APPENDIX B: JUVENILE CHINOOK AND COHO SALMON IDENTIFICATION ACCURACY

Susitna-Watana Hydroelectric Project
(FERC No. 14241)

Study of Fish Distribution and Abundance in the Middle and Lower Susitna River
Study Plan Section 9.6

2014-2015 Study Implementation Report
Appendix B: Juvenile Chinook and Coho Salmon Identification Accuracy

Prepared for

Alaska Energy Authority

SUSITNA-WATANA HYDRO

Clean, reliable energy for the next 100 years.

Prepared by

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1. INTRODUCTION

In 2014, certain licensing participants expressed concern about the amount of sampling error apparent in AEA's fish distribution and abundance studies. This appendix addresses that general concern, and more specifically, the concern that level of error associated with Chinook and Coho Salmon species identifications by AEA's fish study teams was higher than acceptable within the fisheries profession and therefore, compromises the use of the study results to support management decisions. To do so within this appendix, available literature that addresses error in ecological field sampling and fish identifications is summarized. Then, the accuracy of the fish collections from Studies 9.5 and 9.6 Fish Distribution and Abundance in the Upper and Middle and Lower Rivers, respectively, is reviewed, and the efficacy of the QAQC protocol that AEA proposed to improve accuracy is evaluated. Finally and most importantly, the management implications associated with having a known level of uncertainty around species identifications of juvenile Chinook and Coho salmon is discussed.

Although it is not often estimated or even discussed, sampling error is ubiquitous in all ecological sampling and observer bias is widespread in studies that rely upon humans to collect data (Elphick 2008). While it is often assumed that the degree of error can be attributed to a lack of training and experience of observers, studies have shown that training and experience can reduce or change the type of sampling error that occurs (Fitzpatrick et al. 2009), but cannot eliminate observer error (Elphick 2008, Kirsch et al. 2014). Understanding the bias/error associated with ecological data sets allows researchers to take steps to potentially reduce that error, but more importantly, to evaluate the influence of the uncertainty imposed by error might have on the use of the data for management decisions. Since error is unavoidable, it is important to know what implications, if any, the error would have for use of the data.

For fish surveys, field crews are often asked to identify fish to genus or species where possible. Field identification of fishes relies on phenotypes (such as coloration, or fin shape) and meristics (countable traits such as fin rays), and natural variations in these traits can make field identification challenging (Moyle 2002). A literature search for studies that addressed uncertainty in species identification based on phenotypes revealed only one study with freshwater fishes.

A recent experimental study conducted by the USFWS estimated error associated with the identification of fish species in California (Kirsch et al. 2014). This study demonstrated an overall average accuracy of 84 percent for all observers. In addition, although accuracy increased with observer experience (accuracy was approximately 60 percent for inexperienced observers and 80 percent for observers with approximately 18 months of experience in the region), it remained highly variable among observers ranging from 85 to 95 percent for even the most experienced observers (15 years of experience) demonstrating that there is an individual human component to bias that experience and training do not affect. It is important to note, that during this experiment the identification of test specimen had to be agreed upon by four expert California fish scientist and the specimen for which the experts could not agree were excluded from the experiment. Thus, these results might be underestimates of identification error for difficult determinations where considerable overlap in phenotypic variation occurs. This study by Kirsch et al. provides a basis of comparison for documented observer error during fish species identification.

2. ACCURACY OF CHINOOK AND COHO SALMON SPECIES IDENTIFICATION

During 2013 field sampling for Study 9.6, field crews identified that a proportion of the juvenile salmon catch were challenging to identify to species due to high variability in color patterns and meristics that overlapped across species. In particular for juvenile Chinook and Coho salmon, large variation among individuals with respect to species defining characteristics: 1) the spacing of parr marks, 2) the coloration of the leading edge of the fins, and 3) the shape of the anal fin, resulted in a large amount of uncertainty in identifying some fish to species (Figure C-1). To address this concern, in 2014 additional onsite training was provided to field crews, crews were instructed to increase photo-documentation of challenging fish, and laboratory confirmation of field identification through collection of tissue samples for DNA analysis was initiated to provide feedback and evaluate field identification accuracy. Field identifications were reviewed for quality control based on photo documentation and a final QC3 species determination was made.

There are two terms that we can use to characterize the uncertainty in species identifications that were made during 2012-2013 fish surveys. The first term, **sampling accuracy**, characterizes the correctness of the species determinations when the fish are taken from a mixed pool of unknown species, and is calculated as the correct number of Chinook and Coho salmon determinations by the study team divided by the known number of each species in the collection as determined by DNA analysis. The second term is **species-specific accuracy** and describes the error around the study team identifying a species as itself in the field, in other words, identifying a Chinook Salmon a Chinook and, likewise, calling a Coho Salmon a Coho. Species-specific error is determined by dividing the number of correct field identifications for each species by the genetically verified number of that species. This term is important to evaluate because it helps us to understand where the identification error is arising from, such as misidentification of one species, the other, or both.

