

Temporal and Spatial Snapshots of Brown Trout and Rainbow Trout Pescivory and Diet Composition during June -December 2000 in Grand Canyon Corridor of the Colorado River

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Introduction

Non-native brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) have traditionally been targeted as one of the direct causes for population declines in native fish of the Colorado River in the Grand Canyon (Minckley and Deacon 1991). Biologists have often assumed that non-native salmonids are major predators on the endangered Humpback chub (*Gila cypha*), yet little quantitative data exists demonstrating reduced native fish populations are the result of predation by *S. trutta* and *O. mykiss*. Documented cases where salmonids in the Colorado River have native fish remains in their stomach contents exists (Valdez 1993, Valdez and Ryel 1995), however most of these data have inadequate samples sizes, and are piecemealed together from different studies (Valdez and Ryel 1995). Even though the implications of predation on *G. cypha* are great, there has been little effort to specifically design and implement long-term studies to investigating predation on *G. cypha* and other native fish in the Grand Canyon over time and space.

The objectives of this pilot study are two-fold: 1) to develop a methodology to estimate the percent of piscivory on native fish (specifically *Gila cypha*) that occurs both spatially and temporally by *S. trutta* and *O. mykiss*; and 2) to develop a methodology to monitor temporal and spatial variation in the diet of both *S. trutta* and *O. mykiss*. These data are essential to the development of a larger understanding of the bioenergetics of Colorado River fishes in Grand Canyon. The products of this study are a preliminary analysis of diet data from *s. trutta* and *O. mykiss*, methodologies to continue collecting data on predation of native fish and non-native diets, as well as further recommendations on how to improve data collection, analysis and design of future studies on piscivory and diet.

Methods

During the months of June, July, August, September, and December 2000, two non-native fish (brown trout and rainbow trout) in the Colorado River, were collected for stomach content analysis. Fish were captured by electrofishing sweeps along the Colorado River corridor (river mile 18-225) by Arizona Game and Fish Department. Stomachs were catalogued, preserved in formalin or ethanol and stored at the Grand Canyon Monitoring and Research Center. .

Two reaches priority reaches were established based on estimates of native and non-native fish densities (AGFD 2001), assuming that increased predation of native fishes is more likely to occur where higher densities of prey occur. These reaches included a Little Colorado River reach (LCR, river mile 55-75) and a Bright Angel Creek reach (BAC, river mile 85-105). Priority reaches were chosen based on their proximity to well-established populations of Humpback chub (*Gila cypha*) and relative estimates of

populations both non-native trout according to recent studies conducted by Arizona Game and Fish (AGFD 2001). The assumption made when picking these two priority sites were that the LCR reach, designated critical habitat for humpback chub, has a higher *Gila cypha* availability than the downstream BAC reach. Additionally *O. mykiss* abundance is relatively high in the LCR reach (>5000 individuals/mile) conversely; *S. trutta* is more often around the BAC reach according to recent studies by AGF, 2001. (Ten random subsamples were selected from these reaches for detailed analysis of stomach contents for both species during each trip. In some cases there were less than ten samples to choose from, at which time, all samples were taken for detailed analysis. The rest of the stomach samples taken from priority reaches were analyzed specifically for any fish remains.

Pescivory analysis

Stomachs from 395 fish (154 *S. trutta* and 241 *O. mykiss*) from the LCR and BAC reaches were surveyed for fish prey items to estimate percent pescivory. All formalin from stomach samples was poured off in to a toxic waste container. Fish stomachs were rapped in a fine mesh to ensure that water could flow through and that formalin was able to leach out, while keeping all stomach material within. The wrapped fish stomachs (with data tags) were soaked in water for >24hrs with continual water exchange. After most formalin had sufficiently been leached out, fish were taken out of the water and stored in ethanol until dissection. Preparation and analysis of each stomach was 3 day process, although many samples could be prepared at the same time. While dissecting fish stomachs, samples were stored in leaching bucket with continual water exchange. This method reduces noxious fumes while dissecting fish stomachs.

Stomachs were cut open and contents were sorted looking for fish carcass or bones. All fish remains found were preserved for later identification. After all stomachs were surveyed, any fish remains found were carefully sorted under a dissecting microscope. Many of the fish remains were moderately to completely digested (only bones present), therefore they had to be dissected or sorted to find the gill arches (if present) for more positive identification. Gill arches of Cyprinidae (minnows) will have 3-5 well-developed teeth in comparison to Catostomidae (suckers), which will have a row of fine teeth, much like a comb (Minckley 1973, p.37). I used detailed species descriptions (Minckley 1973), plus bones I took from humpback chub and flannel mouth sucker as references. Other key features that were used for identification included the relative size of the peduncle, size of fish scales, and position of mouth. When analyzing data for temporal differences August data was lumped with the September data because of such close proximity of sampling dates. Temporal and spatial differences were analyzed using Kruskal-Wallis and T-tests.

