Genetic Stock Identification of Chinook Salmon Harvest on the Yukon River 2008 and 2009

R&M# 08-08

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1. Introduction:

Summary:

Knowing the origin of Chinook salmon (*Oncorhynchus tshawytscha*) harvested in Yukon River fisheries is important for the successful management of these fisheries. Under the Yukon River Salmon Agreement between the United States and Canada, U.S. fishery managers are obligated to pass a specified target range of Chinook salmon into Canada. This target range is comprised of an escapement goal and a harvest share of the total Canadian-origin run. Monitoring the proportion of Canadian-origin Chinook salmon in fishery harvests from U.S. waters of the Yukon River represents an important tool for successfully meeting those obligations. Yukon River fisheries managers consider test fishery and commercial harvest numbers an important indicator of Chinook salmon run size for inseason management, and postseason genetic analyses have been very effective at distinguishing major stock components in the commercial catch since 2004. Past studies on stock compositions of the commercial harvest in Districts Y-1 and Y-2 have shown that the proportion of Canadian-origin fish may vary significantly within a season, with a contribution ranging from 25% to 69% of the harvest (Templin et al. 2006; DeCovich and Templin 2009). Since 2004, the stock composition of Chinook salmon harvests in the subsistence and commercial Chinook salmon fisheries of the Yukon River has been estimated by genetic stock identification (GSI) techniques based on a comprehensive baseline of DNA markers (Smith et al. 2005a; Templin et al. 2005; Beacham and Candy 2006; Templin et al. 2006a-b; Templin et al. 2008; DeCovich et al. 2010).

Two types of genetic markers, single nucleotide polymorphisms (SNPs) (Smith et al. 2005a; Templin et al. 2006b) and microsatellites (Flannery et al. 2006; Templin et al. 2006a,c; Beacham et al. 2008) have replaced the allozyme baseline developed in the 1990s (Beacham et al. 1989; Wilmot et al. 1992; Templin et al. 2005). The 2004 baseline of 9 SNPs assayed in 23 populations was increased to 17 SNPs (Templin et al. 2006b) and in 2008, the SNP baseline was augmented with additional populations and genetic markers and now consists of 27 populations and 52 SNPs. A subset of this baseline, consisting of all 27 populations and 42 SNPs, was used to provide the stock composition estimates reported in this study. The 42 SNPs used represent all polymorphic loci available for Yukon Chinook salmon populations.

This report describes the mixed stock analysis of the Chinook salmon test fishery catches and subsistence harvests in the U.S. portion of the Yukon River in 2009. We briefly describe the baseline used to analyze fishery samples, the simulations used to verify the accuracy and precision of estimated stock proportions, and report the results of the mixed stock analysis of fishery samples. The stock contribution estimates are provided for 3 hierarchical sets of reporting groups: 1) country of origin (U.S. and Canada), 2) broad scale (Lower Yukon, Middle Yukon,

and Canada), and 3) fine scale (Lower Yukon, Tanana, Upper U.S. Yukon, Canada Border, Pelly, Carmacks and Takhini).

Funding for this project was provided by three sources. The 2009 U.S. Fish and Wildlife Research and Management Fund, projects URM 08-09 and URM 08-08, provided funding to analyze samples from the subsistence harvests from Districts Y-1, Y-3, Y-4, and Y-5. Funds from URM 08-09 were originally intended to be used for analysis of commercial fishery samples from Districts Y-1, Y-2, and Y-5. Since no directed Chinook salmon fishery took place in 2009, these funds were used for the analysis of the subsistence fishery samples from District Y-5. The 2009 Restoration and Enhancement Fund of the Yukon River Panel, project 19N-09, provided funding for the inseason analysis of samples collected from the Lower Yukon Test Fishery (LYTF) and the test fishery associated with the Pilot Station sonar program. This was a pilot study to test the feasibility and application of inseason stock composition estimates for management of Chinook salmon fisheries.

