Original plans to study floodplain habitats on the San Joaquin River and their biotic and abiotic properties, food webs, and production potential throughout the 2014 juvenile Chinook rearing period (February to May) have been temporarily interrupted due to the critical dry conditions. These critical low water conditions have forced the San Joaquin River Restoration Program (SJRRP) and the Friant Water Users Authority to temporally reduce water releases from Friant Dam. These extremely low flow conditions will most likely have a large effect on San Joaquin River ecosystem, limiting available floodplain habitat for juvenile Chinook salmon. Early estimates suggest that this will be the driest year ever recorded.

For this reason, it is mandatory that we sample the river mainstem to see how the food web reacts to these inclement conditions. This year we are planning to sample the mainstem river directly adjacent to our future floodplain sites using similar protocols, which will provide a low-flow (Critical Dry) baseline for future studies on the juvenile Chinook and the river’s food web potential that will be directly relatable. The samples collected alongside the floodplain sites will aid us in subsequent years to determine the nature of mainstem lower trophic levels and production that will ultimately be transported into the floodplains as allochthonous material under restoration flows. Autochthonous organic matter will be produced within the floodplain; however the allochthonous organic matter will be found in the mainstem or terrestrially and then imported into the floodplain through natural processes. Data from this year’s sampling efforts will strengthen our assessment of floodplain production and their benefit as a food web to the juvenile Chinook salmon in the upcoming years.

1 HISTORY AND BACKGROUND

The San Joaquin River historically played host to the largest spring-run Chinook Salmon (Oncorhynchus tshawytscha) run in California, estimated at approximately 200-500,000 adult spawners (SJRRP Reintroduction Strategy (2011)). Due to heavy impacts to the river, including the building of levees, diverting of water for irrigation, pollution, and infrastructure including Friant Dam, the runs of Chinook salmon were completely eliminated. As part of the Settlement reached by the Friant Water Users Authority, U.S. Departments of the Interior and Commerce, and the Natural Resources Defense Council, two restoration goals were set for the San Joaquin River Restoration Program (SJRRP). These are:
RESTORATION: TO RESTORE AND MAINTAIN FISH POPULATIONS IN "GOOD CONDITION" IN THE MAIN STEM OF THE SAN JOAQUIN RIVER BELOW FRIANT DAM TO THE CONFLUENCE OF THE MERCED RIVER, INCLUDING NATURALLY REPRODUCING AND SELF-SUSTAINING POPULATIONS OF SALMON AND OTHER FISH.

WATER MANAGEMENT: TO REDUCE OR AVOID ADVERSE WATER SUPPLY IMPACTS TO ALL OF THE FRIANT DIVISION LONG-TERM CONTRACTORS THAT MAY RESULT FROM THE INTERIM FLOWS AND RESTORATION FLOWS PROVIDED FOR IN THE SETTLEMENT.

Based on these two goals the SJRRP has begun to implement investigations for the restoration of the San Joaquin’s functions. One of these functions is the support of large Chinook salmon runs.

Rearing of juvenile salmonids within the river is an important part of the Chinook salmon life cycle. It was previously concluded, in large river-scale projects (SJRRP 2012), that the river does not have a level of production, the necessary biodiversity of taxa, nor the appropriate range of habitats to support juvenile rearing. This study will seek to provide fine-scale analysis of juvenile Chinook rearing habitat possibilities within the main-stem using studies of organic matter storage and production, primary consumers (i.e. invertebrates), and ultimately food web resolution and habitat mapping.

Primary production and primary consumption are at the base of any food web. The abiotic factors that drive primary production and thus allow for primary consumption are the main drivers of ecosystems. Consumers may also use organic matter (live or as detritus) that is imported from upriver reaches or from riparian vegetation. Consumer functional feeding groups and river food webs are thus quite variable given a broad range of concurrently available organic matter. In aquatic ecosystems, primary producers, (i.e. algae, epiphytes, phytoplankton) and organic matter availability are affected greatly by varying regimes in water velocity, substrate types, light availability, and water chemistry (Hauer 2011).

Higher levels of primary production and organic matter delivery will likely alleviate food limitation on the production of primary consumers (i.e. invertebrates). When abiotic factors allow for organic matter availability and consumption, secondary consumer (i.e. fish) production may flourish. Floodplains have been found to be areas where such high levels of primary production occur following inundation (Ahearn 2006). However, during low flow years, it may be seen that the mainstem river behaves as a floodplain, with reduced velocities, higher temperatures, and increased primary production and organic matter processing. Our study seeks to examine if such conditions exist and will allow for a trade-off, in which the juvenile Chinook salmon can utilize mainstem habitats that are not suitable during normal and high flows, but which become suitable with a draw-down of flow levels.

