# Draft Outline – SAS Report

2016 EA30 Students

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# 1 Introduction

## 1.1 Problem Statement

This report aims to determine whether the study of Biological Oxygen Demand (BOD) and water flow velocity are relevant to future conservation research for the endangered Santa Ana sucker *C. santaanae*. The experiment seeks to gather information on the water quality of a section of the Santa Ana River and use this data to help answer the questions: *Are the biochemical oxygen demand (BOD) levels healthy for sucker*, *do differing levels affect the abundance of individuals in certain sections of the river*, *Are the flow rates in certain sections of the river optimum for sucker populations*, and *do high- or low-flow events affect the number of individuals in the stream?* Because the river is regulated by a water treatment facility, we believe that BOD levels will be very low, increasing with distance from the facility. Because of this, we also expect high dissolved oxygen (DO) content and a high sucker presence. We additionally hypothesize that larger populations of the sucker will concentrate in low-flow areas that are nearby high-flow sections. Through this experiment, we aim to inform Santa Ana sucker conservation efforts and hope to inform action by the nearby water treatment facility.

## 1.2 Background (Literature Review)

A 2012 U.S. Fish and Wildlife Service report on the recovery of the endangered fish noted that specific tolerances to dissolved oxygen have not been determined for Santa Ana Sucker[1]. In general, fish have been known to be at risk of suffocation when exposed to dissolved oxygen (D.O.) levels below 2 mg/L for only short periods of time[2]. The 2012 FWS report also notes that constant water flows are important to the availability of coarse substrate which the Sucker needs to spawn offspring and hide from predators. According to Evans et al. (2005), temporary reduction of flows can significantly reduce the amount of habitat for suckers.[3] Just last month, the Center for Biological Diversity reported that "by halting water releases critical to maintaining surface flows of the Santa Ana River, the Rapid Infiltration and Extraction (RIX) treatment plant is stranding and killing threatened fish." [4]

## 1.3 Objectives

We hope to measure BOD levels and water flow velocity in different areas of the Santa Ana River and correlate those measurements with FWS data about where the Sucker is living in the river.

> Our null hypotheses are H0: Water flow velocity and/or BOD levels do not significantly correlate with prevalence of the Santa Ana Sucker.
>
> Our alternative hypotheses are H1: Water flow velocity and/or BOD levels significantly correlate with prevalence of the Santa Ana Sucker.

If we can reject one or both of our null hypotheses, we can conclude that the study of Biological Oxygen Demand (BOD) and water flow velocity are relevant to future conservation research for the endangered Santa Ana sucker *C. santaanae*.

# 2 Methods

## 2.1 Site Descrition

We evaluated the Santa Ana River between... near Colton, California (Figure 1).

## 2.2 Field Methods

### 5-Day Biochemical Oxygen Demand Test (BOD5)

Approximately 1L of source river water was collected at each of two sites, one upstream location closer to the wastewater discharge facility, and one downstream location (fig. 1). Ideally, these would be transported to the laboratory for analysis within four hours.

### Water Velocity Collection

At each of the corresponding water sample collection sites, water velocity was also measured using a SonTek FlowTracker Handheld Advanced probe, which emits sonar waves at a certain depth in the water column, and based on the feedback (20 pings) gives a velocity reading. Ideally, multiple readings would be taken at each site, after the probe is placed on a flat section of the riverbed where water appears to be flowing net in the same direction.

## 2.3 Laboratory Methods

### BOD5

Ideally within the same day of collection, water samples were analyzed for initial dissolved oxygen content and prepared for 5-day incubation.

- Three different dilutions were used for each of two sites, with source water volumes of 25, 50, and 100 mL.
- A seed suspension was prepared using PolySeed Seed Innoculum, and 4 mL of the solution was added to each 300 mL sample bottle. This solution was also used to create four seed blanks with seed volumes 15, 20, 25, and 30 mL.
- Nitrification inhibitor was created by dissolving 2.0 g allylthiourea (ATU, C4H8N2S) in 1 L distilled water. 0.3 mL of the ATU solution was added to each source water sample, as well as to all seeded samples.