Objectives:

The goal of this project was to provide estimates of the stock composition of the Chinook salmon catches in test fisheries, and harvests in commercial and subsistence fisheries on the Yukon River in 2009. To achieve this goal, the following objectives were to be met:

1) Sample individuals from each test, commercial and subsistence fishery in districts Y-1, Y-2, Y-3, Y-4 and Y-5 as follows:
    i. District Y-1 subsistence – 400 individuals
    ii. District Y-1 commercial (collected from chum salmon-directed fishery) – 200 individuals per period
    iii. District Y-2 commercial (collected from chum salmon-directed fishery) – 200 individuals per period
    iv. District Y-3 subsistence – 250 individuals
    v. District Y-4 subsistence – 250 individuals from each subdistrict
    vi. District Y-5 commercial – 400 individuals
    vii. District Y-5 subsistence – 400 individuals.
2) Analyze samples from 3 pulses of Chinook salmon from the LYTF after the pulses are identified by the test fishery.
3) Analyze samples representing the first two main pulses from the Pilot Station Test Fishery
4) Analyze a representative sample of individuals from each district and period for genetic variation at the SNP loci in the baseline.
5) Estimate the relative contribution of stocks to the test, commercial, and subsistence fisheries of the Yukon River.

2. Methods:

BASELINE

A slightly modified version of the 25 population baseline described in Templin et al. (2008) was used for this analysis. Two populations were added; the Chatanika River in the Tanana River drainage and a mainstem spawning population collected near Minto. The SNP set used was also changed slightly and included 42 loci. In the 2007 and 2008 version of the baseline, if linkage disequilibrium was significant (α=0.05*) in more than half of the collections, we produced

composite phenotypes for each fish by combining the genotypes from these linked markers and treated them as a single locus in further analyses. For 2009, we instead removed one locus from each pair of putatively linked loci.

Simulations were conducted to evaluate the accuracy and precision of the SNP baseline to provide compositional estimates of mixtures of Chinook salmon harvested in Yukon River fisheries. These simulations were used to help assess whether the baseline of allele frequencies at the 26 SNP markers would provide sufficient information to identify individual stocks or groups of stocks (reporting groups) in mixtures. Reporting groups for genetic stock identification of Yukon River Chinook salmon were defined in previous studies based on a combination of genetic similarity, geographic features, and management applications (Flannery et al. 2006).

Reporting groups were defined hierarchically into 3 levels: 1) country of origin, 2) broad scale, and 3) fine scale. The broad scale groups (Lower Yukon, Middle Yukon, and Canada) were the same regions previously used for estimating stock composition of the harvest by scale pattern analysis (JTC 1997). Simulations performed using fine-scale reporting groups represent identifiable sets of populations useful for management and research (Table 1). These groups were previously defined in 2004 (Templin et al. 2006b) when SNPs were first used to estimate the stock composition of the harvest.

Simulations were performed using the Statistical Package for Analyzing Mixtures (SPAM version 3.7, Debevec et al. 2000). Mixture genotypes were randomly generated from the baseline allele frequencies assuming Hardy-Weinberg equilibrium. Each simulated mixture (N=400) was composed entirely of the stock or reporting group under study. When a reporting group mixture was simulated, all stocks in the reporting group contributed equally to the mixture. Average estimates of mixture proportions and 90% confidence intervals were derived from 1,000 simulations. Reporting groups with mean correct estimates of 90% or better are considered highly identifiable in fishery applications (Seeb et al. 2000).

FISHERY COLLECTIONS

Chinook salmon were sampled from the test, commercial, and subsistence fisheries in the U.S. portion of the river (Table 2; Figure 1). Samples were collected randomly during each fishing period during the process of sampling the harvest for age, sex, and length data (DuBois and DeCovich 2008). A fishing period is a designated time during which either subsistence or commercial fishing is allowed. Chinook salmon fishing periods on the U.S. portion of the Yukon River are authorized by ADF&G and the USFWS (Bue and Hayes 2008). Axillary processes were collected and preserved in ethanol. Samples were collected from test fisheries in Districts Y-1 (LYTF) and Y-2 (Pilot Station), commercial harvests in Districts Y-1 and Y-2, and from subsistence harvests in Districts Y-1, Y-3, Y-4, and Y-5. The subsistence samples from District Y-4 were collected from subdistricts Y-4A (Kaltag and Nulato), Y-4B (Bishop Rock and Galena), and Y-4C (Ruby). The subsistence samples from District Y-5 were collected from subdistricts Y-5C in the mainstem Yukon River above Hess Creek, at Rapids, and at Fort Yukon.