Detailed Stomach Analysis

Since detailed stomach content analysis is time intensive a subsample of Ten fish stomachs of each species from the LCR and BAC reaches were taken from each sampling trip (June, July, August,

September, and December 2000 and examined for food items. Stomachs were soaked in water for > 24hrs as explained above.

Fish stomachs were dissected in two parts. A lateral incision was made from the esophagus to the end of the stomach (sphincter muscle). The contents of the first half of the stomach were sorted separately from the second half of the stomach contents. Data from the two different sections were combined for statistical analysis, but the separation of the anterior and posterior sections of the stomach may easily lend insightful information about relative rates of digestion of different prey items and/or temporal patterns of feeding if capture time is recorded.

Food items were broken up into categories (fish, *Gammarus*, simuliids, chironomids, gastropods, terrestrial insects, algae, algae/detritus, parts of diptera larvae). These categories were established based on relative abundance found in stomach samples and categories established by previous monitoring studies estimating food base in the Colorado River (Blinn et al 1993, Valdez and Ryel 1995). Any fish prey found in these detailed stomach contents analysis was not included in percent mean weight comparison of stomach contents, because its relative weight is much larger than all other food items and it skewed the data considerably. Although all fish prey items were reported in the piscivory analysis. All identifiable and intact invertebrates were counted. Head capsules of larvae were lumped into parts of diptera and not counted, due to possible differential digestive rates being slower for hard parts in comparison to soft parts of larvae, which may skew data. Algae included macrophytes and some colonial algae. The algae/ detritus category was composed of unidentifiable biotic material and small unicellular algae matrix. Any fish remains found were carefully rinsed and stored in ethanol for later identification. Sorting of all stomach contents was done with a Leica dissecting microscope using at least 10X optics. To examine proportional contents, wet weights and volumes were taken of stomach contents using a mettler balance and graduated cylinder. Due to the small numbers of some of the categories and relatively small amounts, only counts and wet weights were consistently taken. When analyzing data for temporal differences August data was lumped with the September data because of such close proximity of sampling dates. Count data for food items were not used in these statistical tests. Temporal and spatial differences among food item wet weights were analyzed using Kruskal-Wallis and T-Test respectively. Data were analyzed using Jump in statistical package.

Results

Piscivory analysis

Piscivory occurred in 8% of the totaled 154 brown trout sampled. Piscivory in brown trout was found significantly more often ($p < 0.006$) in the LCR reach (21% out of 33 individuals) in comparison to BAC reach (5% of the 126 ind.). Additionally, there was some variation of observed piscivory over the different months ($p < 0.08$). December was the month with the highest frequency of observed piscivory (Table 1). Piscivory in rainbow trout was considerably less. Out of a total of 241 rainbow trout stomachs collected, only 1 small, unidentified fish larva was found from a fish collected at 64 mile in late August 2000.

The fish prey composition consisted of unidentifiable remains (bones) (1.9% of fish sampled), unidentified salmonids (1.9%), native catostomids (3.8%), and humpback chub (0.6%) (Table 1). In seven of the fish identified gill arches were retrieved and positively identified to family. In most of the cases if fish were present in the stomach, there was no other food item present in the stomach. Turbidity classifications were noted for some of the days that samples were taken (Table 1). There does not seem to be any clear pattern with the frequency of piscivory and water turbidity.

Accounts of *Salmo trutta* piscivory

Date	River mile	Turbidity	<i>S. trutta</i> length	Fish prey	prey wet wt.g	Arches saved
6/7/00	LCR 63	na	340	native sucker	14.1	yes
7/26/00	BAC 88	Low	396	native sucker	37.3	yes
9/1/00	BAC 85	High	365	unidentified	-----	no
9/3/00	BAC 93	High	326	salmonid	4.32	no
12/17/00	LCR 63	Low	403	native sucker	43.1	yes
12/17/00	LCR 63	Low	330	native sucker	35.6	no
12/17/00	LCR 64	Low	308	trout	7.5	no
12/17/00	LCR 65	Low	381	Humpback chub	9.62	yes
12/18/00	LCR 65	Low	492	native sucker	11.8	yes
12/21/00	BAC 88	Low	324	unidentified	-----	no
12/21/00	BAC 88	Low	424	unidentified	-----	no
12/23/00	BAC 98	Low	448	native sucker	50.8	yes

Table 1 . Accounts (date, reach, river mile, and water turbidity) of Salmon *trutta* piscivory from 154 individuals sampled. Reaches include LCR (Little Colorado River, river mile 55-75) and BAC (Bright Angle Creek, river mile 85-105) items are listed with wet weights if available, some prey items were completely digested and consisted of bones only, therefore weights were not applicable. If pharyngeal gill arches were retrieved from the prey item to help the identification, it was denoted.