Figure 1: Google Earth –Example of a map. What's wrong with this image?

- A glucose-glutamic acid (GGA) solution was prepared by dissolving 150 mg each of dry glucose and glutamic acid in 1 L of distilled water, and was added to each of the four seed blanks, as well as the six source water samples. Three GGA blanks were also created with 6 mL of GGA solution in incubation bottles.

- Dilution water was created using 1 mL each phosphate buffer (8.5 g KH2PO4, 21.75 g K2HPO4, 33.4 g Na2HPO*7H2O, and 1.7 g NH4Cl dissolved in 1 L distilled water), Magnesium sulfate solution (4.5 g MgSO4*7H2O dissolved in 200 mL distilled water), Calcium chloride solution (5.5 g CaCl2 dissolved in 200 mL distilled water), and Ferric chloride solution (0.05 g FeCl3*6H2O dissolved in 200 mL distilled water), and added to the six source water samples, four GGA blanks, and three seeded blanks. Three dilution water blanks were also created using the same procedure diluted to 300 mL.

Initial DO readings were to be taken on all blanks and samples using a Thermo Scientific DO Probe with auto-spinning functionality. The bottles were then incubated in a dark area for 5 days, and DO readings were again taken.

## 2.4 Statistical Methods

### Quality Control Checks

Using the seed blanks, glucose-glutamic acid blanks, and dilution water blanks, quality control checks were performed prior to data collection.

- Minimum DO Depletion–Viable samples must have min. DO depletion of 2.0mg/L, and residual DO of at least 1.0mg/L.

- Glucose-Glutamic Acid Check–The resulting average BOD for the 3 GGA blanks (after correction for dilution and seeding) must be 198+/- 30.5mg/L.

- Dilution water check–DO uptake after incubation must not be more than 0.20mg/L and preferably not more than 0.10 (before seed corrections).

  Dilution Water–If dilution water blank exceeds 0.20 mg/L, clearly identify samples in data.

- Seed control–Calculate Seed Control Factor (SCF) using [(D1-D2)*f], where

  D1= initial DO of seed control, mg/L

  D2= final DO after indubation, mg/L,

  f= (vol. seed in diluted sample)/(vol. seed in seed control)

### BOD5

BOD5 was calculated for viable samples according to Standard Methods for the Examination of Water and Wastewater, using the equation BOD5, mg/L= ((D1-D2)-(S)Vs)/P, where

D1= initial DO, mg/L
D2= final DO after incubation, mg/L

# 3 Results

The Seed Control Factor was calculated for the four seed blanks, and the slope of the resulting values and the corresponding seed concentrations was determined. Analysis of the data using quality control parameters indicated that two source water samples met minimum DO depletion standards. Our BOD5 calculations on these data yielded the values:

Upstream BOD5= 22.069 mg/L
Downstream BOD5= 27.3493 mg/L

# 4 Discussion

Our results were skewed due to several hiccups that occurred throughout the process of our experiment. The first time we collected our samples, said samples were not processed on the same day as collection. We donâĂŹt have the initial BOD measurements and so our group had to think outside of the box and backpedal some in order to work backwards and acquire the numbers needed in order to make the vital calculations and check our work. When in the middle of checking the BOD of our samples after the 5 day incubation period we realized that we had not turned on the motor for the previous handful of samples as well as some in our initial stages. In order to accommodate this discrepancy, we decided to include spin v no spin as apart of our variables in our project to see the results we would get since there was such a huge margin between the numbers when the solutions were stirred and when they were not. In order to fill any gaps in our own research and numbers, we have also decided that it would be best that we correlate our results with those collected by the Fish and Wildlife service when they conducted their electroshock tests on the fish.

# 5 Conclusion and Recommendations

Future experiments can improve upon our methods by using the optimum amount of source water and dilution water to get the optimum about of BOD depletion. The ideal BOD depletion reading was given at 25 mL of source water and 275 mL dilution water. It would also be best if it is meticulously made sure of that the BOD is taken of the initial samples. Important to the process is to have every sample spun each time to allow for consistency and as much accuracy as possible with each BOD reading.