The results from the study can be compared with those resolved from future floodplain studies to evaluate differences between mainstem and floodplain production values within those mainstem habitats most likely to provide rearing potential to juvenile Chinook.
1.1 STUDY SITE

The study sites are found on the San Joaquin River, a heavily impacted river with a watershed basin covering approximately 31,800 mi$^2$ and running approximately 366 miles from the high Sierra to the Sacramento-San Joaquin River Delta (NRDC (2007)). Within this preliminary investigation during an assumed critical-low water year, the study will contain 4 sites within Restoration Reaches 1A and 1B, running from River Mile 267.6 down to River Mile 229, 38.6 River Miles. Within Reaches 1A and 1B, study sites were selected based on presence of adjacent floodplain habitat and landowner access permissions (Figure 1.1).

**FIGURE 1.1 MAP OF STUDY SITES WITHIN RIVER RESTORATION REACHES 1A AND 1B**

1.2 STUDY REACH ESTABLISHMENT

Mainstem sites are located adjacent to planned floodplain sites, which were previously set up as part of the SJRRP Floodplain Production Assessment Study in cooperation with the Bureau of Reclamation.

Due to water limitations, the Floodplain Production Study has been temporarily suspended pending an increase in flow levels and water availability. However, because it would be beneficial to the study, we would like to have comparability between floodplain habitats and mainstem habitats, especially to identify main channel rearing potential during a period of severe water reduction. Therefore, we have decided to select mainstem areas, which are found adjacent to the pre-established floodplain sites to sample which will provide baseline information and allow future comparison.

Sites will be established with X and Y Geographic coordinates at each corner for spatial referencing and will run for a length of 100m along the River Left Bank (RLB). Eleven transects will be established evenly spaced within the reach (10m apart). Transects start points will be geo-referenced on RLB and run perpendicularly to river flow. Start and End points will be marked to provide a permanent reference for transects.
1.3 SAMPLING EVENTS

Sampling events will occur at least two times within the normal flood season stretching from late February until late March or early April. Additional sampling may occur with this period to produce more data points for analysis.

1.4 DATA CATALOGING PROCEDURE

A procedure has been laid out to define the cataloging of each sample so as to create a unique catalog number for every sample that is taken. Labeling will follow the example shown in Figure 1.2. Care should be taken to record the number faithfully and without error. Sample Location is the two digit number associated with the percentage of the transect defined by the sample location. For example, 18% of the transect is 18, 91% is 91. Sample Number is used if the sample is split into multiple parts due to large sample volumes.

2 SAMPLING AND COLLECTION PROTOCOLS

2.1 PHYSICAL HABITAT AND WATER QUALITY

2.1.1 SUBSTRATE SIZE

Relationships of macroinvertebrates with substrate structure and heterogeneity are very complex (Barnes et al. 2013). Thus collecting substrate data will not only give us a view of the river’s morphology, but also help determine if these designated stretches of river have the habitat necessary to facilitate and maintain a robust food web (Gore 1985). Methods adapted from Wolman (1954) will help us identify and categorize the substrate. At least 50 substrate particles will be systematically measured at every transect. These
substrate samples will be evenly distributed throughout each transect (~550 samples per study site Figure 2.1) (Wolman 1954).

During the substrate sampling process we will carry a rod to keep sampling objective. We will place the tip of the rod down into the substrate, and once rod touches a piece of substrate we will reach down and pick up the piece of substrate that the rod is touching. This rod technique will help make the substrate sampling collection unbiased. We will take the substrate particle and places it into a size category using the substrates intermediate axis to keep the data uniform (Figure 2.2). There are twelve-size classes we will use to delineate the substrate size. These size classes are: <8.0 mm, 8.0 mm, 16.0 mm, 22.2 mm, 31.8 mm, 44.5 mm, 63.5 mm, 89.0 mm, 127.0 mm, 177.8 mm, 254.0 mm, and >254.0 mm. We will determine substrate size classes by using the gravimeter (Carnet Technology, Terry, MS) (Figure 2.2).

2.1.2 HYDROLOGY

2.1.2.1 VELOCITY, WIDTH, AND DEPTH

River discharge in the study areas will be estimated from the velocity area method adapted from Ode (2007). We will also use discharge data from USGS (US Geological Survey) and CDWR (California Department of Water resources) gauge stations which are located on the San Joaquin River at Donny Bridge, Friant Dam, Highway 41, Skaggs Bridge (Highway 145), Gravelly Ford, and Mendota. The gauge station data can be found online at (http://cdec.water.ca.gov/cgi-progs/queryDaily?).

