Genetic Stock Assessment of Yukon Delta Bering Cisco Harvest

2011 Annual Report

FRMP Study 10-209

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May 3, 2012

Abstract

Bering cisco *Coregonus laurettae* are anadromous salmonids with known spawning populations only in the Yukon, Kuskokwim, and Susitna rivers in Alaska. Rearing Bering cisco are found in the coastal lagoons and estuaries of western Alaska, from Kuskokwim Bay to the Colville River delta, where they are actively harvested in subsistence fisheries. A commercial fishery for Bering cisco was recently initiated at the mouth of the Yukon River and the product is being marketed to the kosher community in New York City. The fishery has been limited to an annual harvest of about 4,500 kg; approximately 10,000 Bering cisco. A much larger allocation has been requested, suggesting that the fishery would expand if permitted to do so. The commercial harvest of Bering cisco at the mouth of the Yukon River is most likely a mix of Kuskokwim and Yukon River populations. Our primary objective here was to estimate the proportional composition of the annual commercial harvest from known Bering cisco populations using genetics methods, the results of which will provide direction to future population monitoring activities. To accomplish this objective, we began development of a genetic baseline for the three Bering cisco populations. Tissue samples were collected from the three spawning populations during 2009, 2010, and 2011. During the 2011 season primers that provide effective baseline markers capable of distinguishing among spawning populations were developed and optimized. Mixed-population samples from the fishery are currently being processed and analyzed. Beginning in 2010, the genetic samples from the north Delta harvest were kept separated from the samples from the south Delta harvest. Because there may be differences in population composition between regions of the Delta, these are the samples that are being processed. Analysis of samples collected during the 2010 season are almost complete and final analysis of the results will be forthcoming within the next few weeks. Processing of the 2011 samples began in late April, 2012. One additional season of genetics collections from the harvest are planned during fall 2012, which will provide three years of mixed population data to evaluate the consistency of population composition in the harvest.

Introduction

McPhail (1966) established Bering cisco *Coregonus laurettae* as a species distinct from Arctic cisco *C. autumnalis* based primarily on geographic distribution and differences in gill raker counts on the lower limb of the first gill arch. Bickham et al. (1997) confirmed McPhail's (1966) taxonomic determination, and also identified both species in the western Beaufort Sea using morphological characteristics and genetic analyses. McPhail (1966) was not aware that neither Bering cisco or Arctic cisco spawned in local rivers of the western Beaufort Sea, but once it became clear that Arctic cisco returned to the Mackenzie River to spawn (Gallaway et al. 1983; Fechhelm and Griffiths 1990), other researchers realized that Bering cisco must similarly return to either the Yukon or Kuskokwim River to spawn as well (Craig 1989; Bickham et al. 1997). Unlike Arctic cisco, spawning migrations of Bering cisco have not been observed in Asia (Chereshnev et al. 2002). However, during a fisheries survey at the mouth of the Chegitun' River, just north and west of the Bering Strait, Chereshnev (1984) documented two rearing Bering cisco, which are the only two ever documented in coastal waters of Asia. They undoubtedly originated in North America.

Spawning populations of Bering cisco are known only from the Yukon, Kuskokwim, and Susitna rivers (Figure 1; Alt 1973; ADF&G 1983d). Subsistence surveys and fisheries inventories of river systems other than the Yukon, Kuskokwim, and Susitna, in south-central and western Alaska have not provided any evidence of additional spawning populations of Bering cisco. Georgette and Shiedt (2005), for example, conducted a comprehensive fisheries subsistence inventory of communities in the Kotzebue Sound region in northwest Alaska and found that people fishing coastal waters recognized and routinely captured Bering cisco while those who limited their fishing efforts to the Noatak, Kobuk, and Selawik rivers never captured Bering cisco. This is consistent with our understanding that Bering cisco in Kotzebue Sound originate in the Yukon or Kuskokwim rivers. Coiley-Kenner et al. (2003) examined wild resource use by residents of Manokotak, Togiak, and Twin Hills, communities on the north side of Bristol Bay. They found that residents identified 20 non-salmon fish species they harvested, including humpback whitefish *C. clupeaformis*, least cisco *C. sardinella*, and round whitefish *Prosopium cylindracium*, but Bering cisco were not among them. This information is consistent with our understanding of the marine distribution of Bering cisco being from Kuskokwim Bay north, with very few individuals ever identified in Bristol Bay. Hildreth and Dion (2006) identified 27 Bering cisco at a picket weir set up at the mouth of Mortensen's Creek, a small drainage on the south side of the Alaska Peninsula. The weir was near the high tide mark and the Bering cisco were identified as immature fish, based on examination of the gonads and feeding condition, that were feeding in the tide water. Blackburn et al. (1980) conducted extensive fisheries surveys in Cook Inlet and found Bering cisco to be common in coastal environments on the west side. They stated that the marine currents through Shelikof Strait were to the southwest, suggesting a mechanism for coastal distribution of Susitna River Bering cisco along the south shore of the Alaska Peninsula. Presumably, Bering cisco observed in Mortensen's Creek (Hildreth and Dion 2006) originated in the Susitna River. These findings are consistent with our understanding that spawning is not occurring in small stream systems and that rearing Bering cisco may be widely dispersed from their river of origin. Many other