Detailed Stomach Analysis

Stomach contents varied between reaches and over the seasons for both non-native salmonids. The most abundant food categories observed for *Salmo trutta*, included *Gammarus* (24% of food weight), simuliid larvae (20%), algae/detritus (17%), macro and microscopic algae (13%) and chironomid larvae (13%). Examination of *S. trutta* diet over the seasons showed a significant difference between the percent weight of snails ($p < 0.0001$), algae/detritus ($p < 0.0001$) and simuliid larvae ($p < 0.0001$) (see Figure 1). When comparing food items for *S. trutta* between the LCR and BAC reaches simuliid larvae were found significantly more often in stomachs of fish in the BAC reach (18%) in comparison to the LCR reach (4%, $p < 0.006$) (see figure 2).

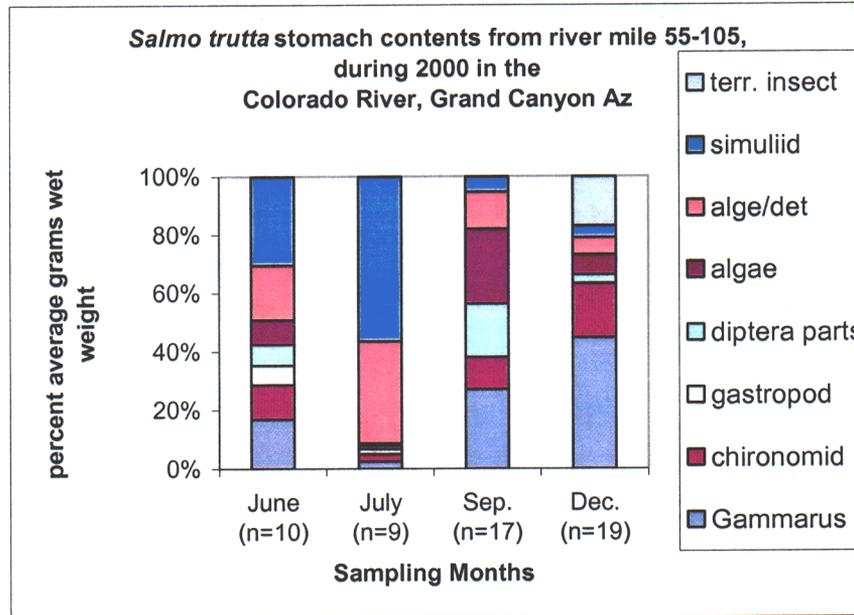


Figure 1. Percent of average grams wet weight of stomach contents in *Salmo trutta* during June 6-12, July 24-28, September (8/28 -9/5), December 16-23, 2000 from Colorado River, Grand Canyon, AZ, river mile 55-105

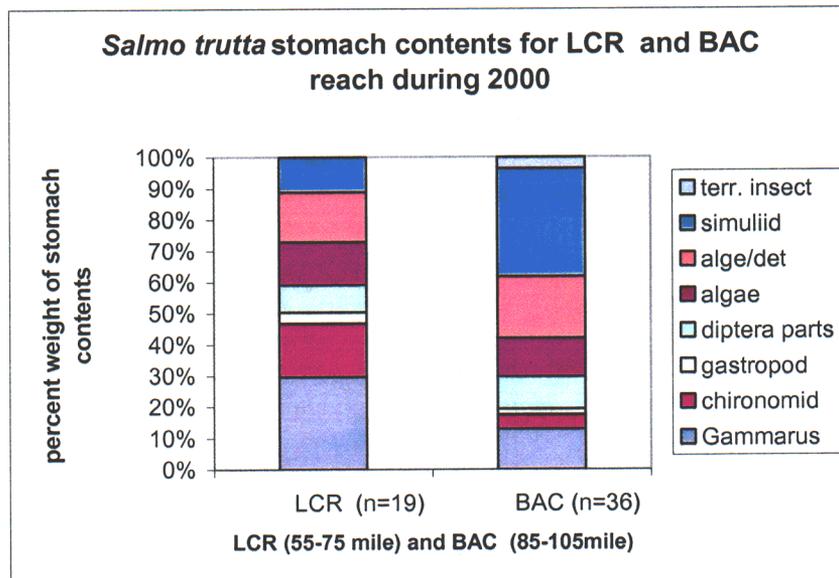


Figure 2. Comparison of percent of average grams wet weight of stomach contents in *Salmo trutta* between Little Colorado River (LCR) reach and Bright Angle Creek (BAC) reach of the Colorado River, Grand Canyon, AZ during June 6-12, July 24-28, September (8/28 -9/5), December 16-23, 2000.