To collect the flow data, we will be using a flow-meter (FH950 Portable Velocity Flowmeter; Hach Company, Loveland, Co). This specific flow-meter uses an electromagnetic sensor to measure the waters velocity in a conductive liquid such as water. The waters velocity is displayed on a digital display as feet per second (ft/s) or meters per second (m/s) (Figure 2.3). The device measures water velocity using fixed point averaging (FPA), which is the average velocity measured over a fixed period of time (CFS uses a 30 second interval).
The velocity data will be collected using a modified version of Ode (2007). At each transect 10 water velocity/depth measurements will be taken. Each flow-meter is attached to a top-set wading rod (Figure 2.4), therefore we will simultaneously take depth measurements while doing the velocity measurements. A small boat will be used to support us in collecting the velocity data in deeper portions of the river. We will string a rope across that river to the opposite bank. This rope will be held approximately 0.3-0.6 m (1-2 ft) above the water surface and will be perpendicular to the flow along the transect. We will then string a measuring tape along the rope, attaching the measurement tape to the rope with clamps. This measuring tape will help us determine the width of the river at each transect. Once the width is assessed, we will take the width and divide that measurement by eleven. This number will be the even distribution of the 10 velocity and depth samples. If the water is more than two feet, water velocity measurements are taken at 20 and 60 percent of the waters depth. If the water is less than two feet deep, the velocity is taken at only 60 percent of the waters depth. When taking the flow measurements, we will make sure to allow 10-20 seconds for the flow-meter to stabilize before recording the data (m/s) (Ode 2007).

2.1.2.2 WATER QUALITY: TEMPERATURE, DISSOLVED OXYGEN, TURBIDITY

Taking water quality measurements is a necessary step in the protocol because temperature and water quality are important to the overall health of rivers and aquatic ecosystems. The water quality data can be directly correlated to the potential of the rivers food web. When water quality declines, major food web disturbances can be seen that are extremely detrimental to the health of the river’s aquatic ecosystem (Smith 2009). Temperature is an important variable because it is a main determinant of the metabolism and thus production of aquatic species (Caissie 2006). The water quality data will also be used to determine if the river is meeting the minimum criterion for water quality standard set forth by the San Joaquin River restoration (SJRRP 2010).

During each sampling period we will take point measurements to determine the temperature, dissolved oxygen (DO), and turbidity. The water quality data will be sampled at the same time as the drift net sampling. We will take the temperature, DO, and turbidity measurements at three transects (Transect 1, 6, 11), at three sample locations (25, 50, and 75% of the transect), and at two different depths (20 and 60% of the waters depth). The DO and temperature will be recorded using a handheld DO meter (YSI Dissolved Oxygen Instrument; Model 550A, Yellow Springs, Oh) and the turbidity will be measured using the portable turbidity meter (Model 2020 Turbidity Meter, LaMott Company, Chestertown, MD).

Water quality instruments will be place at each study site to continuously record the water temperature and light (Hobo® Pendant, Onset Computer Cooperation, Bourne, MA), and perhaps dissolved oxygen. We will place these instruments at organic matter collection locations. This will give us needed spatial and temporal water quality data, while also enhancing the organic
matter (periphyton) sampling data. These continuous data loggers can be downloaded in the field by using a waterproof shuttle (Hobo® U-DTW-1, Onset Computer Cooperation, Bourne, MA), thus allowing the devices to continuously take samples. Along with the point and continuous water quality data, we will utilize data from USGS and CDWR river gauge stations, to confirm and strengthen the field data collection as previously mentioned in the hydrology section.

2.1.2.3 WATER DEPTH FLUCTUATION

Pressure transducers will be rotated among study sites. The transducer will be in a stationary location; secured to the substrate of the river. When placing the transducer at the selected site, we will take a depth measurement for the initial data reading. Installation of pressure transducers will be according to the manufacturer’s specifications. We will download this data at the same time the continuous temperature and DO readings are downloaded. The output data of the pressure transducers will be compared to the two-dimensional modeling data to determine if the models are consistent with the observed pressure reading.

2.1.2.4 TOPOGRAPHIC CHANNEL BATHYMETRY AND TWO-DIMENSIONAL MODELING

TWO-DIMENSIONAL MODELING

Using the data obtained from the physical habitat sampling we will create a two-dimensional model of the rivers hydrodynamics. This model will be obtained by using methods adapted from Steffler and Blackburn (2002). The two-dimensional analysis will make it possible to model the study depth, flow, sediment transport, and water quality over a range of flow regimes. This modeling can help determine the rivers habitat suitability over different flow (Steffler 2002).

TOPOGRAPHIC CHANNEL BATHYMETRY

Bathymetry will be mapped using a combination of light and radar (LiDAR) remote sensing data from the Bureau of Reclamation and bathymetric surveys collected by our team. LiDAR data will be merged with points to generate a TIN surface with a vertical accuracy of 0.05 - 0.5 meters. Average point densities across the site will range between 0.5 - 2.0 m depending on topographic complexity.

