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**Microsatellite Identification of Sockeye Salmon Rearing
in the Bering Sea During Summer 2016**

by

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Abstract: Stock composition of Sockeye Salmon (*Oncorhynchus nerka*) caught in the southern central Bering Sea during a Japanese research cruise in the summer of 2016 was estimated through an analysis of microsatellite variation. Variation at 14 microsatellites was analyzed for immature Sockeye Salmon, and a 415-population baseline spanning Japan, Russia, Alaska, Canada, and Washington State was used to determine the stock composition of the fish sampled. Alaskan-origin Sockeye Salmon were the most abundant in the catch of immature individuals, comprising 85.3% of all Sockeye Salmon caught, with the catch dominated by Sockeye Salmon of Bristol Bay origin (80.0%). Canadian-origin salmon accounted for an average of 3.3% of the catch, while Russian-origin Sockeye Salmon accounted for 11.3% of the catch, with 382 individuals of the catch genotyped.

Introduction

The application of DNA-based genetic markers to salmon stock identification studies has provided greater resolution of stock composition relative to other biological markers (Beacham et al. 2005) such as scale pattern analysis (Ishida 1989), parasites (Bennett et al. 1998), and otolith characters (Sohn et al. 2005). High resolution stock composition of Sockeye Salmon mixed-stock samples to lake or river of origin is crucial to our understanding of their population-specific responses to recent climatic regime shifts in the north Pacific Ocean (Welch et al. 2000; Mueter et al. 2002). DNA markers provide a natural tag with which the origin of all individual fish within a sample can be determined if required (Beacham et al. 2005). This is in marked contrast to physical tags, where recovery rate of individually marked juvenile salmon fish is typically well under 1% (Hartt and Dell 1986; Trudel et al. 2009). By employing DNA-based genetic markers for stock identification, larger scale sampling of juvenile salmon in the ocean can be undertaken in order to determine migration routes and areas of marine residence.

The initial application of DNA-based genetic markers to estimation of Sockeye Salmon stock composition in the Bering Sea was reported by Habicht et al. (2010). By applying a set of 45 single nucleotide polymorphisms (SNPs) to identify population structure in a Pacific Rim distribution of populations, Habicht et al. (2010) defined eight regional stocks of Sockeye Salmon in the baseline, with a single eastern Gulf of Alaska (EGOA) stock comprised of Sockeye Salmon from southeast Alaska, British Columbia, and Washington. This EGOA stock, which comprised a mixture of Canadian and American populations, was reported to comprise up to 10% of immature Sockeye Salmon sampled in the south central Bering Sea during September of 2002 and 2003. Beacham et al. (2011) reported that stock composition of juvenile Sockeye Salmon captured between 15 July to 9 August 2009 during a research cruise conducted with the Japanese research vessel *Hokko-maru* in the central and northern Bering Sea was 86% Alaskan origin, 10% Russian origin, and 4% Canadian origin. Beacham et al. (2016) expanded the study of Beacham et al. (2011) to include analysis of variation in size and stock composition of immature Sockeye Salmon caught during Japanese research cruises in the south central

Bering Sea in 2009, 2011, 2012, 2013, and 2014. In this report, we outline the size and stock composition of immature Sockeye Salmon caught in the south central Bering Sea during the 2016 Japanese research cruise with the *Hokko-maru*.

Methods and Materials

Sample collection and analysis

Juvenile Sockeye Salmon were captured between late July to early August during a research cruises conducted with the Japanese research vessel *Hokko-maru* in the central and southern Bering Sea during 2016. The cruise was designed to conduct the annual survey of Japanese stocks of Chum Salmon (*O. keta*) in the southern Bering Sea, with cruise tracks and details of the cruise outlined annually by Japanese investigators (eg. Morita et al. (2011)). Details of the trawl nets and sampling regime were also outlined by Morita et al. (2011). Juvenile Sockeye Salmon captured were weighed to the nearest 10 g, fork length measured (nearest mm), a tissue sample collected for subsequent analysis of genetic variation. Fish age was determined from scales by staff of the Hokkaido National Research Fisheries Institute, Japan Fisheries Research and Education Agency, Japan. Age reporting followed the method outlined by Koo (1962). An individual designated as x.1 spent 1-3 winters rearing in fresh water (x years), and one winter in the ocean, having been sampled during the summer in their second ocean year. Similarly, individuals identified as x.2 reared for two winters in the ocean, with subsequent capture and sampling during their third summer of ocean rearing. Individuals identified as x.3 were in their fourth summer of ocean rearing. Tissue samples were preserved in 100% ethanol, and sent to the Molecular Genetics Laboratory at the Pacific Biological Station of Fisheries and Oceans Canada in Nanaimo. Fourteen microsatellites (Beacham et al. 2005) were surveyed with an ABI 3730 capillary DNA sequencer, and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard as outlined by Beacham et al. (2005).