Samples from the LYTF were collected over the course of the Chinook salmon run (approximately June 1 to July 15, 2009) from all three mouths of the Yukon River using set gillnets with 8.5-inch mesh. Pilot Station samples were collected from approximately June 9 through July 19, 2009, using a broad array of mesh size drift gillnets, ranging from 2.75-inch to 8.5-inch stretch mesh. An attempt was made to sample distinct pulses of Chinook salmon passing through the test fisheries. Pulses are identified by increases in catch per unit effort (CPUE) for a sustained period of 3-5 days followed by a substantial decrease in CPUE. Tissues were clipped from each salmon, placed in individually labeled plastic tubes, and preserved in denatured ethanol. Samples were flown to the ADF&G Gene Conservation Laboratory (GCL) in Anchorage, analyzed and reported on within 36 hours of receipt at the GCL.

For collections from both the Pilot Station Test Fishery and subdistrict Y-5C subsistence fishery at Rapids, samples were stratified temporally postseason. Sample sets were defined to be representative of catch proportion estimates while maintaining minimum sample size requirements.

Samples were collected in subdistrict Y-4A from Kaltag and Nulato, and in subdistrict Y-4B from Bishop Mountain and Galena. Stock composition estimates were calculated for these fisheries both by location (i.e., Kaltag and Nulato separately) and also by subdistrict (i.e., Kaltag and Nulato pooled). Tanana Chiefs Conference (TCC) collected most of the District Y-4 and Y-5 samples used in this analysis.

LABORATORY METHODS

Genetic data were collected from the fishery samples as individual multi-locus genotypes for 52 SNPs. However, the same version of the baseline with 26 SNPs used in 2006 and 2007 (Templin et al. 2008) was used in 2008, and only genotypes from the 26 SNPs were used to analyze the fishery samples in this study (Table 3). This reduced set of SNPs, when compared to the original set of 51 SNPs assayed in 2006, was determined to provide acceptable levels of accuracy and precision while providing substantial cost savings. More SNPs were assayed in this study because recent advancements in laboratory technology reduced the cost per genotype, and it is no longer cost effective for the GCL to run only 26 SNPs. The current platform supports genotyping either 48 samples and 48 assays or 96 samples and 96 assays, with the lowest cost per genotype obtained when all 48 by 48 or 96 by 96 reactions are performed. Therefore, 48 SNPs were assayed for this study, and an expanded baseline using 48 SNPs will be used for future Yukon River Chinook salmon GSI studies.

Genomic DNA was extracted using a DNeasy® 96 Tissue Kit by QIAGEN® (Valencia, CA).[1] Chinook salmon samples were genotyped using a BioMark 48.48 Dynamic Array (Fluidigm http://www.fluidigm.com/biomark_genotyping.htm). The BioMark 48.48 Dynamic Array contains a matrix of integrated channels and valves housed in an input frame. On one side of the frame are 48 inlets to accept the sample DNA from each individual fish, and on the other are 48 inlets to accept the assays for each of the SNP markers. Once in the wells, the components

[1] Product names used in this report are included for scientific completeness, but do not constitute a product endorsement.

are pressurized into the chip using the NanoFlex 4-IFC Controller. The 48 samples and 48 assays are then systematically combined into 2,304 parallel reactions. Each reaction was conducted in a 6.75 nL volume consisting of 1xTaqMan Universal Buffer (Applied Biosystems), 1.5 U AmpliTaq Gold DNA Polymerase (Applied Biosystems), 9 mM of each polymerase chain reaction (PCR) primer, 2 mM of each probe, 1xDA Assay Loading Buffer (Fluidigm), 12.5xROX (Invitrogen), and 0.01% Tween-20. Thermal cycling was performed on a BioMark IFC Cycler as follows: an initial denaturation of 10 min at 95°C followed by 50 cycles of 92° for 15 s and 60° for 1 min. The Dynamic Arrays were read on a BioMark Real-Time PCR System after amplification and scored using BioMark Genotyping Analysis software (Fluidigm).

The SNP data collected were individual diploid genotypes for each locus. Long term storage of the data is in an Oracle database, *LOKI*, supported and maintained by ADF&G. In addition, genotype data were stored as output text files on a network drive. The data on this network are backed up nightly.