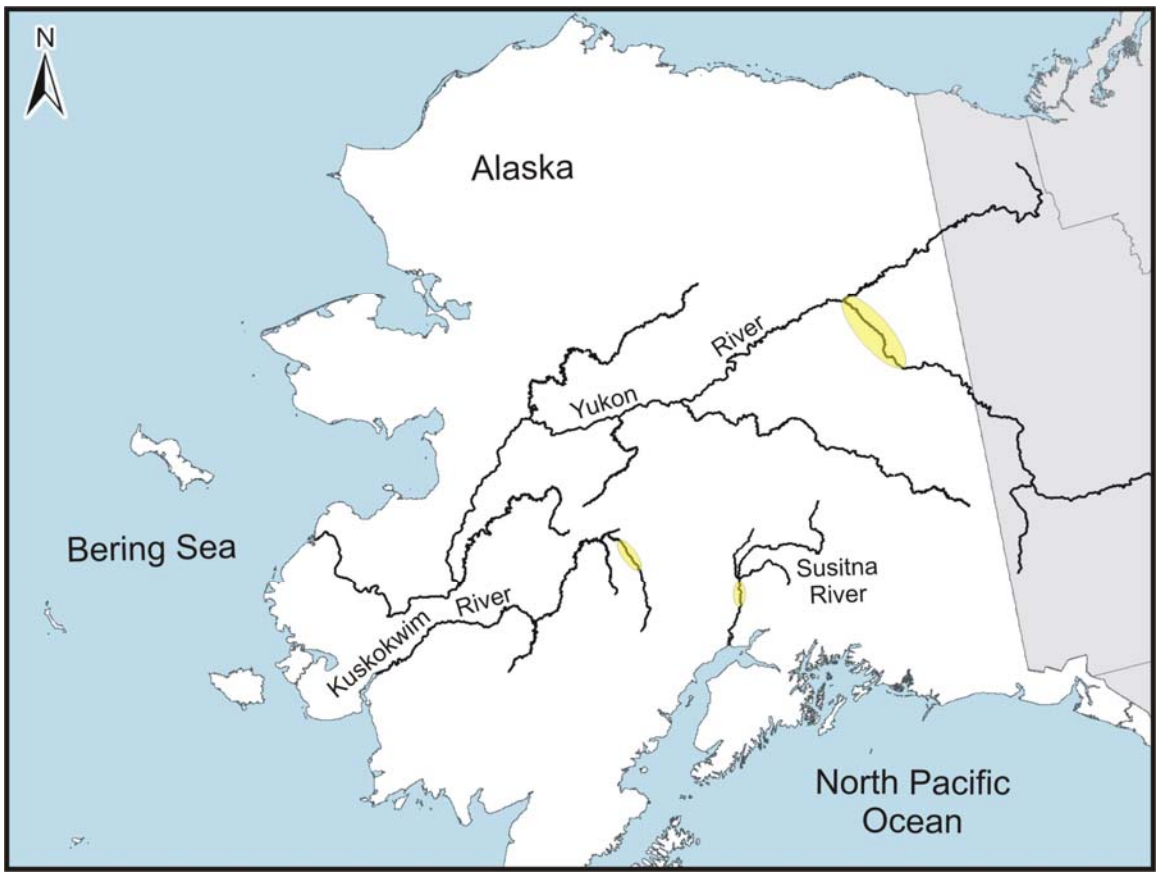

Figure 1. Approximate locations of the spawning areas of the three known populations of Bering cisco worldwide (yellow ellipses).

surveys of coastal and river drainage fisheries and fish inventories of river systems are available and none are in conflict with our understanding of there being just three large rivers of origin for Bering cisco.

