location to inject CO₂ should be evaluated, as it could be added directly into the secondary channel or at the front of the bypass tubes. Adding dry ice directly to the secondary channel would be easier to complete and safer for employees, although it may only remove a portion of the fish residing within the system. It is recommended that a dose of > 200 mg/L be used for this test to ensure that fish in the two areas are quickly affected and removed.

Atmospheric monitoring is needed during future tests to determine the effect of dry ice treatment on atmospheric CO₂ levels in the secondary channel and verify that these levels do not exceed the Occupational Safety and Health Administration (OSHA) permissible exposure limits (5,000 mg/L [9,000 mg/m³] time-weighted average, 30,000 mg/L [54,000 mg/m³] short-term exposure limit; DHHS 2007). Carbon dioxide concentrations and pH should also be monitored in the canal downstream of the TFCF during and after predator removals to verify that levels return to pre-treatment conditions. The worst case scenario should be tested first, as the presence of CO₂ may be very dilute. This condition occurs when only one pumping unit is used for water export at the JPP during winter.

These recommended investigations, as well as others that have yet to be determined, will be necessary in order to effectively implement the use of elevated CO₂ concentrations to remove predatory fish from the bypass tubes and secondary channel at the TFCF on a weekly basis, as required by Action IV.4.1 of the 2009 NMFS Biological Opinion.

ACKNOWLEDGMENTS

We would like to thank Joel Imai and the TFCF fish diversion workers for accommodating this research activity and for assisting with the CO₂ predator removal pilot test in the bypass tubes and secondary channel at the TFCF. We are grateful to René C. Reyes for technical support, assistance with data collection and review of the document. We would like to thank Bradd B. Bridges, Michael R. Trask, and Don F. Faris for their assistance with data collection and other technical support provided during the course of this project. Editorial review provided by Donald E. Portz, Zachary A. Sutphin, Jerry Morinaka, Jarod Hutcherson, and 2 anonymous reviewers was also greatly appreciated. Funds for this study were provided by Reclamation Mid-Pacific Region office and were administered by Ronald G. Silva as the former Tracy Fish Facility Improvement Program (TFFIP) manager. We would also like to give thanks to the current TFFIP manager, John C. Dealy, for his continued support of this project.

REFERENCES

- Aasen, G.A. 2012. *Fish salvage at the Tracy Fish Collection Facility during the 2011 water year*. Contract Number R0785504. California Department of Fish and Game, Bay-Delta Region, Native Fishes and Fish Facilities Monitoring Unit, Stockton, California.
- Ackerman, P.A., J.D. Morgan, and G.K. Iwama. 2005. *Anesthetics*. (Supplement to the Canadian Council on Animal Care [CCAC] guidelines on the care and use of fish in research, teaching and testing).

- Bell, G. 1987. *An outline of anesthetics and anesthesia for salmonids, a guide for fish culturists in British Columbia*. Canadian Technical Report of Fisheries Aquatic Sciences No. 1534.
- Black, E.C., F.E.J. Fry, and V.S. Black. 1954. *The influence of carbon dioxide on the utilization of oxygen by some freshwater fish*. Canadian Journal of Zoology. 32:408–420.
- Bowser, P.R. 2001. *Anesthetic options for fish*. Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York.
- Brauner, C.J. and D.W. Baker. 2009. *Patterns of acid-base regulation during exposure to hypercarbia in fishes*. Pages 43–63 in M.L. Glass and S.C. Wood, editors. *Cardio-Respiratory Control in Vertebrates: Comparative and Evolutionary Aspects*. Springer Dordrecht Heidelberg London New York.
- Bridges, B., R.C. Bark, and M.D. Bowen. (in press). *Predator impacts on fish salvage at the Tracy Fish Collection Facility for adult delta smelt and juvenile Chinook salmon*. Tracy Fish Collection Facility Studies , U.S. Bureau of Reclamation, Mid-Pacific Region and Denver Technical Service Center.
- DHHS (Department of Health and Human Services). 2007. *NIOSH Pocket Guide to Chemical Hazards*. Publication No. 2005-149, DHHS (NIOSH), Washington D.C.
- Downing, K.M. and J.C. Merkens. 1957. *The influence of temperature on the survival of several species of fish in low tensions of dissolved oxygen*. An. Appl. Biol. 45 (2): 261–267.
- Fausch, K.D. 2000. *Reducing predation mortality at the Tracy Fish Test Facility: Review and analysis of potential solutions*. Tracy Fish Collection Facility Studies, Volume 12, Bureau of Reclamation, Mid-Pacific Region, Denver Technical Service Center and Colorado State University.
- Goodman, J., L. Bradbury, and J. Murphy. 2008. *Dry ice*. Human Wonder Research. http://www.appstate.edu/~goodmanjm/rcoe/hwr/science/dry_ice/dry_ice.html. (July 2013).
- Gourdon, J. 2003. *Fish and aquatic amphibian anesthesia*. Standard Operating Procedure 110.01, Cornell University Center for Animal Resources and Education, Ithaca, New York.