Consolidated topographic data sets will be input into the bed file creation and substrate attributes collected via pebble counts will be converted to roughness values as described by Gard (2006). We will use water surface elevations and flow transects to calibrate the model and river hydrodynamics that will be simulated for a range of inundation under hypothesized restoration flow conditions. For the analysis, we will select three flow levels (i.e., low, moderate, and high relative to mean depths and velocities) to evaluate changes in juvenile rearing habitat conditions within the main channel from February through June (juvenile Chinook rearing period).
2.2 ORGANIC MATTER (FINE PARTICULATES & PERIPHYTONT)

2.2.1 BACKGROUND:

Organic Matter from primary producers is found in one of two forms, as fine particulate organic matter (FPOM) and periphyton. FPOM can be further classified into two forms: Fine Benthic Organic Matter (FBOM), which has been deposited onto the substrate, and seston, which remains suspended in the water column. Periphyton is a matrix of biotic and abiotic material usually comprised of a combination of algal, bacterial, fungal, and microzoan colonies. Periphyton is found attached to substrates in most aquatic systems (Hauer 2011).

Primary producers are generally the primary source of organic matter comprising the base of aquatic food webs (Hauer 2011). The methods below explain the proper selection, sampling, cataloging and processing procedures for each of these three forms of organic matter in likely habitats for juvenile Chinook in both floodplain and mainstem reaches.

2.2.2 COLLECTION METHODS:

Sampling for organic matter within a reach will occur at each discreet sample site (25%, 50%, 75%) along the 1st, 6th and 11th of the 11 transects of the sampling reach). However, because non-null values on edges will be required for appropriately mapping the reaches, if either the 25% or 75% sample sites are also too deep or aphotic researchers will move along the transect to the first spot at which light at the substrate is >1% of surface light. These will become the new permanent discreet sample points for the transect.

2.2.2.1 FINE BENTHIC ORGANIC MATTER

Researchers will pre-label a Whirl-Pak bag for each FBOM sample following the cataloging protocols in Section 1.

If water is shallow enough (~30 cm), researchers will place a bottom-less five gallon (19L) bucket onto the substrate slowly so as not to disturb the substrate. Once a seal has been made against the substrate, with a quick, twisting motion researchers will dig the bucket into the substrate to a depth of 13 cm. Once the bucket is firmly in place, researchers will remove any large debris, including cobbles, and organic debris from the area inside the bucket, shaking them inside the water to ensure that any fine particles deposited on them are suspended in the water. They will then measure and record the depth of the water in the bucket in centimeters.

Next, using a hand rake, they will stir into the substrate confined by the bucket to a depth of 5 cm for 1-minute; this will suspend any FBOM in the water column. While the FBOM is suspended, an approximately 500-mL grab sample will be taken in the appropriate pre-labeled Whirl-Pak bag. Samples will be stored in a cold dark place (cooler full of ice) for transport back to the lab. Processing of samples will be completed in the lab using the Processing Procedures (Section 2.2). Processing should be done as soon as possible, preferably day of sample collection.
Sampling for FBOM can also be accomplished using the gravel coring method from Moulton (2002) (pg. 19) to facilitate sampling at depths greater than the height of the 19L bucket.

2.2.2.2 SESTON (ADAPTED AND MODIFIED FROM LAZORCHAK (2000))

Seston and drifting invertebrates will be sampled concurrently using a modified dual plankton net (500μm mesh) (MDPN) assembly(Figure 2.5) and will occur at the 25%, 50% and 75% sampling stations on the 1st, 6th, and 11th transects of the reach.

Researchers will pre-Label Whirl-Pak bags following the cataloging protocols in Section 1; 18 bags per reach. Depending on the depth of the River at sampling, they will either wade or use a craft on the DOWNSTREAM side of the transect cable. At the 25% sampling mark, researchers will measure the depth from the surface of the water to riverbed. Then they will adjust the MDPN, such that the distance from the bottom of the platform to the bottom of the second net is 20cm less than the total depth of the river at the sample site. They will record the starting reading of the flowmeter (Flowstart). Then they lower the MDMN into the water at the 25% width mark and secure in place to the transect wire. Researchers will set a stop watch to begin timing for 10 minutes. After 10 minutes, they will raise the assembly. They will record the final flowmeter reading for each net. Using stream water on the outside, they will rinse any matter down the sides of the nets, being careful not to pour any water into the inside of the net. They will ensure each collection jar contains at least 500 ml of water. Unscrewing the collection jars from the end of the nets, researchers will pour the two samples into the whirl-pak bag labeled for appropriate sample. These steps are repeated for all sample sites. The samples will be stored in a cold dark place (cooler with ice) for transport to the laboratory for processing. Processing should be done as soon as possible, preferably day of sample collection.