The results of the genetic analysis from 1,226 fish confirmed that fish crews had an overall sampling accuracy when identifying Chinook and Coho salmon of 86 percent (Table C-1), with 84 percent and 90 percent sampling accuracy for Chinook and Coho salmon, respectively. Genetic analysis also showed that species-specific accuracy was one-directional in 2013 (Table C-2). In 2013, observers identified true Chinook Salmon with high species-specific accuracy of 96 percent (only 12 out of 320 verified Chinook Salmon were called Coho Salmon); but, they erroneously identified 122 out of 290 verified juvenile Coho Salmon as Chinook resulting in a species-specific accuracy rate of 57 percent. The species-specific error numbers indicated that it was the incorrect assignment of Coho Salmon that caused the problem in 2013. Photographic QAQC confirmed that it was the variation of distinguishing characteristics of Coho Salmon and how they overlapped with those used to distinguish Chinook Salmon that caused the error in 2013.

In 2014, species-specific accuracy remained stable for Chinook Salmon and improved dramatically for Coho Salmon, such that no directional error was evident. The 2014 species-specific accuracy rates were 95 percent for Chinook Salmon (22 errors out of 403 verified Chinook) and 96 percent for Coho Salmon (7 errors out of 186 verified Coho). This improvement likely is related to additional training and feedback provided to field crews about specific characteristics of Coho Salmon in the Middle Susitna River as well as implementation of photographic QAQC of field identification as is discussed below.

It is important to note that this problem was isolated to the Middle River below Devils Canyon and in the Lower River. The juvenile salmon collected in the Middle River within Devils Canyon (between Impediment 1 and 3) and above Impediment 3, as well as in the Upper River were phenotypically distinct and were assigned as Chinook Salmon with 100 percent accuracy (Table C-3) in all survey years 2012-2014. This high level of accuracy was likely related to the facts that 1) there were no Coho Salmon collected in any of the samples within and above the Canyon and there was no co-occurrence of juvenile Pacific Salmon upstream of Impediment 1 in Devils Canyon, although this was not known with certainty prior to initiation of AEA's recent field surveys and genetic sampling.

Even within the Middle and Lower River Segments, the results of genetic analysis show that the species identifications were similar to or greater than accuracy levels reported elsewhere (USFWS 2014) except in two Middle River Segment reaches, MR-6 and MR-7 (Table C-3). Importantly, the lowest accuracy of 33 percent, evident in MR-7, was based on a small sample size of nine genetically verified Chinook Salmon that came from two habitats: the Oxbow side channel and a side slough at PRM 117. This information points to localized areas where the phenotypic variation among juvenile salmon is high and poses challenges for species identification. Photographic review of juveniles collected in Oxbow side-channel showed the fish to be in the process of smoltification and confirmed the difficultly in species identification due to a lack of distinguishing characteristics. This was the only reach where photographic review was less than 90 percent accurate when compared to genetically verified specimen (Table C-4).

3. EVALUATION OF AEA'S PHOTOGRAPHIC QA/QC FOR FIELD IDENTIFICATIONS OF JUVENILE SALMONIDS

In 2014, AEA developed and filed with FERC a proposed protocol entitled *Fish Distribution and Abundance in the Upper and Middle/Lower Susitna River (Studies 9.5 and 9.6): Draft Chinook and Coho Identification Protocol* (R2 2014) to improve the accuracy of species determinations for juveniles of these two salmon species. The protocol consists of five components: 1) site-specific training in areas where these species have co-occurred and identification has proven challenging; 2) standardized genetic verification across habitats; 3) collection of up to 20 voucher specimen of each species for meristic analysis by field crews; 4) collection and senior review of photographs for all undifferentiated Pacific salmon and all PIT-tagged Chinook and Coho salmon and 5) development of a Susitna specific identification guide for use by field crews. In 2013, an evaluation of the photographic QA/QC was implemented on 317 juvenile salmon that had both photos and genetic tissue samples taken at the time of capture. A comparison of the 2014 QC3 species determination (final study team determination after photographic quality control of field identifications) showed that species identification from photo QA/QC was accurate for 96 percent of the Chinook Salmon identifications and 98 percent for Coho Salmon (Table C-4). This high level of accuracy supports the use of photographic review for verifying field identifications in AEA's future studies downstream of Devils Canyon. Further support for AEA's proposed protocol comes from Moyle (2002) who recommended the use of photographic review of recently caught field specimen and collection of voucher specimens to improve fish identification accuracy and account for natural variation in morphology and human perception.

