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Species Identification of Salmon Seized from the Chinese Vessel *Run Da*

by

Christine M. Kondzela, Jacqueline A. Whittle, Charles M. Guthrie III, Hanhvan T. Nguyen, Sharon L. Wildes, and Jeffrey R. Guyon

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Alaska Fisheries Science Center, Auke Bay Laboratories
17109 Point Lena Loop Road, Juneau, Alaska 99801, USA

and

Lane M. Munroe, John S. McKenzie, and Donovan K. Antalik

U.S. Coast Guard
District 17, Enforcement Branch
709 West 9th Street
Juneau, Alaska 99802, USA

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Introduction

On June 16, 2018, U.S. Coast Guard personnel from the cutter *Alex Haley* boarded the Chinese-flagged fishing vessel *Run Da* at approximately 43° 30.3'N and 160° 57.9'E (Fig. 1). Approximately 80 tons of salmon and 1 ton of squid were found onboard[1]. The location of where the fish were caught is unknown. The salmon were frozen whole into large blocks of fish and the blocks bagged. It was estimated that most of the salmon were chum salmon (*Oncorhynchus keta*), 35–40 cm in length. Other fish included at least 3 Chinook salmon (*O. tshawytscha*), 5 tuna (species unknown), and approximately 10 sharks (species unknown). Two of the Chinook were 71.8 cm and 75.6 cm in length. Tissue samples from a subset of the salmon were collected by U.S. Coast Guard personnel for genetic analysis by the Auke Bay Laboratories.

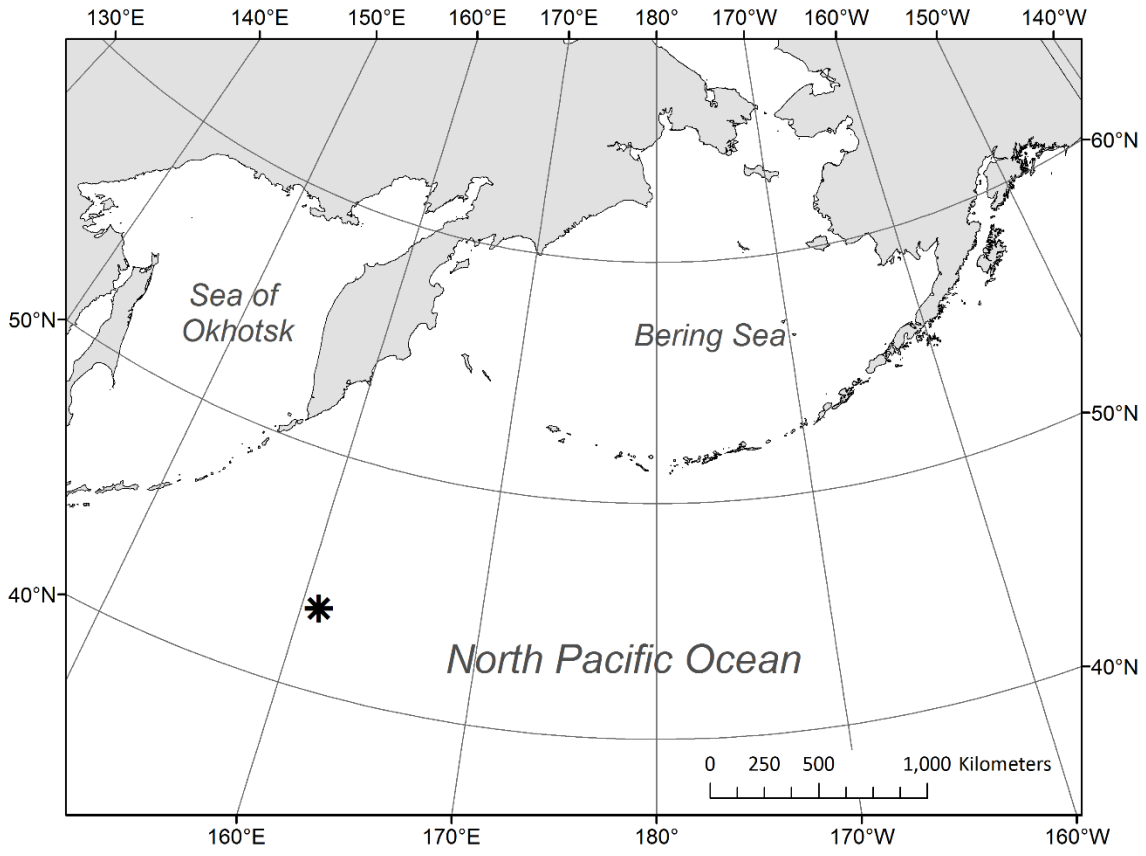

Figure 1. Location (*) of the Chinese vessel *Run Da* in the North Pacific Ocean when boarded by personnel from the U.S. Coast Guard cutter *Alex Haley* on June 16, 2018.

[1] https://content.govdelivery.com/accounts/USDHSCG/bulletins/1f94a1a

Materials and Methods

Sample Collection

Two samples were collected per bagged block of salmon from different locations in the fish hold. A genetic sample collection kit with a NPAFC collecting protocol was used to store samples in 18 ml plastic tubes in 99.8% isopropyl alcohol. The U.S. Coast Guard retained custody of the 52 samples until they were shipped by a commercial shipper from Kodiak, Alaska to Juneau, Alaska. Upon receipt of the samples at the Auke Bay Laboratories, the alcohol was decanted, the tissue samples were transferred to 2 ml screw-cap tubes, and frozen at -70°C. Most of the samples contained a piece of skin and fin tissue. Any excess tissue was placed back into the large tubes and frozen at -70°C.