FIGURE 2.5 THE MODIFIED DUAL MILLER NET DESIGN SHOWING A) FRONT PROJECTION, B) SIDE PROJECTION, AND C) TOP PROJECTION.
Quantitative Periphyton will be sampled from 56 cm² clay tiles installed at the 25%, 50%, and 75% sampling sites on the 1st, 6th, and 11th transects. Qualitative samples for SIA will be taken from natural substrates at each sampling site.

Clay tiles will be washed for 48 hours in a 2N H₂SO₄ solution prior to installation. Next nine clay tiles will be installed onto one masonry unit at each sampling site using zip ties. Before going into the field researchers pre-label whirl-pak bags according to the cataloging procedures in Section 1. Each discreet sample site will require three tile samples: one for chlorophyll-α, one for ash-free dry mass, and one for stable isotope analysis. Bags are labeled according to the protocols in Section 1. Each discreet sample will also require one natural qualitative periphyton sample. This will be removed from the natural substrate using one of two methods depending on substrate size.

At each sampling site researcher will fill the appropriately labeled whirl-pak bag for each quantitative sample (chl.-α, AFDM, SIA) with 500 ml of filtered water. They will then remove a tile from the matrix of tiles on the block and place it into one of the whirl-pak bags for each quantitative sample. Whirl-paks will be stored in a cold dark place (cooler with ice) for transport to the laboratory for processing. Processing should be done as soon as possible, preferably day of sample collection.

Depending on substrate type one of the two methods will be used to sample periphyton from natural substrates.

For cobble dominated transects, researchers will select three good sized relatively flat, exposed cobbles. They will place the cobbles in a pan with 500 mL of water. They will scrape off the periphyton growth from the light-exposed side of the cobble and into the water in the pan. Once all the periphyton from the side has been scraped, researchers will use aluminum foil to cover the scraped part of the cobble, attempting to get the foil as flat as possible. Researchers will use scissors to trim the aluminum foil to exactly match the scraped area of the cobble. These procedures are repeated for each cobble, rinsing each one in the same pan to form an aggregate sample. They will place trimmed aluminum foil in enveloped labeled identically to the Whorl-Pak. They will pour the slurry of periphyton from pan into the whirl-pak, making sure to get all of the organic material. Store the whirl-paks in a cold dark place (cooler with ice) for transport to the laboratory for processing. Processing should be done as soon as possible, preferably day of sample collection.

For fine sediment area, researchers will use the modified PVC gravel core sampler (modified from Moulton 2002) to sample three locations near the block with mounted tiles. The PVC core sampler will be submerged into the substrate until the substrate reaches the threads on the inside. They will then carefully tighten the cap to create a seal. Researchers then insert the flange to cut off the first 5cm of substrate. Researchers will lift the core sampler out of the water and release the sample into a pan filled with 500 ml of water. This will be repeated three times, aggregating all samples into the pan. Researchers will suspend periphyton in the pan by carefully agitating and swirling the pan. Then they will quickly pour the water with the suspended periphyton into the pre-labeled whirl-pak bag. Researchers will store the whirl-paks in a cold dark place (cooler with ice) for transport to the laboratory for processing. Processing should be done as soon as possible, preferably day of sample collection.
This project will use a semi-quantitative benthic macroinvertebrate sampling method (Moulton 2002). This sampling method will help us relate our invertebrate data to the overall production in the mainstem study area.

The collection of benthic macroinvertebrate (BMI) samples in our study reaches will use methods that are adapted from Ode (2007). The sampling in Ode (2007) was created to use the same transects which are used to define the physical characteristics of the river. By using the same transects as the physical habitats we can get a more complete view of the benthos in the river without targeting specific substrate or habitat (Ode 2007). We will next employ a subsampling method that will use the same three transects as the periphyton, seston, and FBOM measurements; which allows for correlations to be assessed.

We will take measurements at three transect (Transect 1,6,11) and two sample locations on those transect (25 and 75 percent of the transect) (Figure 2.6). We will make sure to sample from the downstream to the upstream transect, in order to prevent macroinvertebrates from flowing onto transects that have yet to be sampled (Ode 2007).