Finally, the value of AEA's field protocol extends beyond reducing any misidentification of juvenile Coho or Chinook salmon. In some habitats, such as Slough 6A, 2013 field crews were only able to identify large numbers of juvenile salmonids to genus, based on phenotypic characteristics, and thus called them Undetermined Pacific Salmon (SAMs). This resulted in SAMs in preliminary datasets. Use of photographic QA/QC has allowed AEA to re-classify the majority of these SAMs. For example, in Slough 6A the number of SAMS was reduced from 335 to 14 after implementing the QA/QC verification protocol. Through application of AEA's QA/QC protocol, presence of both juvenile Coho and Chinook salmon has been positively documented within many habitat features surveyed including upland sloughs with active beaver dams (Table C-5). Finally, application of AEA's QA/QC verification protocol confirmed results documenting age 2 juvenile Chinook Salmon rearing in the Middle River Segment of the Susitna River. Because this age class has not been evident in previous sampling, licensing participants expressed concern during the October 2014 Initial Study Report meetings that the larger sized juvenile Chinook Salmon collected by field crews in 2013 were misidentified Coho Salmon and questioned the accuracy of the 2013 field data. AEA's protocol allowed for confirmation of the presence of larger sized, age 2 Chinook Salmon, documenting new information about the life history diversity of this species in the Middle River Segment. Application of AEA's species identification protocol has proven valuable at reducing observer error, and substantiating findings from 2012-2014 fish studies.

4. MANAGEMENT IMPLICATIONS

As discussed above, all ecological sampling has inherent error and studies that rely upon humans to collect data will have observer bias. Understanding this error is important, but the significance of the error is determined by considering how the error may affect use of the study results. How might observer bias in calling a juvenile Coho Salmon a juvenile Chinook Salmon affect an analysis of AEA's potential impact and subsequent management decisions? To address this, the ecology of juvenile Chinook and Coho salmon in the Susitna River basin needs to be understood. The following description is based largely on analysis of photographically and genetically-verified juvenile Chinook and Coho salmon from FDA 2013 and 2014 databases (Study 9.6), and analysis of juvenile Chinook and Coho salmon identified in 2013 and 2014 and recorded in the HSC database (Study 8.5).

Data from 2013 and 2014 field studies suggest that juvenile Chinook and Coho salmon in the Middle and Lower Segments of the Susitna River show considerable overlap in ecological niches during the open water period. Data from fish sampling indicate that these juveniles occupy similar habitats within the Middle Susitna River and co-occurred in 87 percent of the habitat features where species identification were verified (Table B-5). In addition, site-specific habitat suitability criteria data collected by Study 8.5 show that juvenile Chinook and Coho salmon are keying in on similar habitat conditions such as shallow water depths, and water velocities less than 0.5 feet per second and temperature (Figure B-2). Within these habitats, the isotopic data collected under Study 9.8 indicates that these fish rely upon similar food resources both across habitats and across seasons (Figure B-3). Finally, the size distributions of the genetically-verified juvenile Chinook and Coho salmon show considerable overlap in size (Figure B-4) and support AEA's finding from scale analysis that both species exhibit life history diversity that includes freshwater rearing for more than one year (Figure B-5). Based on these data from the Middle River Segment, many juvenile Chinook and Coho salmon are rearing in the same habitats during open-water periods, are exposed to similar microhabitat conditions, depend upon similar food resources, grow to similar sizes, and are of similar ages while rearing in mainstem freshwater habitats.

Given the ecological similarities between juvenile Chinook and Coho salmon in the Middle and Lower Susitna River during the open-water period and the low accuracy with identifying Coho Salmon in some areas in 2013, AEA will combine data collected on Chinook and Coho salmon from 2013 and 2014 collections to characterize the distribution, relative abundance and habitat associations of these two juvenile salmon species when evaluating Project impacts. Where appropriate, AEA also will make use of the verified field identifications to look for species-specific patterns in growth and movements. Evaluations of Project effects using a pooled juvenile Chinook/Coho salmon data may overestimate the distribution, abundance and movement timing for individual species. However, overestimating each species' habitat use or range of movement timing would support more protective measures than could be justified for each species individually. Draft HSC are being developed that may show small differences between juvenile Chinook and Coho salmon (Study 8.5), especially when considering both open-water and ice periods. Final effects analyses may consider an approach where protection of habitats occupied by both juvenile Chinook and Coho lifestages is based on the lifestage that is most susceptible to effects of Project operations. AEA is confident in the integrity of study results and their ability to support a rigorous evaluation of potential Project impacts and where appropriate, development of Protection, Mitigation, and Enhancement measures for these ecologically similar life stages.

5. LITERATURE CITED

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6. TABLES

Table B-1. Accuracy of 2012-2014 QC3 species identification as determined by genetic analysis of tissue.

QC3 Species ID*	N	Species-Genetic Determination				% Correct
		Chum Salmon	Chinook Salmon	Coho Salmon	Sockeye Salmon	
Chinook Salmon	854	3	721	126	4	84.4%
Coho Salmon	371		35	334	2	90%
Pacific Salmon, Unspecified	1		1			-
Total Samples	1,226	3	757	460	6	86.10%

*Includes species identification changes for 53 fish in 2014 data set after review of 317 photos.

Table B-2. Accuracy of QC3 species identification by month and year. Accuracy was determined by genetic analysis tissue samples from N fish.