The most frequent food sources for *O. mykiss* included simuliid (51% of stomach contents weight), algae/detritus (23%), terrestrial insects (18%) and *Gammarus* (3%). *Oncorhynchus mykiss* diet varied significantly over time in percent weight of stomach content of simuliid larvae ($p < 0.0001$). Simuliid larvae

are more often found in *O. mykiss* stomach contents during June (36% of stomach content weight), July (27%) and December (34%) in comparison to September (5%) (see figure 3). In addition, diet of *O. mykiss* varied significantly between reaches LCR and BAC in the following food categories: *Gammarus* (10%, 0.7%, $p < 0.02$) and chironomid larvae (6%, 0.6%, $p < 0.016$) (see figure 4).

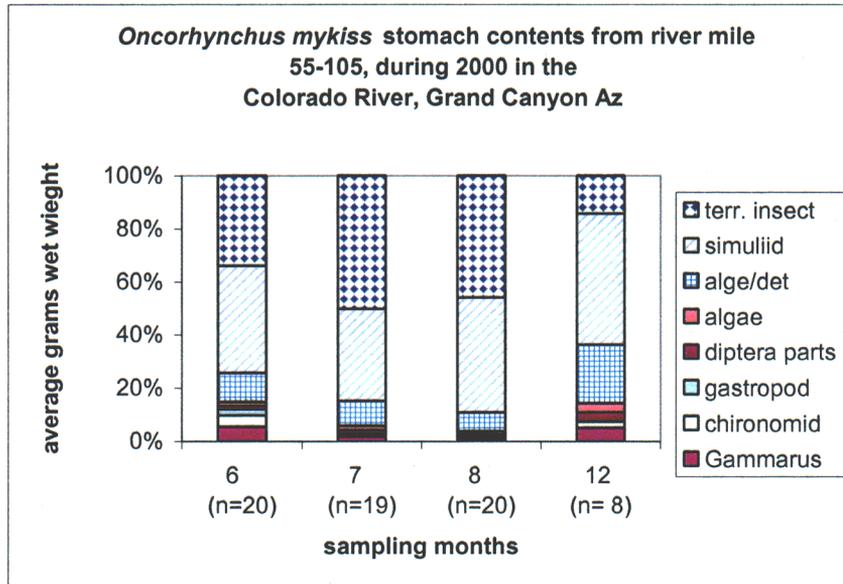


Figure 3. Percent of average grams wet weight of stomach contents in *Oncorhynchus mykiss* during June 6-12, July 24-28, September (8/28–9/5), December 16-23, 2000 from Colorado River, Grand Canyon, AZ, river mile 55-105

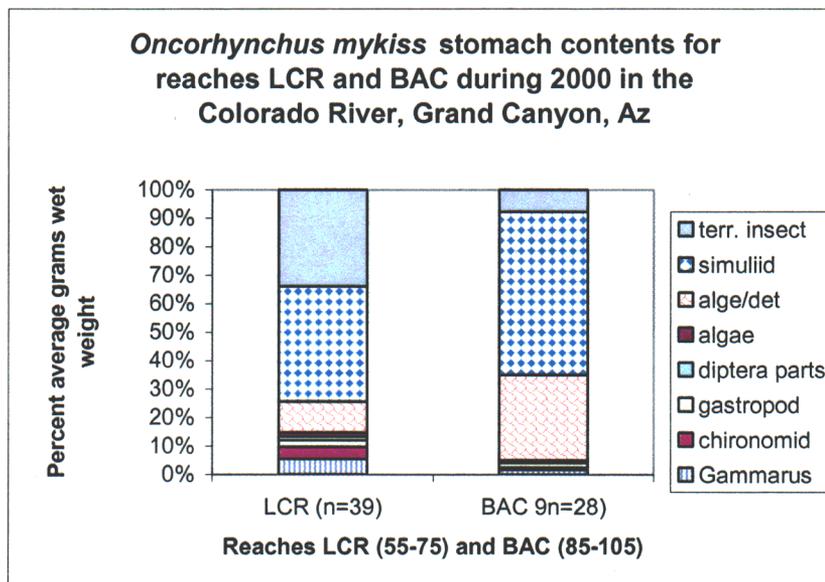


Figure 4. Comparison of percent of average grams wet weight of stomach contents in *Oncorhynchus mykiss* between Little Colorado River (LCR) reach and Bright Angle Creek (BAC) reach of the Colorado River, Grand Canyon, AZ during June 6-12, July 24-28, September (8/28–9/5), December 16-23, 2000.