In the event that one of our BMI sampling locations falls on a spot that is either deeper than 100 cm or the light-meter at the substrate reads less than 1% of surface light, then the BMI sampling will be moved to the nearest spot towards shore that has 1% or greater light that reaches the substrate. This new sampling location must get a percentage marker to be factored in when doing the data analysis. To get this percentage the researchers will use the formula given below (Equation 2).

\[
X - D / W = \text{New Sample Location} \\
X = \text{Distance from shore} \\
D = \text{Distance moved from } X \\
W = \text{Width of the river}
\]

Once we have identified the first point at 25 percent of the transect width we will use a D-frame net (Figure 2.7) which is the most commonly used gear for lotic macroinvertebrate sampling across the united states monitoring agencies (Carter and Resh 2001). The D-frame kick-net that will be used has a width of 30.5 cm (12 inches), height of 25.4 cm (10 inches), and a 500 μm mesh net to collect macroinvertebrates. D-frame net capture methods will produce a 30.5 cm² sample area of the riverbeds substrates at each sample location, totaling 1.83 m² per reach (Ode
To easily define the area in which will be sampled, a PVC pipe guide (30.5 cm x 30.5 cm) will be lowered to the substrate to be used as a template. This PVC guide will help outline the area in which we will sample (Moulton 2002). In this defined sample area we will now begin the sampling procedures. We will start by grabbing larger organisms and placing them into the D-net. Next we will use our hands to grab larger substrate (i.e. cobble, gravel, pebbles, etc.) and rub the substrate in front of the D-net opening (if flow allows), thus any BMI will flow into the net. After the smaller substrate has been rubbed into the D-net we will proceed to use our hands, feet, or hand-rakes to dislodge any remaining BMI. While doing this procedure continue dragging the D-net through the sample area. If the D-net is kept moving through the sample area during this procedure more organisms will be collected and the BMI which has already been collected will not escape. This portion of the BMI sampling should last for 30 seconds. Once we have completed this last step they should pull the D-net out of the water quickly, with the opening always pointed upstream (to keep BMI from flowing out of the net). Once out of the water we will wash the samples to the bottom of the net with a water bottle. Transfer the sample from the D-net to a 500mL jar by inverting the D-net into the jar. This should only be done over a white tray in order to make sure nothing is lost during the transfer. Once a majority of the sample has been moved to the jar, use forceps to get any organisms left in the net. Large debris such as rocks and twigs can be removed while in the field, however every item that is removed must be inspecting for BMI before being discarded (Ode 2007).

When transferring the BMI from the nets to the sample jar, never fill the jar more than two-thirds of the capacity. After the sample has been completely transferred, add 95% ethanol (EtOH) to the jar to preserve the organisms. This process of only filling the jars about two-thirds of the total capacity ensures the ethanol will cover the sample completely, preserving all the organisms for laboratory sampling on a later date (Ode 2007). The jar must be labeled using the labeling technique found in the Data Labeling section.

Trophic efficiency between fish and invertebrate prey can be influenced by factors such as the distribution of prey sizes in addition to overall prey biomass and taxonomic composition for example. We will therefore analyze the size distribution of all invertebrate prey across the main-stem study reaches, using normalized size spectra (Blumenshine et al. 2000) and the Pareto distribution (Moore 2011). After all the data has been inventoried the benthic invertebrate numbers along with density will be standardized to an aerial basis (m⁻²) (e.g. Smock 2006).

2.3 LAB PROCESSING METHODS

2.3.1 SECTION 1. CHLOROPHYLL-A AND PHAEOPHYTON ANALYSIS

Chlorophyll-a and Phaeophytin densities (µg/cm²) will be analyzed using modified EPA Method 446.0 (Arar 1997), summarized here.

All laboratory-processing procedures should be conducted in subdued lighting to protect the chl.-a and phaeophytin samples.

Filtration Prior to Extraction
Prior to extraction, samples labeled for chlorophyll-a and phaeophytin analysis will be filtered onto clean Whatman GF/F filters (pore size = 0.7µm). Each sample will be re-suspended and
split into two equal aliquots. Each aliquot will be filtered using the filtration specifications found in Section 8.0 of EPA Method 446.0. If necessary, we will isolate, label, and store filters in a dark freezer set to -20°C.

**Extraction of Chlorophyll-a and Phaeophyton-a**

We will calibrate the spectrophotometer using 95% EtOH, this has a higher extraction rate than that of 90% acetone and will be used in place of acetone in all procedures. Following calibration, samples will be extracted and analyzed following the methods detailed in Arar 2007 (Sections 11 and 12). Post daily extraction, a reagent blank will be filtered and extracted to determine if there is any laboratory contamination and to provide a control for analysis of results. Results will be analyzed according to the methods described in 12.2 to correct for phaeopigments.