QUALITY CONTROL METHODS

The following measures were implemented to ensure the quality and consistency of data produced by laboratory procedures:

1) Each individual was assigned a unique accession identifier. When DNA was extracted and analyzed from each individual, a sample sheet was created that linked each individual's code to a specific well in a uniquely numbered 96-well plate. This sample sheet accompanied the individual through all phases of a project, minimizing the risk of misidentification of samples.
2) Genotypes were assigned to individuals using a double-scoring system. Two researchers designated allele scores for each individual.
3) Approximately 8% of individuals, 8 samples from each 96-well DNA extraction plate, were reanalyzed for all SNPs. This provided a measure of reproducibility and discrepancy rates, and allowed for correcting any errors created during the processing of individual plates. Failure rates were calculated, representing the number of samples that did not amplify during PCR, averaged over all loci.
4) The final data were checked for duplicated multi-locus genotypes for indication of errors caused prior to extraction of the DNA. When duplicate genotypes were found, the genotype was attributed to the first individual, and subsequent individuals with the same genotype were removed from the analysis.
5) The data have been permanently stored in an Oracle database, *LOKI*, administered by ADF&G.

MIXED STOCK ANALYSIS

Stock composition estimates for the stock groups of management interest were generated using BAYES (Pella and Masuda 2001). Individual population estimates were first calculated, and then summed into reporting regions (stocks). The 90% confidence interval for all group contribution

estimates was computed from the posterior distribution of stock composition estimates generated during the estimation procedure. Each mixture was run for three chains, each with a length of 10,000, with the first 5,000 iterations discarded as the burn-in. A flat prior was used for all sample sets.

Stock composition estimates were reported for three hierarchical levels when sample sizes were > 200 as follows: 1) country of origin (U.S and Canada), 2) broad scale (Lower Yukon, Middle Yukon, and Canada), and 3) fine scale (Lower Yukon, Tanana, Upper U.S. Yukon, Canada Border, Pelly, Carmacks and Takhini). When sample sizes were < 200, only the first two levels of the hierarchy were reported. Increasing the resolution to three reporting groups in the U.S. (Lower River, Tanana, and Upper Koyukuk/Upper U.S. Yukon) has been supported by simulation studies of the baseline (Templin et al. 2006a).

3. Results:

COLLECTIONS

During 2009, 5,343 Chinook salmon were sampled as part of 13 collections from test, commercial, and subsistence fisheries in the U.S. portion of the Yukon River drainage (Table 2; Figure 1). Test fishery catches were sampled from the Dall Point Test Fishery located in the coastal district, LYTF located in District Y-1, from the Pilot Station test fishery located in District Y-2, and from the Eagle sonar test fishery located near the U.S. /Canada border. Samples from Eagle were shipped to the CDFO genetics lab in Nanaimo B.C. for processing, but are included in Table 2 for completeness. Sampling was conducted in two commercial fishing periods in District Y-1. In 2009, all lower Yukon River commercial fishing targeted chum salmon, and samples were taken from the incidental harvest of Chinook salmon; since the incidental sale of Chinook salmon was prohibited, only 11 total fish were sampled in Y-1 and 10 in Y-2. Mesh sizes in this fishery were restricted to 6 inches or less for the purpose of targeting chum salmon. No samples from commercial harvests were analyzed in this study due to insufficient sizes.

Subsistence harvests were sampled in Districts Y-1, Y-3, Y-4, and Y-5. Samples from the District Y-4 subsistence harvest were taken from Kaltag and Nulato (subdistrict Y-4A), Bishop Mountain and Galena (subdistrict Y-4B), and Ruby (subdistrict Y-4C). Samples from District Y-5 subsistence fisheries were collected from subdistricts Y-5C in the mainstem Yukon River above Hess Creek, at Rapids, and at Fort Yukon.

LABORATORY / QUALITY CONTROL ANALYSIS

Of the fishery samples, a total of 3,361 individuals were analyzed for allelic variation at 42 SNPs (Table 3). The quality control analysis demonstrated an overall discrepancy rate of 0.26%, which, if one assumes an equal error rate in the original and quality control genotyping process, represents an error rate of 0.13%.The overall genotyping failure rate was 1.49%, and ranged from a low of 0.17% for the samples collected at Pilot Station to a high of 3.07% for the samples collected from the District Y-1 commercial fishery harvest.