- Hargreaves, J., and M. Brunson. 1996. *Carbon dioxide in fish ponds*. Southern Regional Aquaculture Center, SRAC Publication No. 468.
- Helfman, G.S., B.B. Collette, and D.E. Facey. 1997. *The Diversity of Fishes*. Blackwell Science, Inc., Massachusetts.
- Liston, C., C. Karp, L. Hess, and S. Hiebert. 1994. *Summary of the fish predator removal program and intake channel studies, 1991–1992*. Tracy Fish Collection Facility Studies, Volume 1, Bureau of Reclamation, Mid-Pacific Region and Denver Technical Service Center.
- Murphy, B.R. and D.W. Willis. 1996. *Fisheries Techniques*. Second Edition. American Fisheries Society, Bethesda, Maryland.
- NMFS (National Marine Fisheries Service). 2009. *Biological opinion and conference opinion on the long-term operations of the Central Valley Project and State Water Project*. Endangered Species Act Section 7 Consultation, National Marine Fisheries Service, Southwest Region.
- Reyes, R. 2013. Tracy Fish Collection Facility, Byron, California, personal communication.
- Senese, F. 2010. *Why does the solubility of gases usually increase as temperature goes down?* General Chemistry Online! <http://antoine.frostburg.edu/chem/senese/101/solutions/faq/temperature-gas-solubility.shtml>. (November 2013).
- Southgate, P. 2005. *The importance of measuring carbon dioxide in aquaculture*. Fish Vet Group. <http://www.thefishsite.com/articles/103/the-importance-of-measuring-carbon-dioxide-in-aquaculture>. (February 2011).
- Wurts, W.A. and R.M. Durborow. 1992. *Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds*. Southern Regional Aquaculture Center, SRAC Publication No. 464.

APPENDIX 1

Chemical and Physical Properties of Carbon Dioxide

Table A1-1.—Physical constants of carbon dioxide (Asia Industrial Gases Association, 2009)