Genetic analysis

DNA was extracted from the 52 samples with DNeasy Blood and Tissue Kits (QIAGEN, Inc., Germantown, Maryland)[2] as described by the manufacturers and processed with a QIAcube HT (QIAGEN, Inc.). Extracted DNA was stored in 96-well elution plates at -20ºC.

The suite of 13 microsatellite loci represented in the coastwide chum salmon genetic baseline (Beacham et al. 2009a) were amplified for each sample with the polymerase chain reaction (PCR) in three multiplexed panels. Each PCR amplification was conducted in a 5 µL volume containing ~10 ng of DNA, 2.5 µL QIAGEN Multiplex PCR Mastermix, 0.2 µM of each primer, and RNase-free water. Primer sequences for the 13 loci have been described in the following publications: *Oki2* (Smith et al. 1998), *Oki100* (Beacham et al. 2009b), *Omm1070* (Rexroad et al. 2001), *Omy1011* (Spies et al. 2005), *One101*, *One102*, *One104*, *One111*, *One114* (Olsen et al. 2000), *Ots103* (Beacham et al. 1998), *Ots3* (Greig and Banks 1999), *OtsG68* (Williamson et al. 2002), and *Ssa419* (Cairney et al. 2000). Thermal cycling for the PCR amplification of DNA fragments was performed on a dual 384-well GeneAmp PCR System 9700 (Life Technologies, Inc., Carlsbad, CA). *Oki2* was amplified separately with the following protocol and the post-PCR product was pooled with that of the other loci in Panel A: initial denaturation at 94ºC for 5 min, then 37 cycles at 94ºC for 20 s, 52ºC for 1 min, and 72ºC for 1 min, followed by a final polymerization step at 72ºC for 5 min and then held at 15ºC until removal from the thermocycler. The other loci in Panel A (*Omm1070*, *Omy1011*, *One102*) were amplified with the following protocol: initial denaturation at 95ºC for 15 min, then 16 cycles at 94ºC for 30 s, 60ºC (-0.5ºC per cycle) for 90 s, and 72ºC for 1 min, then 24 cycles at 94ºC for 30 s, 52ºC for 90 s, and 72ºC for 1 min, followed by a final polymerization step at 60ºC for 30 min and then held at 15ºC until removal from the thermocycler. Loci in Panel B (*Oki100*[3], *One101*,

[2] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.
[3] *Oki100* was amplified separately and the post-PCR product was pooled with that of the other loci in Panel B prior to analysis on the ABI 3130*xl*.

One111, *Ots3*, *Ssa419*) and Panel C (*One104*, *One114*, *Ots103*, *OtsG68*) were amplified with the following protocol: initial denaturation at 95ºC for 15 min, then 34 cycles at 94ºC for 30 s, 60ºC for 90 s, and 72ºC for 1 min, followed by a final polymerization step at 60ºC for 30 min and then held at 15ºC until removal from the thermocycler.

Samples from the PCR reactions were diluted into 96-well plates for analysis with a 16-capillary, 36 cm array on the Applied Biosystems (ABI) 3130*xl* Genetic Analyzer as follows: 1 µL diluted PCR product (1:25 of Panels A and C; 1:50 of Panel B), 8.6 µL Hi-Di™ formamide, and 0.4 µL GeneScan™ 600 LIZ® size standard (Life Technologies, Inc., Carlsbad, CA). Samples were denatured at 95ºC for 3 min, cooled to 4ºC and then analyzed on the ABI 3130*xl*. Output from the ABI 3130*xl* was reviewed with GeneMapper® 5.0 software (Life Technologies, Inc., Carlsbad, CA) to determine genotypes.

When the results of the microsatellite analysis indicated that species other than chum salmon were likely present, a single-nucleotide polymorphism (SNP) assay to identify salmon species (Habicht et al. 2019) was used with samples of known salmonid species to determine the salmon species of each sample.

Results and Discussion

Most of the samples collected from the fishing vessel *Run Da* were pink salmon. The species identification SNP assay identified one chum salmon, four Chinook salmon, and 47 pink salmon. The microsatellite analyses also identified one chum salmon. Many microsatellite loci developed from one salmonid species can be used for genetic analyses in other species (Cairney et al. 2000, Scribner et al. 1996). We determined that one Chinook was sampled twice because identical genotypes were observed for the 6 loci that could be genotyped with the microsatellite markers used for the chum salmon coastwide baseline, indicating that there were 3 unique Chinook salmon in the collection of samples. Additional analysis of the Chinook samples with the suite of 43 Chinook SNPs (Templin et al. 2011) used for mixed-stock analyses (e.g., Guthrie et al. 2018) confirmed this result.

The small number of chum and Chinook salmon samples preclude estimating the probable stocks of origin, an analysis that is usually best done with larger sample sizes, for example, more than 100 samples. Although there is no publically available coastwide DNA baseline for pink salmon, when such a baseline becomes available, we anticipate analyzing the pink salmon samples from the *Run Da* for stock identification. Past genetic stock analyses with allozymes (Noll et al. 2001), a genetic marker type no longer in use in salmonid research, a regional-scale population structure study with microsatellite markers (Beacham et al. 2012), and recent development of pink salmon SNPs (Tarpey et al. 2018) support the premise that DNA markers will at a minimum identify the samples as originating from Asia or North America.

Based on the range of non-Chinook fish lengths (35–40 cm) and assuming the fish were caught in the same year that the vessel was intercepted, the pink salmon were from the even-year broodline and would have returned to their rivers of origin that summer or fall to spawn. The location of where the salmon were caught by the *Run Da* is not known, but if the salmon catch occurred near the western North Pacific interception location of the vessel, then the pink salmon were likely of Asian origin based on known pre-spawning ocean distributions (Radchenko et al. 2018, Myers et al. 1996).

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