Distribution data from within the Yukon, Kuskokwim, and Susitna rivers suggest that there is a single main-stem spawning population in each river. Fisheries surveys of Yukon River tributary streams such as the Andreafsky (Alt 1981), Innoko (Alt 1983), Bonasila, Anvik, Khotol, Nulato (Alt 1980), Koyukuk (Alt 1978; Wiswar 1994; Brown et al. 2007; Andersen 2007), Melozitna (Alt 1981), and Nowitna (Alt 1985) found other coregonid species present but not Bering cisco. A CPUE fishwheel operated by ADF&G in the Tanana River near Nenana has a video system (Daum 2005; Borba 2007) that collects images of every fish captured. A subsample of video images has been examined each year since it began operating in 2003 and while other coregonid species are routinely observed, no Bering cisco have been seen. By contrast, Bering cisco is the most numerous of the coregonid species in a similar video monitored CPUE fishwheel on the Yukon River upstream from the mouth of the Tanana River (Figure 2). Alt (1973) reported Bering cisco in the Porcupine River, but they were captured near its confluence with the Yukon River and not farther upstream. More recent sampling in upstream reaches of the Porcupine River drainage (Brown et al. 2007), including the Black River (Brown and Fleener 2001), indicate that Bering cisco do not continue migrating up the

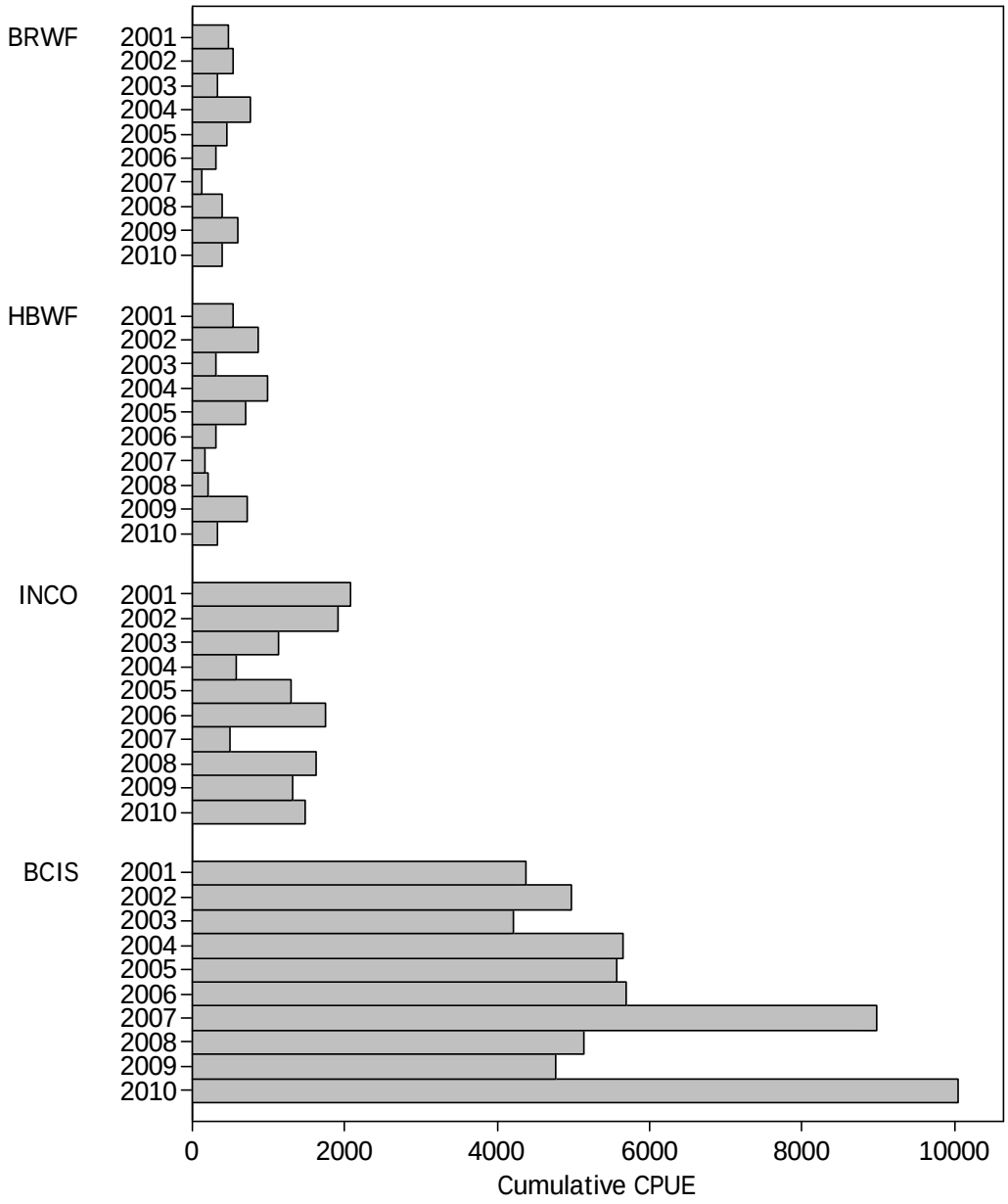

Figure 2. Cumulative CPUE (sum of all daily catches from mid-June through late September) from 2001 through 2010 of broad whitefish (BRWF), humpback whitefish (HBWF), inconnu (INCO), and Bering cisco (BCIS) at Rapids sampling fishwheel, 1,200 km upstream from the Yukon River mouth. Data provided by Stan Zuray, Rapids Research Center.