### 2.3.2 BIOMASS AND IDENTIFICATION PROCESSING METHODS

#### 2.3.2.1 FBOM BIOMASS PROCESSING

In the lab, re-suspend the 500-mL grab sample and pour 100mL through a 1mm sieve and then filtered onto pre-ashed, pre-weighed (MassF) Whatman GF/F glass fiber filters (pore size = 0.7μm). Retain the remainder of the sample for SIA analysis and chl-a analysis. Filters are then dried at 70°C for 24 hours. After drying, cool filters in a desiccator and weigh to the nearest 0.1mg, and record under pre-combusted weight (MassNC). Place dried and weighed filters into muffle furnace at 500°C for an hour. Samples are then cooled, rewetted, and re-dried at 70°C for 24 hours. After re-drying, cool in a desiccator and then weigh and record the new weight under post-combusted weight (MassPC). Determine and record Percent of Organic Mass (%organic) by ignition by dividing the difference in masses (Massorganic) by the total mass. Next determine the volume of the water sampled from within the bucket by using the formula $V_{bucket} = (531cm^2)h$, where $531cm^2$ is the area of the inside of the bucket, and $h$ is the depth of the water inside the bucket. The total amount of FBOM (mg/cm²) is determined as follows:

$$\%_{\text{Sample by Volume}} = \frac{100mL}{V_{bucket}}$$

$$\text{Amount}_{\text{Organic}} = \frac{\text{Mass}_{\text{Organic}}}{\%_{\text{Sample by Volume}}}$$

$$\text{Amount}_{\text{Total Organic}} (mg/cm^2) = \text{Amount}_{\text{Organic}}/531cm^2$$

$$\text{Amount}_{\text{Inorganic}} = \frac{(\text{Mass}_{\text{PC}} - \text{Mass}_{F})}{\%_{\text{Sample by Volume}}}$$

$$\text{Amount}_{\text{Total Inorganic}} = \frac{\text{Amount}_{\text{Inorganic}}}{531cm^2}$$

**EQUATION 2: FORMULA USED TO DETERMINE DENSITIES OF ALGAL MATERIAL**

#### 2.3.2.2 SESTON BIOMASS PROCESSING

In the lab, separate the invertebrates from the seston by pouring the sample through a 250μm mesh. Rinse the mesh with filtered water to ensure all the seston has passed through the mesh. Invertebrates should be placed into a jar of 95% EtOH and labeled appropriately as drifting invertebrates to be processed appropriately. Split the remaining seston sample into three aliquots, one for mass sampling, one for SIA analysis, and one for chl-a analysis. Using the methods described above for FBOM processing, determine the %organic and %inorganic matter and total sampled density (mg/L).
2.3.2.3  PERiphyton Biomass Processing

In the lab, process samples labeled for biomass analysis using the methods described above for FBOM, resulting in mg/cm² sample densities.

2.3.2.4  Invertebrate Biomass Processing

In the lab, samples obtained from both benthic and drift environments will be processed separately, but in the same fashion. Using keys from Merritt and Cummins (1996) sort, identify, and enumerate all organisms to the lowest taxonomic level with the following exceptions:

- Chironomidae identified to Subfamily
- Non-Insects identified to Family or Order

During identification, record the length of each individual for use in length mass regression analysis. After identification, enumeration and length measurements are taken for all individuals from a sample, average all lengths for each taxon. Then, for macro-invertebrates, using the length-mass regressions from Benke et al. (1999) determines the average mass of each taxon. After this process has been completed, see Section 2.2.2ii for further processing for SIA.

2.3.3  Section 3. Stable Isotope Analysis (Adapted From Heady (2013) And Berto (2013))

2.3.3.1  Stable Isotope Analysis for FBOM, Seston And Periphyton

The UC-Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/) will conduct all stable isotope analyses. Sample preparation includes prior to transport to the UC-Davis SIF include:

- Re-suspend samples of each type of primary producer, filter 150ml of each sample onto pre-combusted Whatman GF/F filters (pore size = 0.07μm), place into pre-labeled vials with the correct sample number. Filtered samples will be stored at -20°C in the dark before sending to UC-Davis for analysis.

2.3.3.2  Stable Isotope Analysis for Invertebrates

Analysis by the UC Davis SIF will performed separately on both benthic and limnetic invertebrates (protocols in Appendix A). After the sorting, classification, and measuring has been done (Section 2.2.1.iv), individuals from a single sample are added to a washed and dried mortar with small chunks of dry ice (CO₂), then thoroughly ground to lyophilize the samples and create a fine lyophilized powder. The lyophilized powder is transferred to 5mm x 9mm tin capsules until target mass (0.7 ± 0.05mg) is reached. Sealed capsules are placed in the cells of a 96-well cell culture plate.
3 RESULTS, ANALYSIS, AND DELIVERABLES

3.1 FOOD WEB RESOLUTION

Following SIA analyses, the food webs between primary producers and consumers will be established. Due to limits in sampling, juvenile Chinook will not be present in sampling results and therefore cannot be present for foodweb analysis. However, the eventual resolution of juvenile Chinook food-webs in both mainstem and floodplain habitats is at the center of these food-web resolutions.