BASELINE ANALYSIS

Linkage disequilibrium within each collection yielded significant results in >90% of collections at 2 marker pairs: *Ots_FGF6A* and *Ots_FGF6B*; and *Ots_HSP90B-100* and *Ots_HSP90B-385*. The second marker in each pair *Ots_FGF6B* and *Ots_HSP90B-385* was removed from subsequent analyses.

SIMULATIONS

All fine-scale reporting regions had mean correct allocations of >90% for the 100% simulation tests (Table 4).

MIXED STOCK ANALYSIS

Test Fishery

Difficult fishing conditions in the LYTF made it difficult to detect specific pulses of Chinook salmon. Therefore, the decision was made to shift some of the inseason analysis efforts to the Pilot Station test fishery for the purpose of characterizing the stock composition of the run.

An attempt was made to identify the first pulse of Chinook salmon entering the LYTF, and samples collected through June 17 (Big Eddy) and June 18 (Middle Mouth) were flown to the GCL and analyzed (Figure 2). The conclusion of the first pulse was identified by a decline in the daily catch from a high of 54 on June 16, and 296 samples caught through June 18 were flown back to Anchorage and analyzed. Stock composition estimates indicated that the proportion of Canadian-origin Chinook salmon present in the LYTF from June 3 through June 18 was 63% for Big Eddy (Table 5) and 53% for Middle Mouth (Table 6).

The conclusion of a second pulse through the LYTF was detected by a decline in the daily catch from a high of 70 on June 18 and 204 samples caught between June 18 and June 22 were flown to the GCL and analyzed (Figure 2). Stock composition estimates indicated that the proportion of Canadian-origin Chinook salmon present in the LYTF for this time period was 63% for Big Eddy (Table 5) and 45% for Middle Mouth (Table 6).

Samples from the Pilot Station test fishery caught through June 15 were also flown to Anchorage and analyzed concurrently with the samples from the first pulse at the LYTF. Early season high water conditions made sampling the first part of the run difficult at the LYTF and Pilot Station, and early arriving fish were likely missed at both locations. In general, sampling at Pilot Station was conducted in proportion to the passage estimate determined by the sonar project (Figure 3). Stock composition estimates indicated that the proportion of Canadian-origin Chinook salmon present in Pilot Station test fishery for this time period was 70% (Table 7; Figure 4).

An additional two inseason analyses were done on samples from Pilot Station. Pulses were difficult to detect in this fishery, and samples were flown back to Anchorage based on the availability of a USFWS aircraft making scheduled flights between Pilot Station and Anchorage. The strata used for these inseason analyses are shown in Figure 3 and represent the samples caught between June 9-15 (Stratum 1), June 16-24 (stratum 2) and June 26-29 (stratum 3). Stock composition estimates indicated that the proportion of Canadian-origin Chinook salmon present in the Pilot Station test fishery for these strata ranged from 70% in stratum one to 43% in stratum 3 (Table 7; Figure 4).

The estimates for each of the four separate inseason analyses were available within 36 hours of receipt of samples at the GCL.

Upon completion of the ADF&G Pilot Station sonar project, daily passage estimates were available for the entire season, and more samples were available from Chinook salmon representing the fourth quarter of the run. After reviewing this information, new strata were constructed (Figure 5). Once the additional samples were assayed, stock composition estimates were re-calculated for the new strata. The Canadian-origin Chinook salmon present in the Pilot Station test fishery ranged from a high of 68% in stratum 1 to a low of 17% in stratum 4 (Table 8; Figure 5).

Commercial

No samples from commercial fisheries were analyzed due to insufficient sample sizes.

Subsistence

In the District Y-1 subsistence fishery, 63% of the harvest samples were comprised of Canadian populations (Table 9). The Pelly region was the largest contributor to the Canadian component at 22%. Of the U.S. contribution, the largest component was estimated to be from the Lower Yukon (25%), while populations from the Tanana River contributed 23% of the harvest. The harvest from the Y-3 subsistence fishery showed a lower proportion of Canadian populations (26%), while the Lower Yukon component was larger than in District Y-1 (51%).