Porcupine River. They are very numerous in the upper reaches of the Yukon Flats during early October, their spawning season (Brown 2000), are occasionally captured in fish wheels near the Alaska Yukon border (Brown et al. 2007), and are rarely captured as far upstream as the community of Dawson (deGraaf 1981). During sampling activities on

the Kuskokwim River, Alt (1973) found Bering cisco in main-stem habitats and in the South Fork Kuskokwim River, but not farther upstream and not in other tributary systems. Comprehensive sampling of the Susitna River during the fisheries investigations associated with the Susitna River hydroelectric project studies conducted in the early 1980s identified a spawning migration of Bering cisco that remained in the main stem and spawned along gravel bars over a 12 or 15 km reach located downstream from the confluence of the Chulitna, Susitna, and Talkeetna rivers (ADF&G 1983a, 1983b, 1983c, 1983d). These data suggest that Bering cisco follow similar life history strategies in all three rivers where spawning populations are known to exist.

Bering cisco are specifically harvested in coastal villages of western and northwestern Alaska and are a bycatch species in Pacific salmon *Oncorhynchus* spp. fisheries in the Yukon and Kuskokwim rivers. Crawford (1979), for example, reported substantial winter harvests of numerous coregonid species including Bering cisco in several lower Yukon River communities. Similarly, Lavine et al. (2007) described under-ice fishing for Bering cisco in the Kuskokwim Bay communities of Quinhagak and Goodnews Bay. Stickney (1984) provided detailed information on Bering cisco fisheries for the Yukon Kuskokwim Delta communities of Hooper Bay and Kwigillingok. Runfola (2011) reported that Bering cisco were the most abundant coregonid species available to residents of Scammon Bay, a community on the central Yukon Kuskokwim Delta. He further contended that Bering cisco were preferred over all other fish species and that great quantities of them were harvested in summer and winter. Georgette and Shiedt (2005) described the fishing practices of people in the Kotzebue Sound area including their harvests of Bering cisco in the coastal habitats but not in freshwater habitats. Bering cisco are taken in fish wheels in the Yukon and Kuskokwim rivers (Stokes 1985; Daum 2005; Brown et al. 2007) but they pass through gillnets set for Pacific salmon. While subsistence accounts like those above document fisheries for Bering cisco, quantitative harvest data are unavailable in State harvest records because whitefish other than inconnu are not identified to species (Brase and Hamner 2003; Hayes et al. 2008).

Because the commercial fishery for Bering cisco at the Yukon River mouth has the potential to grow and possibly impact subsistence fisheries and the Bering cisco populations that support them, we sought to develop a monitoring program for the populations contributing to the fishery. Achievement of this overall goal requires an understanding of the proportional contribution of the three known populations to the commercial harvest. In this project, we are using genetics techniques to provide estimates of the proportional contributions of the three known Bering cisco populations to the commercial fishery. Specific steps to accomplish this objective include; 1) the development of genetic markers for the species, Bering cisco; 2) development of a genetic baseline characterizing the three known spawning populations; and 3) conducting mixed-stock analysis of subsamples from the fishery to estimate the contributions of baseline populations to the Yukon Delta commercial harvest.

Methods

Genetic tissue collections

Yukon River—Genetic collections of Bering cisco from the Yukon River took place at the Rapids sampling fishwheel site during 2009 and 2010. Pre-spawning Bering cisco are present at Rapids when the sampling project begins in mid-June each year and the migration continues through August (Figure 3). The spawning migration must begin under the ice at the mouth of the river but the details of this have not been described. Several pulses of relative abundance are apparent during most years. The pulses are thought to be composed of rearing groups from locations at successively greater distances from the Yukon River mouth. For example, individuals rearing in the Yukon River delta would enter the river before those rearing in Golovin Lagoon on the Seward Peninsula, which would enter before those rearing in Kotzebue Sound estuaries, etc. The migration tapers off to very low levels by early September. Gonadosomatic index (GSI) data indicate that early and late pulses of Bering cisco are all preparing to spawn at the same time, which appears to be in mid-October (Figure 4; Brown 2000), suggesting that there is no temporal aspect to population separation. None-the-less, sampling for genetic tissue was systematically distributed across the run at a rate of approximately 20 samples per week. In 2009 sampling began in mid-July and concluded in mid-September and in 2010 it began in mid-June and concluded in early August. Pelvic fin clips were stored in ethanol and labeled by species, location, and date of collection.