3.2 PREY HABITAT SUITABILITY INDICES

A Habitat Suitability Index (HSI) will be developed using the two-dimensional modeling and results from organic matter sampling. As stated earlier this two-dimensional modeling takes into account depth, flow, sediment types, and water quality. We will use these models to determine the HSI for the key prey species in our study site, as determined by established prey preferences (Rondorf 1990, Tiffan 2014, Chittaro 2014). The HSI will help determine how the key prey species will react in different flow regimes. These models are necessary because the distribution and abundance of the key prey species are affected by any shifts in hydrology or morphology of the river. This data will be important to further compare the mainstem sampling to the floodplain habitat in subsequent studies (Wu 2006).

3.3 JUVENILE SALMON HABITAT SUITABILITY INDICES

The current juvenile Chinook indices for the San Joaquin River as found in the Minimum Floodplain Habitat Area Study (SJRRP 2012) contain values for depth, velocity, and cover. Following HSI development for each prey item, we will combine them with the HSI for juvenile Chinook, which was developed by the San Joaquin River Restoration Program, to create new suitability values which will incorporate prey production and availability. The combination of both habitat and prey values will allow us to score individual cells within the river based on estimated invertebrate production as well as habitat suitability will aid in more robust bioenergetic modeling (Kawai 2014) and river restoration efforts.

3.4 BIOENERGETIC MODELS

Following all of the above, bioenergetic models can be resolved to produce extremely robust models of juvenile Chinook growth and survival within areas. Such models will be able to be used in comparing floodplains and mainstream habitats relative to their ability to support juvenile Chinook rearing. These models will also allow restoration managers to direct restoration towards areas that are bio-energetically unfavorable, in an effort to produce high spawn to adult survival ratios.
3.5 DATA ANALYSES

The inclusion and replicability of independent variables will be contingent on site selection and hydrology. Therefore, specific hypotheses and models will be developed as the environmental conditions of the first study year become apparent. The current plan is to explore sources of variation and pattern in the data using suitable methods such as ordination and regression trees. These exploratory methods will allow for the treatment of issues such as collinearity and autocorrelation that will greatly improve our ability to construct a viable set of models that can be evaluated with model comparison approaches.

The tasks include variables that are not necessarily independent (e.g. water depth & velocity) or univariate. In these cases, we will apply multivariate analyses such as constrained ordination (‘response’ variables with ‘environmental’ variables) and Mantel tests, which would apply to how food web structure varies with environmental variables for example.

Under the current plan, sites will be random factors and sampling events (e.g. early & late) will be fixed effects. The methods specify the numerous dependent variables which can be analyzed independently or in subsets such as through direct gradient ordination with standardized response variables, when response units are continuous variables with different units. Variables such as water depth, velocity, temperature, etc. will be used as covariates in or independent gradients depending on the specific hypothesis.
REFERENCES


Appendix A: Stable Isotope Methodology: UC-Davis Stable Isotope Facility

(http://stableisotopefacility.ucdavis.edu/13cand15n.html)

Carbon (\(^{13}\text{C}\)) and Nitrogen (\(^{15}\text{N}\)) Analysis of Solids by EA-IRMS

The SIF provides \(^{13}\text{C}\) and \(^{15}\text{N}\) isotope analyses of solid materials, such as soils, sediments, plant and animal tissues, etc., using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS). We analyze both \(^{13}\text{C}\) and \(^{15}\text{N}\) in the same sample, or we can use a CO\(_2\) trap and measure \(^{15}\text{N}\) only in the case of very low N materials like wood.

Analysis

Most solid materials (excluding glass fiber filters, soils, and sediments – see below) are analyzed for \(^{13}\text{C}\) and \(^{15}\text{N}\) isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples are combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides are removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flows through a water trap (magnesium perchlorate) and an optional CO\(_2\) trap (for N-only analyses). N\(_2\) and CO\(_2\) are separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS.

Glass fiber filters, soils, and sediments are analyzed for \(^{13}\text{C}\) and \(^{15}\text{N}\) isotopes using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples are combusted at 1000°C in a reactor packed with copper oxide and lead chromate. Following combustion, oxides are removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flows through a water trap (magnesium perchlorate). N\(_2\) and CO\(_2\) are separated using a molecular sieve adsorption trap before entering the IRMS.

During analysis, samples are interspersed with several replicates of at least two different laboratory standards. These laboratory standards, which are selected to be compositionally similar to the samples being analyzed, have been previously calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). A sample’s preliminary isotope ratio is measured relative to reference gases analyzed with each sample. These preliminary values are finalized by correcting the values for the entire batch based on the known values of the included laboratory standards. The long term standard deviation is 0.2 permil for \(^{13}\text{C}\) and 0.3 permil for \(^{15}\text{N}\).

The final delta values, delivered to the customer, are expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and Air for carbon and nitrogen, respectively. For information on delta notation and the international references, please refer to a stable isotope reference such as Sharp, Z. (2005) *Principles of Stable Isotope Geochemistry* (Prentice Hall).