Chemical Name:	Carbon dioxide	
Synonym:	Carbon anhydride, carbonic acid gas, carbonic anhydride, dry ice	
CAS Registry Number:	124–38–9	
	U.S. Units	SI Units
Chemical formula	CO ₂	CO ₂
Molecular weight	44.01	44.01
Vapor pressure ¹⁾ at 2 °F (–16.7 °C)	302 psig	2082 kPa
Specific gravity of the gas at 70 °F (21.1 °C) and 1 atm	1.522	1.522
Solid to gas expansion ratio (specific volume of the gas) at 70 °F (21.1 °C) and 1 atm	8.741 ft ³ /lb	0.5457 m ³ /kg
Density of the gas at 70 °F (21.1 °C) and 1 atm	0.1144 lb/ft ³	1.833 kg/m ³
Density of the liquid saturated at 2 °F (–16.7 °C)	63.3 lb/ft ³ (8.46 lb/gal)	1014 kg/m ³
Density of solid (dry ice) at 1 atm and –109.3 °F (–78.5 °C)	97.6 lb/ft ³	1563 kg/m ³
Sublimation temperature at 1 atm	–109.3 °F	–78.5 °C
Critical temperature	87.9 °F	31.1 °C
Critical pressure	1070.6 psia	7381.8 kPa
Critical density	29.2 lb/ft ³	468 kg/m ³
Triple point	–69.9 °F at 75.1 psia	–56.6 °C at 518 kPa, abs
Latent heat of vaporization at 2 °F (–16.7 °C)	119.0 Btu/lb	276.8 kJ/kg
Latent heat of fusion at 1 atm and –69.9 °F (–56.6 °C)	85.6 Btu/lb	199 kJ/kg
Latent heat of sublimation at 1 atm and –109.3 °F (–78.5 °C)	245.5 Btu/lb	571.0 kJ/kg
Specific heat of the gas at 77 °F (25.0 °C) and 1 atm	0.203 Btu/(lb)(°F)	0.850 kJ/(kg)(°C)
C _p		
C _v	0.157 Btu/(lb)(°F)	0.657 kJ/(kg)(°C)
Ratio of specific heats (C _p /C _v) at 59 °F (15.0 °C)	1.304	1.304
Solubility in water, vol/vol at 68 °F (20.0 °C)	0.90	0.90
Viscosity of saturated liquid at 2 °F (–16.7 °C)	0.287 lb/(ft)(hr)	0.000119 Pa·s
¹⁾ All psig values are referenced to 14.696 psia (101.325 kPa, abs).		

Asia Industrial Gases Association. 2009. *Carbon dioxide*. AIGA 068/10 Globally Harmonised Document. <http://www.asiaiga.org>. (September 2012).

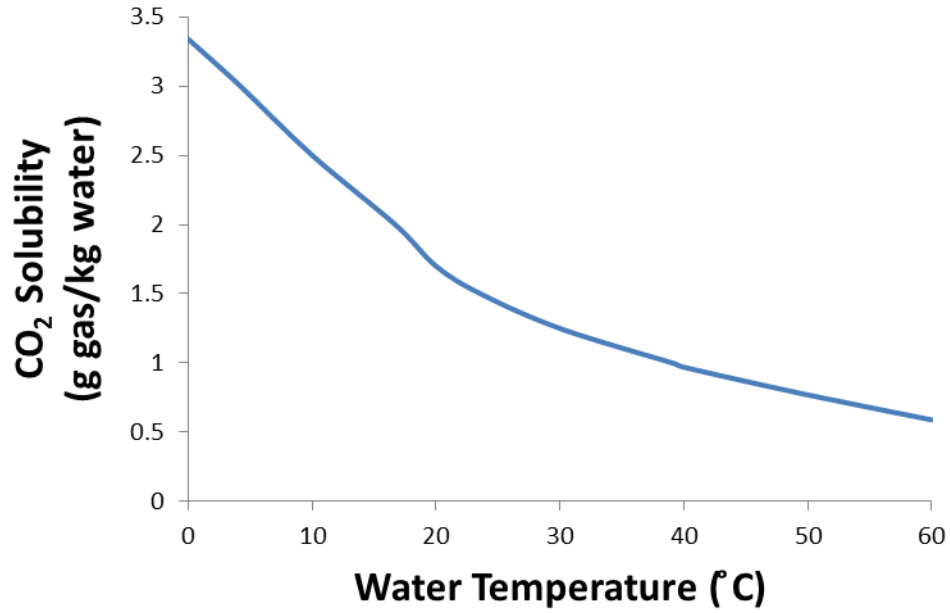


Figure A1-1.—Solubility of carbon dioxide in water as a function of temperature at standard atmospheric pressure (reproduced from Engineering Toolbox 2012).

Engineering Toolbox. 2012. *Carbon dioxide gas solubility chart*.
http://www.engineeringtoolbox.com/gases-solubility-water-d_1148.html.
(September 2012).