The estimated contribution of Canadian populations to the subsistence harvest in District Y-4 varied from a high of 59% in Kaltag (subdistrict Y-4A) to a low of 16% in Ruby (subdistrict Y-4C) (Tables 11-13; Figure 7). As with the commercial harvest in the lower river, the Carmacks region comprised the greatest portion of the Canadian estimate in subdistricts Y-4A and Y-4B (there were insufficient samples for fine-scale analysis of subdistrict Y-4C).

Samples were collected at Rapids throughout the run. Out of 701 collected, 411 samples representing two pulses detected at the fishwheels were analyzed. The Canadian contribution to the subsistence harvest in subdistrict Y-5C, was 95% for both the first strata at Rapids (June 21 - July 1) and Fort Yukon. The Canadian contribution to the second strata at Rapids (July 16 - July 31) was 82%. The Canadian contribution to the subsistence sample harvested from the mainstem Yukon River above Hess Creek was 70% (Table 14).

4. Discussion:

The 2009 Yukon River Chinook salmon run abundance was below average. The preseason outlook predicted a below average to poor Chinook salmon run, and it was clear that projected Chinook salmon run abundance would not support normal subsistence harvests in Alaska (approximately 50,000 Chinook salmon), meet escapement goals in Alaska, and meet the interim management escapement goal (IMEG) of >45,000 fish in Canada agreed to and adopted by the Yukon River Panel. Despite low overall run strength estimated at Pilot Station sonar early in the run, inseason GSI information on the Canada-bound proportion of the run identified a stronger presence of these stocks than had been documented during 2007 and 2008.

The preseason management plan called for no Chinook salmon-directed commercial fishery and a closure of subsistence fishing activity on the first pulse of mainstem Chinook salmon. Inseason,

fishery managers further reduced subsistence fishing activity after the first pulse of Chinook salmon. Although difficult for Alaskan fishermen, these restrictions ensured most escapement goals were met, including border passage of Canadian-origin Chinook salmon above the minimum required by treaty agreement. However, the delay in subsistence fishing activity affected the genetic composition of subsistence harvests compared to recent years.

The proposed objective of sampling the three pulses of Chinook salmon passing through the LYTF was not achieved. The performance of the LYTF was affected by high water and heavy debris. Ice dams moving down the river caused substantial flooding along a substantial portion of the Yukon River. This flooding coincided with the start of the Chinook salmon run. Once it was determined that pulses would be undetectable in the LYTF, efforts were shifted to analyzing catches from the Pilot Station test fishery. Post-season examination of daily fish passage at Pilot Station revealed that the initial inseason strata could be modified to create four new strata with the fourth strata represented by all available samples through the end of the season. These strata were intended to represent the four quartiles of the run passing through the Pilot Station test fishery. The major difference between the inseason and post-season estimates is the dramatic increase in Lower Yukon stocks in the fourth strata. This result was expected based on previous years' GSI studies showing the bulk of the Canadian-origin component of the run to pass through the lower river by the mid-point of the run (Templin et al. 2006, Templin et al. 2008, DeCovich and Templin 2009).

As in 2007 and 2008, harvest locations were available for District Y-4 subsistence samples. TCC also expanded sampling efforts to include the subsistence fishery located along the mainstem Yukon River near Hess Creek. The Canadian proportion was 16% in the sample from Ruby, and the Middle Yukon component was 78%. This is similar to the results of the 2007 analysis, where the Tanana component dominated the Ruby sample. It is hypothesized that since the bulk of the fish sampled at Ruby come from the south bank, Chinook caught at this location are following the plume of water generated by the Tanana River. Unfortunately, the 2009 sample size from Ruby was not large enough to estimate the fine-scale stock groupings. With a larger sample size from Ruby, it would be possible to estimate the Tanana component, which is included in the Middle Yukon group.

The Canadian proportion was high in all subdistrict Y-5C samples. The lowest Canadian component (70%) was estimated from the mainstem sample near Hess Creek. This seems reasonable since this sample is the furthest downstream sample of all the subdistrict Y-5C samples, thus increasing the potential that U.S. populations could be present in the harvest.

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Wholecheese, Larry Wholecheese, and James Willams. District Y-5 subsistence samples were collected by Pat Moore in Tanana, Stan Zuray in Rapids, and the Tanana Chiefs Conference above Hess Creek and in Fort Yukon.

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