Kuskokwim River—Genetic material of Bering cisco from the Kuskokwim River were collected opportunistically from an ADF&G sampling fishwheel located near Lower Kalskag during 2006, 2009, and 2010, and from a sampling expedition into the South Fork Kuskokwim River during late September 2010. The South Fork Kuskokwim River is thought to be the only Bering cisco spawning destination in the drainage (Alt 1973). Migration timing in the Kuskokwim River has not been studied, but presumably it is similarly protracted and variable as in the Yukon River. The sampling fish wheel was operational for various periods of time during the 2006, 2009, and 2010 seasons, with the longest operational period in 2010, from early June to late September. Because of the unknown migration timing and the opportunistic nature of the fish wheel collections they could be considered neither randomly nor systematically distributed across the run, but nothing about the collection method introduced any known sources of bias either. The sampling expedition into the South Fork Kuskokwim River took place in late September when most pre-spawning Bering cisco were expected to be at or near their spawning destinations. Sampling was conducted with small mesh gillnets (76 mm stretch mesh) and beach seines. The combined samples from the fish wheel and the spawning destination provided two alternative sampling methods and improved the odds of collecting an adequate number of samples for baseline development. In addition, the two groups, the fish wheel samples taken from the spawning migration and the South Fork Kuskokwim River samples from the spawning destination, might permit a genetic test of the two sample groups for consistency with our hypothesis of a single spawning population in the drainage.

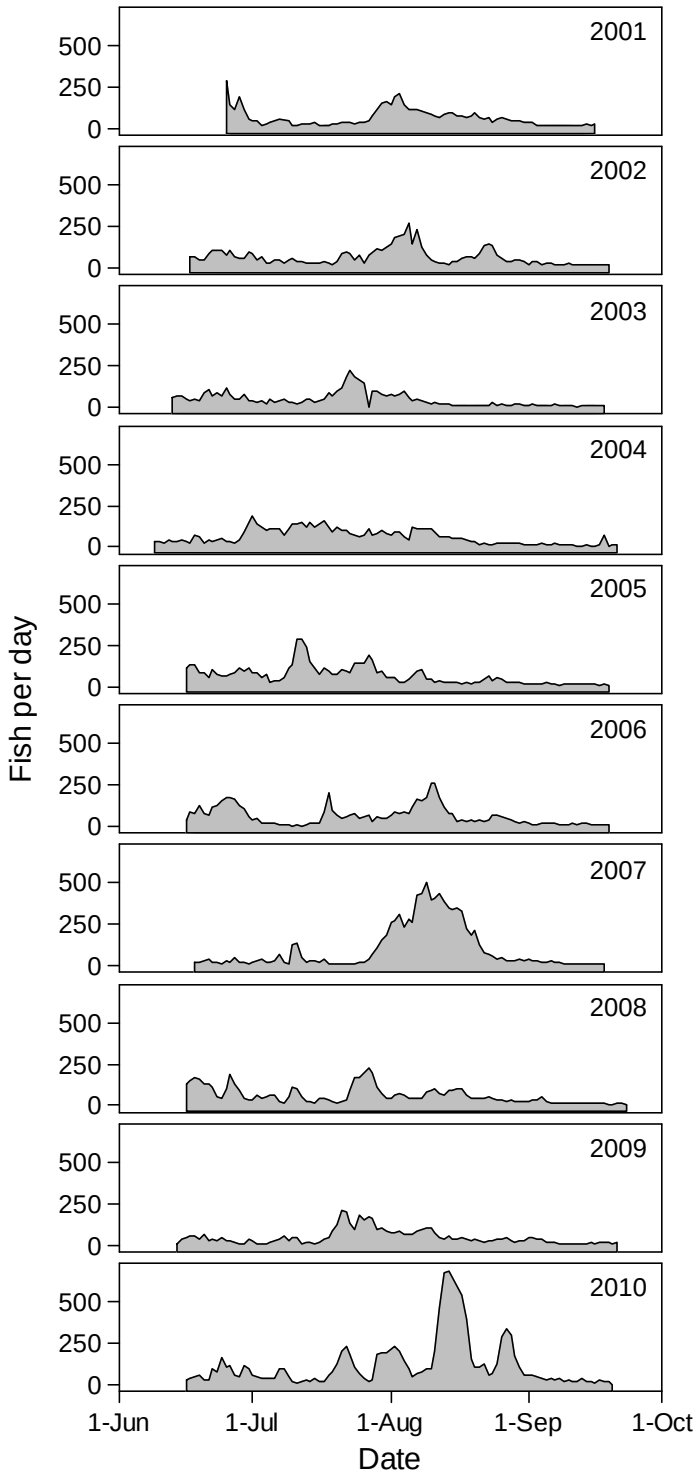

Figure 3. Relative abundance (CPUE = fish · day^{-1}) of Bering cisco at the Rapids sampling fish wheel, approximately 1,200 km upstream from the Yukon River mouth, during the years 2001 through 2010. Data provided by Stan Zuray, Rapids Research Center.