APPENDIX 2

Interrelationships Between Carbon Dioxide,
Temperature, pH, and Total Alkalinity

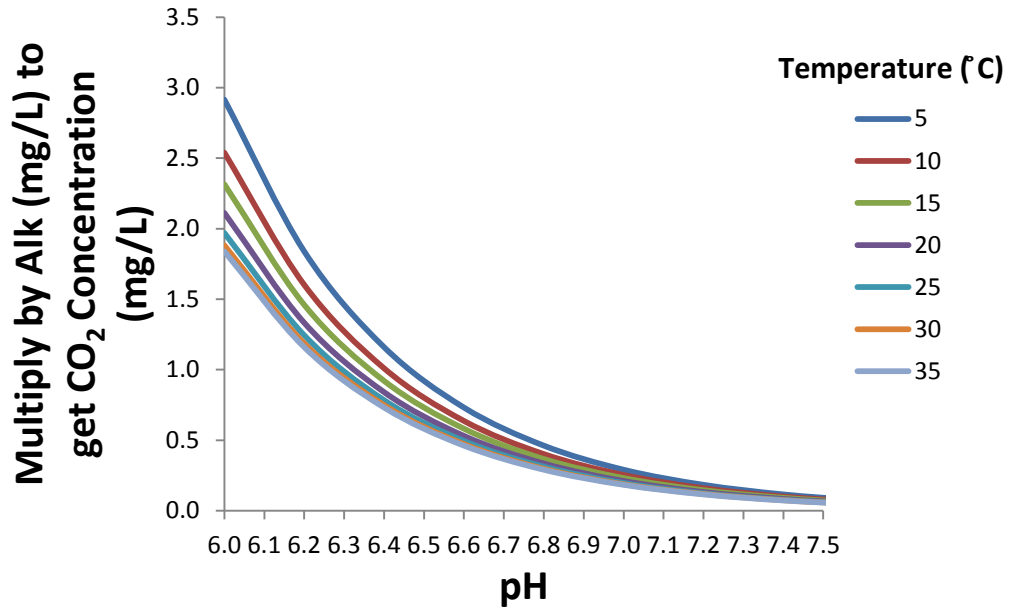


Figure A2-1.—Estimation of carbon dioxide concentration with respect to temperature, pH, and alkalinity (reproduced from Wurts and Durborow 1992).

Wurts, W.A. and R.M. Durborow. 1992. *Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds*. Southern Regional Aquaculture Center, SRAC Publication No. 464.