Year and Month of QC3 ID*	Chinook Salmon				Coho Salmon				Both Species	
	N	Number of Coho	Number of Chinook	% correct	N	Number of Chinook	Number of Coho	% correct	N	% correct
2012 Total	**35**		**35**	**100%**					**35**	**100%**
Aug-12	35	0	35	100%	0				35	100%
2013 Total	**430**	**122**	**308**	**72%**	**170**	**12**	**158**	**93%**	**600**	**78%**
Mar-13	6	2	4	67%	37	0	37	100%	43	95%
Apr-13	22	3	19	86%	2	0	2	100%	24	88%
Jun-13	124	51	73	59%	43	6	37	86%	167	66%
Jul-13	97	14	83	86%	8	0	8	100%	105	87%
Aug-13	116	36	80	69%	42	5	37	88%	158	74%
Sep-13	64	15	49	77%	26	0	26	100%	90	83%
Oct-13	1	1	0	0%	12	1	11	92%	13	85%
2014 Total	**389**	**7**	**381**	**97%**	**201**	**22**	**179**	**89%**	**591**	**94%**
Apr-14	19	2	17	89%	0				19	89%
May-14	33	0	33	100%	39	6	33	85%	72	92%
Jun-14	48	4	44	92%	75	9	66	88%	123	89%
Jul-14	33	`1	32	97%	0				33	97%
Aug-14	167	0	167	100%	37	2	35	95%	204	99%
Sep-14	49	0	49	100%	45	3	42	93%	94	97%
Oct-14	41	0	41	100%	5	2	3	60%	46	93%
Grand Total	**855**		**689**	**84%**	**371**		**337**	**90%**	**1226**	**86%**

*Includes species identification changes for 53 fish in 2014 data set after review of 317 photos.

Table B-3. QC3 Species ID accuracy by geomorphic reach; percent accuracy was determined by genetic analysis of tissue samples from N juvenile fish.

Geomorphic Reach	QC3 Species ID				Total	
	Chinook Salmon		Coho Salmon			
	N	% Correct	N	% Correct	N	% Correct
UR-2	65	100%			65	100%
UR-4	141	100%			141	100%
UR-5	5	100%			5	100%
UR-6	17	100%			17	100%
MR-1	4	100%			4	100%
MR-2	3	100%			3	100%
Impediment 3 PRM 164.8						
MR-4	14	100%			14	100%
Impediment 1 PRM 155.1						
MR-4	151	100%			151	100%
MR-6	219	71%	83	77%	303	73%
MR-7	9	33%	36	89%	45	78%
MR-8	142	63%	176	97%	318	82%
LR-2	85	88%	73	95%	158	91%
LR-3	2	100%			2	100%
Total	857	84%	368	90%	1226	86%

Table B-4. 2014 species ID photo review quality control as determined by comparing photo-based species determination with genetic analysis of tissues from N fish.

Geomorphic Reach	Chinook Salmon		Coho Salmon		Sockeye Salmon		Total	
	N	% Correct	N	% Correct	N	% Correct	N	% Correct
UR-2	2	100%					2	100%
UR-4	9	100%					9	100%
UR-5	1	100%					1	100%
UR-6	15	100%					15	100%
MR-1	4	100%					4	100%
MR-2	3	100%					3	100%
MR-4	105	100%					105	100%
MR-6	54	94%	17	88%	2	0%	73	90%
MR-7	3	0%	19	100%			22	86%
MR-8	24	88%	30	100%			54	94%
LR-2	14	100%	15	100%			29	100%
Grand Total	**234**	**96%**	**81**	**98%**	**2**	**0%**	**317**	**96%**

Table B-5. Documented co-occurrence of verified juvenile Chinook and Coho salmon in 53 of 60 Middle and Lower River habitat features.