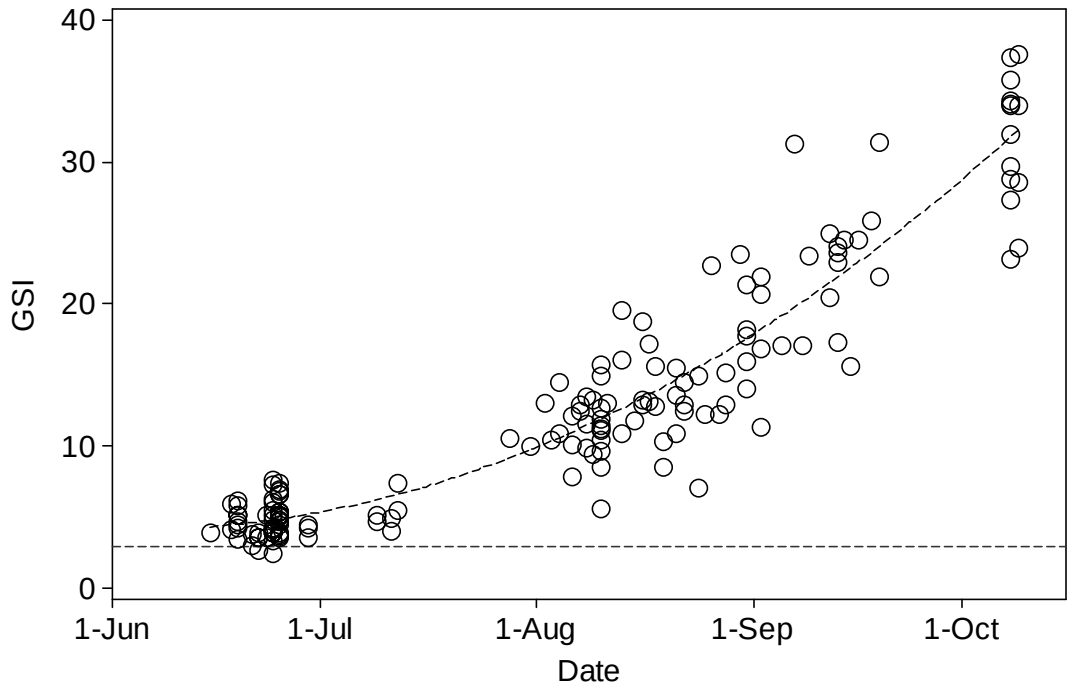

Figure 4. Gonadosomatic index of pre-spawning Yukon River Bering cisco captured in summer during their upstream spawning migration and during the fall spawning season in the upper Yukon Flats. The curved dashed line is the least squared linear regression of $\log_{10}$ transformed GSI data versus the Julian date of capture, which has an R^2 value of 88.6%, indicating that a large fraction of GSI variability can be explained by the date of capture. The horizontal dashed line is at GSI = 3, a level that non-spawning fish rarely exceed.

Susitna River—Genetic material of Bering cisco from the Susitna River was collected opportunistically during 2009, 2010, and 2011 from ADF&G sampling fish wheels located in the lower Susitna River, and from sampling expeditions in October 2009 and 2010 in the spawning region near the mouth of Montana Creek, which was identified during the Susitna River hydroelectric project (ADF&G 1983d). Fish wheel sampling crews collected pelvic fin clips from all Bering cisco they encountered. Sampling expeditions into the Bering cisco spawning reach captured Bering cisco with beach seines and electroshocking boats. Because the Susitna River hydroelectric project provided compelling evidence that there was a single Bering cisco spawning population in the drainage (ADF&G 1983a), the genetic samples from fish wheel and spawning ground collections were pooled into a single baseline collection.