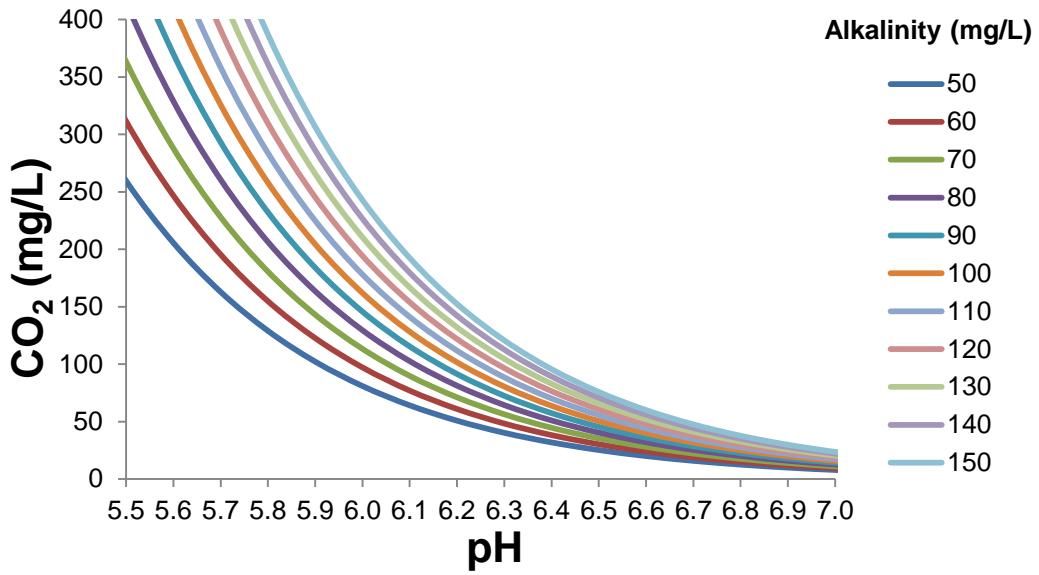


Figure A2-2.—Graph used by pond managers to estimate carbon dioxide concentration in water when total alkalinity and pH values are known (reproduced from Hargreaves and Brunson 1996).

Hargreaves, J. and M. Brunson. 1996. *Carbon dioxide in fish ponds*. Southern Regional Aquaculture Center, SRAC Publication No. 468.

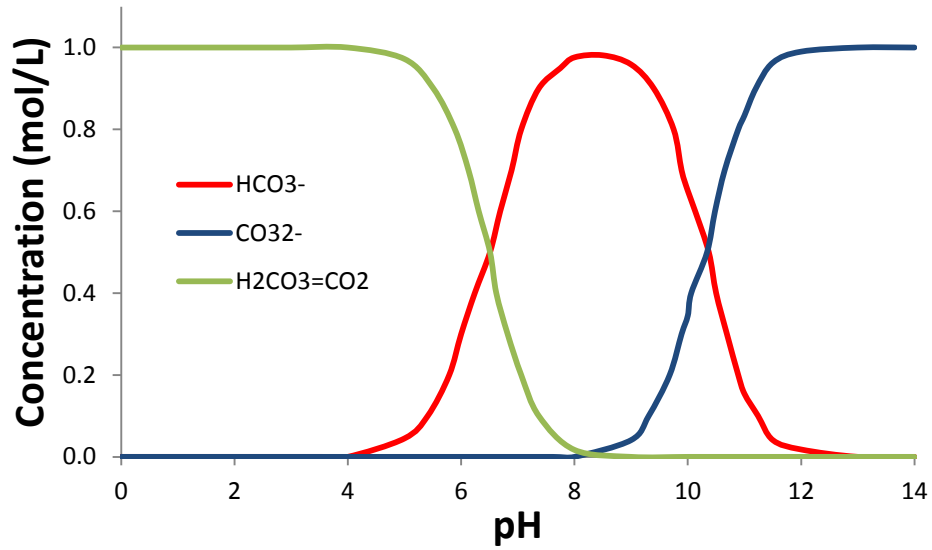


Figure A2-3.—Mole fraction of carbon dioxide species in relation to pH of water (reproduced from Ozone Solutions 2010). HCO₃⁻ = HCO₃⁻, CO₃²⁻ = CO₃²⁻, H₂CO₃ = H₂CO₃, CO₂ = CO₂.

Ozone Solutions. 2010. Ozone Solutions Inc. <http://ozoneinfusion.com/wp-content/uploads/2010/04/CO2-and-PH-Graph2>. (September 2012).

Appendix 3

Stages of Anesthesia

Table A3-1.—Stages of Anesthesia (reproduced from Bowser 2001)

Stage	Descriptor	Behavioral Response of Fish
0	Normal	Reactive to external stimuli; opercular rate and muscle tone normal
1	Light sedation	Slight loss of reactivity to external stimuli; opercular rate slightly decreased; equilibrium normal
2	Deep sedation	Total loss of reactivity to all but strong external stimuli; slight decrease in opercular rate; equilibrium normal
3	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactivity only to strong tactile and vibration stimuli
4	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
5	Loss of reflex reactivity	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes
6	Medullary collapse (stage of asphyxia)	Opercular movements cease; cardiac arrest usually follows quickly

Bowser, P.R. 2001. *Anesthetic options for fish*. Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York.