Geomorphic Reach	Feature Name	Macrohabitat Type	Chinook Salmon Present	Coho Salmon Present
MR-5	FA-151 Portage Creek Plume	Main Channel-CWP	Yes	Yes
MR-5	FA-151 Portage Creek Mouth	Tributary Mouth	Yes	Yes
MR-5	FA-151 Portage Creek	Tributary	Yes	Yes
MR-5	FA-151 MC	Main Channel	Yes	Yes
MR-6	Slough 14	Upland Slough	Yes	Yes
MR-6	PRM 137 US	Upland Slough	No	Yes
MR-6	PRM 134 US	Upland Slough	No	Yes
MR-6	PRM 130 US	Upland Slough	Yes	Yes
MR-6	Jack Long Creek	Tributary	Yes	Yes
MR-6	FA-144 Slough 21 US	Upland Slough	Yes	Yes
MR-6	FA-144 Slough 21 SS	Side Slough	Yes	Yes
MR-6	FA-144 Slough 20	Upland Slough	Yes	Yes
MR-6	FA-144 Side Channel 21	Side Channel	Yes	Yes
MR-6	FA-141 Slough 19	Upland Slough	Yes	Yes
MR-6	FA-141 Slough 17 BW	Upland Slough-Backwater	Yes	Yes
MR-6	FA-141 Slough 17	Upland Slough	Yes	Yes
MR-6	FA-141 SC	Side Channel	Yes	Yes
MR-6	FA-141 MC	Main Channel	Yes	Yes
MR-6	FA-141 Indian River Mouth	Tributary Mouth	Yes	Yes
MR-6	FA-141 Indian River CWP	Main Channel	Yes	Yes
MR-6	FA-141 Indian River	Tributary	Yes	Yes
MR-6	FA-138 Upper Side Slough 11	Side Slough	Yes	Yes
MR-6	FA-138 Slough Slough 13	Upland Slough	Yes	Yes
MR-6	FA-138 Slough Slough 12	Upland Slough	No	Yes
MR-6	FA-138 Slough 11	Side Slough	Yes	Yes
MR-6	FA-128 US	Upland Slough	Yes	Yes
MR-6	FA-128 Slough 8A	Side Slough	Yes	Yes
MR-6	FA-128 Skull Creek Mouth	Tributary Mouth	Yes	Yes
MR-6	FA-128 Skull Creek	Tributary	Yes	Yes
MR-6	FA-128 Side Channel 8A	Side Channel	Yes	Yes
MR-6	Curry DMT	Main Channel	Yes	Yes
MR-7	PRM 117 SS	Side Slough	Yes	Yes
MR-7	PRM 113 US	Upland Slough	No	Yes
MR-7	FA-115 Unnamed Trib 115.4	Tributary	Yes	Yes
MR-7	FA-115 Slough 6A BW	Upland Slough-Backwater	Yes	Yes
MR-7	FA-115 Slough 6A	Upland Slough	No	Yes

Geomorphic Reach	Feature Name	Macrohabitat Type	Chinook Salmon Present	Coho Salmon Present
MR-7	FA-113 Unnamed Trib 113.7	Tributary	Yes	Yes
MR-7	FA-113 Slash Creek	Tributary	Yes	Yes
MR-7	FA-113 Oxbow I US	Upland Slough	No	Yes
MR-7	FA-113 Oxbow I SS	Side Slough	Yes	Yes
MR-7	FA-113 Oxbow I MC	Main Channel	No	Yes
MR-7	FA-113 Gash Creek	Tributary	Yes	Yes
MR-7	Chase Creek	Main Channel	Yes	Yes
MR-8	PRM 106.9 TKA Station DMT	Main Channel	Yes	Yes
MR-8	PRM 106 US	Upland Slough	Yes	Yes
MR-8	FA-104 Whiskers Unnamed Side Slough	Side Slough	No	Yes
MR-8	FA-104 Whiskers Slough	Side Slough	Yes	Yes
MR-8	FA-104 Whiskers Creek	Main Channel	Yes	Yes
MR-8	FA-104 Slough 3B	Side Slough	Yes	Yes
MR-8	FA-104 Slough 3A	Upland Slough	Yes	Yes
MR-8	FA-104 SC	Side Channel	Yes	Yes
MR-8	FA-104 MC	Main Channel	Yes	Yes
Lower River PRM 102.4				
LR-1	Birch Creek	Tributary	Yes	Yes
LR-2	Montana Creek	Tributary	Yes	Yes
LR-2	Montana Creek Mouth	Tributary Delta	Yes	Yes
LR-2	Sheep Creek Slough	Upland Slough	Yes	Yes
LR-2	Slough near Montana Creek	Upland Slough	Yes	Yes
LR-2	Susitna Main Channel near Montana Creek	Main Channel	Yes	Yes
LR-2	Susitna Side Channel near Montana Creek	Side Channel Complex	Yes	Yes
LR-3	Little Willow Creek	Tributary	Yes	Yes

7. FIGURES

Figure B-1. Examples of morphological variability among juvenile Chinook Salmon (left) and Coho Salmon (right) parr from the Susitna River and lower tributary reaches between PRM 80 and PRM 160.5. Species identification was verified through genetic analysis.