Commercial fishery collections—Larry DuBois (ADF&G) supervised the sampling program for the commercial whitefish fishery. He and his sampling staff collected large numbers of fin clips from the fishery during 2009, 2010, and 2011. Bering cisco harvested in late September in the north (Kotlik) and south (Emmonak, Alakanuk, and Nunam Iqua) Delta regions were purchased and shipped to Anchorage in several shipments that were undifferentiated mixtures of north and south Delta captures, although

according to ADF&G records, the majority of the harvest typically comes from the north Delta. The capture location, north or south Delta, of Bering cisco harvested in October 2010 was known to the samplers and location-specific genetic samples were therefore collected. Similarly, capture location was known for the 2011 samples taken in September. In 2009 fish were shipped in large totes, each containing several hundred fish, while in 2010 fish were shipped in boxes, each containing about 50 fish and weighing approximately 23 kg. Sampling was conducted on every shipment. When sampling from the totes, Bering cisco were simply selected from the surface layer of fish without regard for size until a target number of fish were sampled. Size is relatively uniform, ranging from about 30 to 40 cm fork length, so size selectivity is not thought to be an issue. Sex could not be determined until gonads were exposed so there was no sex selectivity to the sampling. When sampling from boxes, a box was selected from the top of a stack and all Bering cisco within the box were sampled. These samples are certainly representative of the harvest. Samples from the commercial fishery will be collected from the 2012 harvests to complete that component of the project.

Genetics analyses

Develop and test genetic baseline— a suite of microsatellite loci were assembled for testing using both loci from other whitefish species (e.g., Olsen et al. 2007) and novel loci developed using Bering cisco DNA. All polymorphic loci were evaluated using the backward elimination procedure in the program BELS (Bromaghin 2008) to identify the suite that provided the greatest accuracy and precision for mixed-stock analysis (MSA). The genetic baseline was developed using more than 200 samples each from the Yukon, Kuskokwim, and Susitna River spawning populations. Accuracy and precision of the selected loci was further tested using the program SPAM 3.7 (Debevec et al. 2000). This program uses parametric bootstrap resampling to simulate 1000 artificial mixtures (N=400) for each population based on that population's allele frequencies and assumptions of Hardy-Weinberg and gametic phase equilibrium. These 100% mixtures were analyzed with the conditional maximum likelihood (CML) method, with parametric bootstrapping of the baseline, to estimate mean allocation and precision. For perfect baseline performance, the mean contribution estimate for each population will approximate 100%, however, estimates of approximately 90% are considered robust for mixture analysis (Seeb and Crane 1999).

Genotyping—Total genomic DNA was extracted from fin tissue (~25mg) using proteinase K with the DNeasy™ DNA isolation kit (Qiagen Inc. Valencia, CA), quantified with fluorometry and diluted to a standard concentration. An MJResearch DNA Engine® thermal cycler was used to perform polymerase chain reactions (PCR) in 10 µl volumes; general conditions were: 2.5 mM $MgCl_2$, 1X PCR buffer (20 mM Tris-HCl pH 8.0, 50 mM KCl), 200 µM of each dNTP, 0.40µM fluorescently labeled forward primer, 0.40 µM unlabeled reverse primer, 0.008 units *Taq* polymerase, and 1 µl of DNA (30ng/µl). Standard thermal cycling conditions were: initial denaturation cycle of 94°C for 3 min, followed by 94°C for 1 min, 50-62°C for 1 min (locus-specific annealing temperature), 72°C for 1 min, with a final single cycle of 72°C for 10 min. One-half µl of PCR product was electrophoresed and visualized with the Applied Biosystems 3730 Genetic Analyzer utilizing a polymer denaturing capillary system. Microsatellite allele sizes were estimated and scored by the computer program GeneMapper® version 4.0. Applied

Biosystems GeneScan™-600 LIZ® size standards, 20-600 bases, was loaded in all lanes as an internal lane standard. All scores were verified manually. Alleles were scored by two independent researchers, with discrepancies resolved by replicating the analysis for the samples in question and repeating the double scoring process until scores matched (unresolved scores were excluded from further analysis).

Commercial Fishery Mixed-Stock Analysis—Mixture samples (N=200-250) from the Bering cisco commercial fishery in the Yukon River were collected in 2009, 2010, and 2011. Additional fishery samples will be collected in 2012. The proportion of Yukon, Kuskokwim, and Susitna River Bering cisco in these samples will be estimated using baseline data and the Bayesian mixture modeling method as implemented in the program Bayes (Pella and Masuda 2001). Previous work by the authors and others indicate that the Bayesian method provides greater accuracy than CML (Pella and Masuda 2001; Koljonen et al. 2005). Bayesian techniques do not lend themselves to simulation studies because of computational time; thus, CML simulations will be used as a conservative guide when testing the baseline (see genetic baseline above).

Results

Genetic tissue collections

Baseline and mixed-population tissue samples were successfully collected between 2009 and 2011. Well over 200 baseline samples were collected from the Yukon, Kuskokwim, and Susitna River populations (Table 1). Sampling personnel in all three river drainages pursued these collections over multiple years. Large numbers of mixed-population samples from the commercial fishery were similarly collected, with the harvest location, north or south mouth, identified for the October 2010 and September 2011 samples. It is our intention to collect genetic tissue samples from the commercial fishery harvest again in fall 2012, consistent with the original investigation plan. These baseline and mixed-population samples will enable us to accomplish our goal of estimating the proportional contributions of the three known Bering cisco populations to the commercial fishery during three annual seasons.