Summary and Recommendations

This study provides evidence of *Salmo trutta* preying on native fish in the Colorado River, yet predation on *Gila cypha* is less than previously documented. Frequency of *S. trutta* predation on *G. cypha* for this study was considerably less (0.6% of 154 stomachs) than Valdez and Ryel 1995 reported (10.4% of 48 stomachs). Valdez and Ryel 1995 sampled non-native fish around the immediate area of the LCR mouth (dates of sampling, and methods for identification of digested material were not documented). Additionally, this study presents data that suggests that rainbow trout is not a direct threat of predation on *G. cypha* in contrast to common ideas. These findings are supported by the relatively few reports of humpback chub remains in *O. mykiss* stomach contents (*see references in Valdez and Carothers 1998, p129*). However, there is documentation of predation on *G. cypha* and other native fishes by other large non-native fish. **If a complete understanding of predation pressure on native Colorado River fishes is to be attained, the scope of sampling should be expanded to include: black bullhead catfish, channel catfish, striped bass, brown trout and rainbow trout.** This recommendation is based on previous studies that indicate almost any omnivore with a gape size large enough to ingest fish will if given the chance (Valdez and Carothers 1998).

A long-term study, monitoring the predation on native fish and diet of non-natives could provide useful information to managers, if it is carried out in a systematic and efficient way. **I suggest designating certain reaches of the river as priority sites as done in this study. Sampling should include at least 10 individuals of each non-native species for a particular date. Repeating this sampling during the spring, early summer, monsoon season and winter will give some idea of seasonal variability.** However, these are only snapshots of diet in a particular time and place. **Other tools for evaluating diet should be used in conjunction with stomach contents.** Stable isotopes (carbon, nitrogen and sulfur) can be useful in looking at important food sources over a lifetime. **If muscle tissue is taken from fish for isotopic analysis, it should be associated to a stomach sample to look at variability between the two diet analysis methods.**

All samples preserved should have a data tag with it that provides accessible information about the sample (date, time, river mile, collection gear, species, water turbidity, weight, length of fish, and id number). The stomachs collected in this study seemed to be of second priority, which was apparent when trying to organize the stored specimens and relate the electronic data to the collected samples. Approximately 99% of the samples had data tags associated with them, however the tags were rolled up and placed inside two whirlpacs, making the data completely inaccessible. When cataloguing and subsampling from the population of samples, each of the >1000 samples had to be reopened, data tags retrieved, and placed in a manner that it could be read from the outside of the whirlpacs. This sounds like a petty issue, but it took ~80 hrs (additional exposure to formalin) to complete. If the tags were unrolled and placed in a viewable position while in the field, this would save time. Additionally, about 5% of the samples did not include all the data specified. The electronic copy of data associated with the samples was often

inaccurate, with fish samples recorded that were not present and samples present that were not recorded. Columns of data were staggered down 3-4 rows in some places. **While human error is inevitable, having a quality control /data checker would help eliminate some of these issues.**

One of the most useful collections of data that should accompany this type of study is food item availability. Coordinating food base monitoring to coincide fish stomach collections would be very helpful in analyzing data and looking for interesting patterns in diet. Additionally, native fish abundances would be useful for fish prey availability data.

Data analysis for stomach contents composition can be more complicated when investigating multiple independent variables (spatial, temporal, abiotic conditions, and prey availability), which, can contribute to prey composition shifts. Picking key variables that may be responsible for diet shifts before the study is important to the study design. **Variables that may be of interest to managers include: Date, time, temperature, habitat (backwater, pool, riffle, cut bank), turbidity, river mile, collecting method, fish catch composition.** The method of quantifying prey items can influence results. Quantifying prey items (weight, volume, or counts) has shown to result in more reliable data in comparison to traditional point counts (rare, common, abundant), which is more subjective (Marrero and Lopez-Rojas 1995). **Recent studies indicate that to get the most information about patterns observed or not yet teased out, one should consider using Principle Component Analysis (De Crespín de Billy et al. 2000).**

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