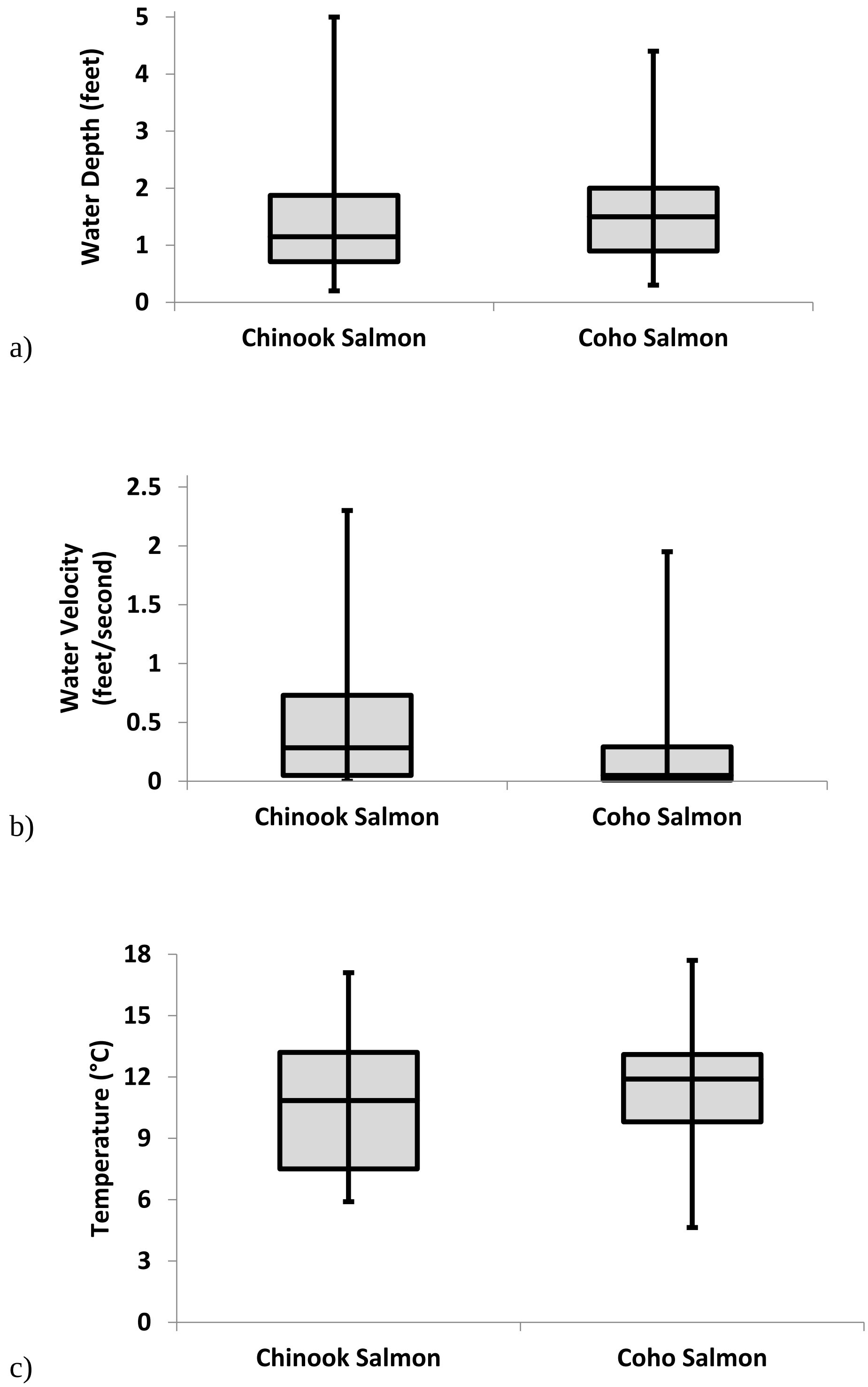

Figure B-2. Distributions of the Susitna River habitat suitability criteria data for the open-water period (median, 25% and 75% interquartile, range) collected for juvenile Chinook and Coho salmon: a) water depth criteria, b) velocity criteria, and c) temperature criteria. (source: 2013 and 2014 habitat suitability criteria microhabitat database http://gis.suhydro.org/SIR/08-Instream_Flow/8.5-Fish_and_Aquatics_Instream_Flow/).