Genetics analyses

Microsatellite genetic markers were developed using Bering cisco individuals from the Yukon River to create a “library” of species-specific genetic markers. A total of 47 potential markers were tested from the library resulting in 18 highly polymorphic loci that were successfully optimized. This final suite of 18 microsatellites was used to run all baseline samples. In the initial genetic processing of samples from the three baseline locations, QC was conducted to exclude samples of poor DNA quality and those that were genetically identified as non-Bering cisco. The baseline data was then analyzed using genetics software to determine the level of genetic differentiation between the three B. cisco populations. Preliminary results indicate that there is statistically significant genetic divergence for all pairwise comparisons of the Yukon, Kuskokwim, and Susitna populations. These results allowed for simulation analysis to determine if enough genetic differentiation among stocks exists to accurately estimate the stock composition of Bering cisco harvested in commercial catches. All estimates from the simulations were

above the 90% threshold (the robust standard for mixture analysis), suggesting that we will be able to use MSA to estimate contributions of populations of commercial harvest samples.

Commercial harvest samples have begun to be processed and analyzed. The 2010 collections that were specific to the north Delta (*n* = 500) and south Delta (*n* = 260) have been processed and the results are currently being analyzed. In addition, a sub-sample (*n* = 96) from the in-river, winter fishery that took place in January 2010 at Mountain Village have also been processed. The Mountain Village samples were not part of the original project proposal but they provided an opportunity to test the assumption that all Bering cisco in upstream reaches of the Yukon River are members of the Yukon River population, perhaps beginning their spawning migration for the next fall. The results from the 2010 commercial harvest samples and the Mountain Village winter samples should be forthcoming within a few weeks. Subsamples from the 2011 collections from the north Delta (n = 500) and south Delta (n = 500) are being prepared for analysis in the laboratory at this time.

Table 1. Details of Bering cisco genetic sample collections from the three known spawning populations (baselines) and from the commercial fishery at the mouth of the Yukon River (mixed-population).

Collection	Year	Annual *n*	Total *n*
Yukon River baseline	2009	146	346
	2010	200	
Kuskokwim River baseline	2006	30	294
	2009	40	
	2010	224	
Susitna River baseline	2009	34	214
	2010	120	
	2011	60	
Mixed-population samples from the fishery	2009	400	2,970
	2009 (December)	80	
	2010 (January)	100	
	2010 (September)	630	
	2010 (October)	500 (North Delta)	
		260 (South Delta)	
	2011 (September)	500 (North Delta)	
		500 (South Delta)	

Discussion

The initial genetic analysis revealed that Bering cisco spawning in the Yukon and Kuskokwim rivers are genetically distinct populations. This is the first evidence of genetic population structure in this species. These results underscore the need to evaluate the Bering cisco commercial fishery in the lower Yukon River to determine the extent to which each population contributes to and is impacted by the harvest. The final phase of this study will evaluate harvest samples to estimate the proportional contribution of the Yukon and Kuskokwim populations and assess if the contribution varies in space and time.

Acknowledgements

This project would not have been possible without the contributions of many individuals and organizations across the vast geographic area encompassed by these collections. The U.S. Fish and Wildlife Service, Office of Subsistence Management, provided funds for this project under agreement FRMP 10-209. Many of the samples were collected opportunistically by people working in the drainages of interest so no funds were requested. Many samples were collected a year or more before funds became available simply because of an interest in the project. The ADF&G has played a critical role in this project, providing important baseline samples from the Kuskokwim and Susitna River populations, and mixed-population samples from the commercial fishery. Special thanks go out to ADF&G employees Kevin Schaberg and his crews at the Kalskag fish wheel on the Kuskokwim River, Richard Yanusz and Pete Cleary and their crews on fish wheels in the lower Susitna River, John Burr and Andy Gryska and their crew on the Susitna River spawning area, and Larry DuBois and his sampling crews for their work with the commercial fishery. Additionally, we appreciate the cooperation that Bill Templin and others at the ADF&G Gene Conservation Laboratory have shown in making the genetic samples from the commercial fishery available to staff at the USFWS Conservation Genetics Laboratory. David Orabutt and Mike Thalhauser of the Kuskokwim Native Association collected a large number of the baseline samples from the Kuskokwim River. And Stan Zuray and his youth sampling crew from Tanana collected the baseline samples for the Yukon River. There are many more individuals who contributed directly or indirectly to these efforts and we appreciate them all.

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