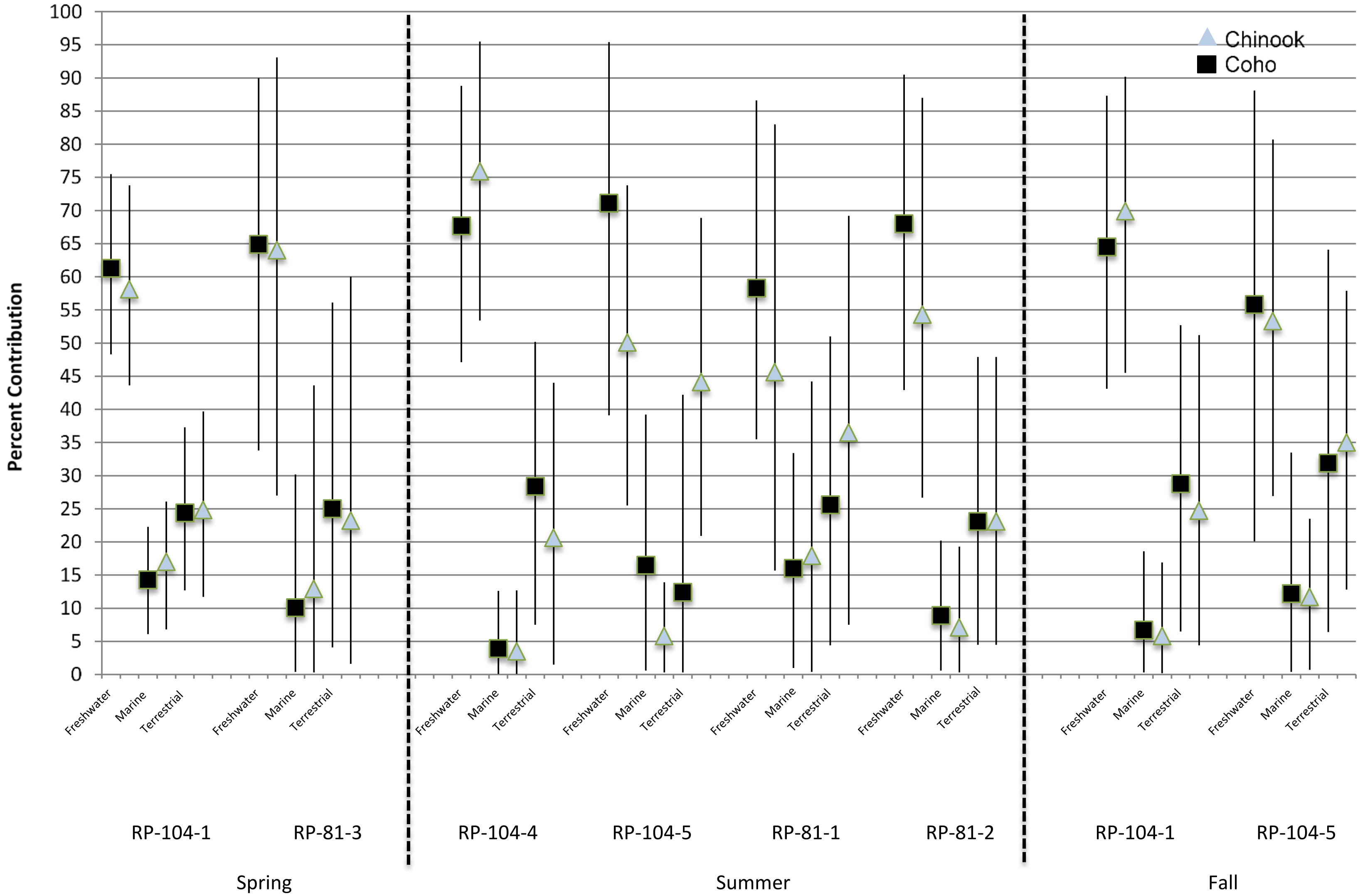

Figure B-3. Results of 2014 isotopic model showing contributions from freshwater, marine, and terrestrial food sources to juvenile Chinook and Coho salmon by site and season (Source: R2 and UAF 2015; Tables 5.4-4, 5.4-5, and 5.4-6).

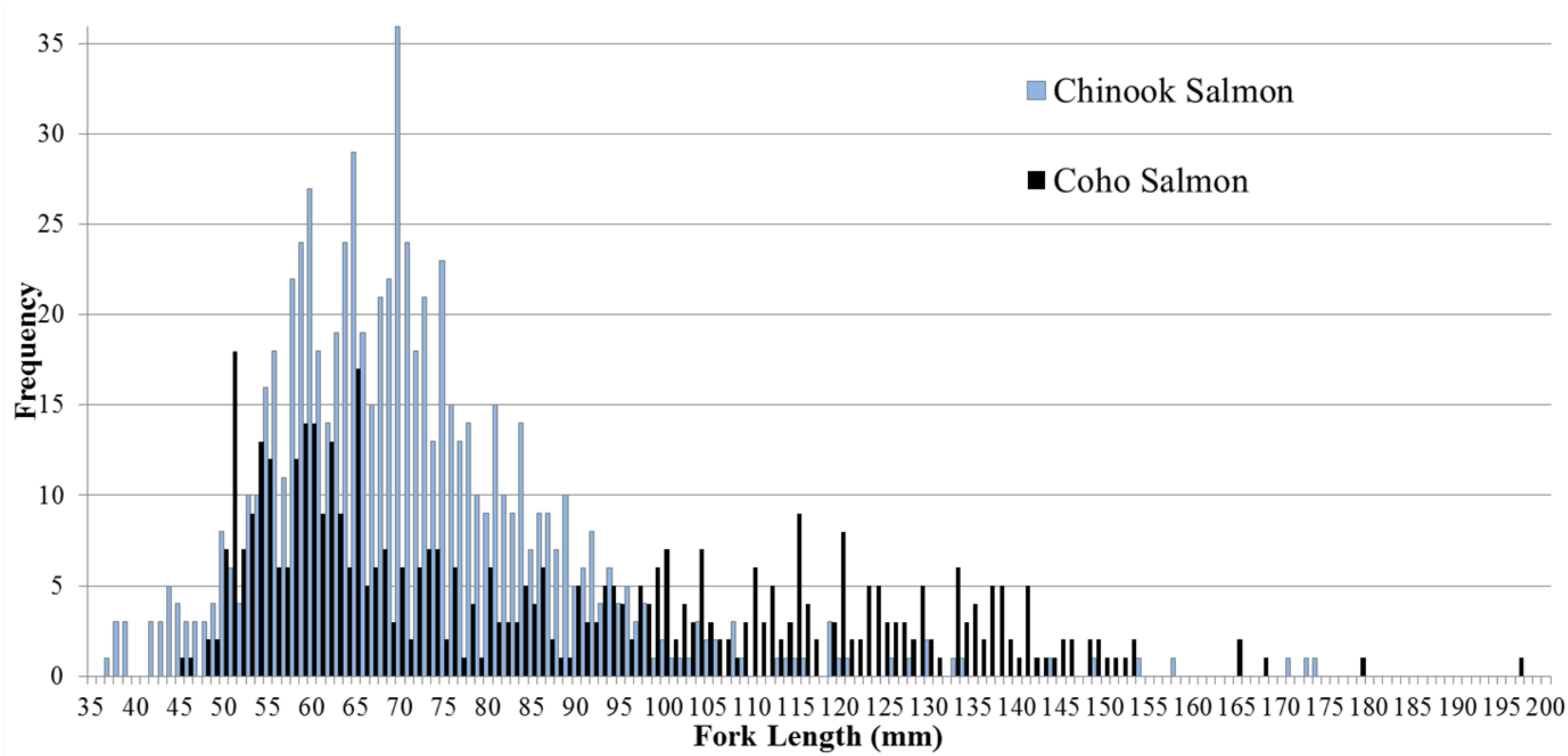

Figure B-4. Size distributions of genetically-verified juvenile Chinook and Coho salmon for the Middle and Lower Susitna Rivers, 2013-2014.

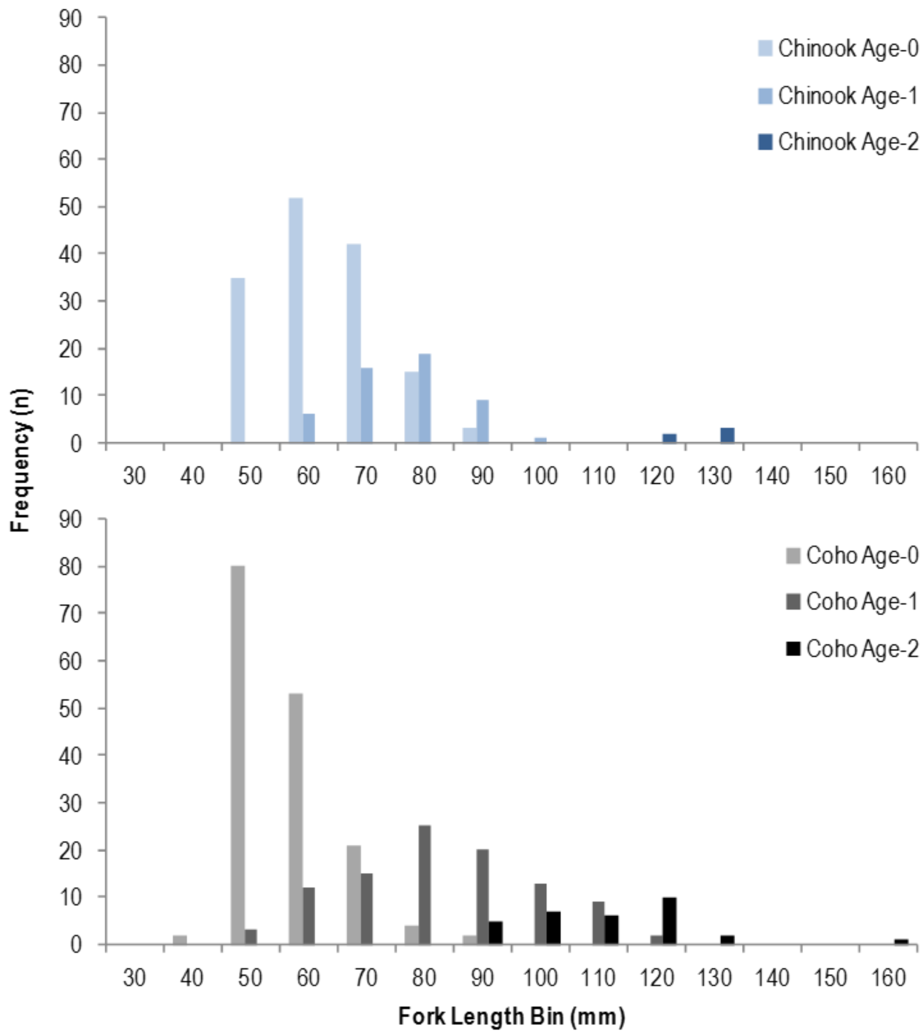

Figure B-5. Age at length of genetically-verified Chinook and Coho salmon based on scale analysis (Source: R2 and UAF 2015; Figures 5.4-5 and 5.4-6).