Baseline populations

The baseline used for estimation of stock composition consisted of a survey of about 73,000 Sockeye Salmon from 415 populations from Japan, Russia, Alaska, Canada, and Washington as outlined by Beacham et al. (2016). Baseline populations were organized into 50 reporting groups as outlined by Beacham et al. (2016).

Estimation of stock composition in mixed-stock samples

Analysis of the samples of juveniles was conducted with a Bayesian procedure (BAYES) as outlined by Pella and Masuda (2001), with a modified version of the program developed as a C-based program (Neaves et al. 2005). In the analysis, eight 20,000-iteration Monte Carlo Markov chains of estimated stock compositions were produced, with initial starting values for each chain set at 0.90 for a particular population which was different for each chain. Estimated stock compositions were considered to have converged when the shrink factor was < 1.2 for the eight chains (Pella and Masuda 2001). The last 1,000 iterations from each of the eight chains were then combined, and for each fish the probability of originating from each population in the baseline was determined. Individuals were assigned to a specific population which was estimated to

have the highest probability of correct assignment. Allocations were made to 415 individual populations, and these were summed to provide estimates to 50 regional stock groups (Beacham et al. 2016). Regional stock groups were not listed in Table 1 if estimated stock composition of the reporting group was zero.

Results

Location, size, and age of catch

The Sockeye Salmon analyzed were typically captured between 53° N and 59° N and 175° E and 175° W in the central Bering Sea (Figure 1). Ocean age x.1 individuals were separated from ocean age x.2 and x.3 individuals in fork length, with a bimodal distribution observed in observed fork length (Figure 2). Age x.1 individuals were typically less than 440 mm. Mean fork length of x.1 individuals was 385 mm (n=214, SD=27 mm) for individuals caught in 2016. Mean length of age x.2 individuals was 481 mm (n=159, SD=28 mm) for individuals caught in 2016, and mean fork length of x.3 individuals was 513 mm (n=12, SD=24 mm).

Body weight distributions were similarly well defined between age x.1 and x.2 individuals, with x.1 individuals typically less than 1,000 g in weight, reflective of the distribution of body weight (Figure 3). Mean weight of age x.1 individuals was 714 g (SD=157 g) for individuals caught in 2016. Mean weight of age x.2 individuals was 1,461 g (SD=271 g) for individuals caught in 2016, and mean weight of age x.3 individuals was 1,798 g (SD=330 g).

Stock composition of Bering Sea samples

Alaskan-origin Sockeye Salmon were the most abundant in the annual catch of immature individuals in the central Bering Sea. Bristol Bay origin immature Sockeye Salmon comprised 80.0% of all Sockeye Salmon caught during the 2016 cruise, with Alaskan-origin salmon comprising 85.3% of the catch (Table 1). Canadian-origin Sockeye Salmon accounted for 3.3% of the catch during 2016. Russian-origin salmon accounted for 11.3% of the catch during 2016. Sockeye Salmon from Bristol Bay dominated the catch of Alaskan-origin salmon, with those from the Egegik River drainage the largest contributor to the catch, with an annual contribution of 26.3% of individuals estimated to be of Egegik River origin (Table 1). Sockeye Salmon from the Nushagak River drainage were estimated to be the next most important contributor to the catch, comprising 15.3% of the salmon sampled. Sockeye Salmon from the Ugashik River drainage were estimated to comprise 9.3% of the sample. Sockeye Salmon from the Kvichak River drainage were estimated to comprise 7.4% of immature Sockeye Salmon sampled during the cruise, those from the Naknek River 6.7%, and those from the Wood River 6.2% of the individuals sampled (Table 1).

Canadian-origin salmon were estimated to originate primarily from the Central Coast and river drainages to the north in British Columbia, and comprised 3.3% of the research catch (Table 1). Russian-origin Sockeye Salmon caught in the central Bering Sea during 2016 comprised 11.3 % of the immature Sockeye Salmon sampled (Table 1). The

most abundant stocks contributing from Russia originated from Kuril Lake (5.8%), Karaginsky Bay (2.2%), and southeastern Kamchatka (1.9%)

Discussion

Mean fork length of the age x.1 immature Sockeye Salmon sampled in the south central Bering Sea during 2016 was larger than observed in six previous years of sampling. For example, the mean fork length of age x.1 individuals was 385 mm (SD=27 mm) in 2016 in comparison with mean fork lengths of 348 mm (SD=26 mm), 349 mm (24 mm), 344 mm (33 mm), 349 mm (24 mm), 363 mm (34 mm), and 372 mm (26 mm) for individuals caught in 2009, 2011, 2012, 2013, 2014, and 2015, respectively (Beacham et al. 2016). However, mean length of age x.2 individuals sampled during 2016 (481 mm, SD = 28 mm) was similar to those sampled in previous years, with mean lengths observed of 476 mm (27 mm), 468 mm (27 mm), 463 mm (32 mm), 462 mm (49 mm), 472 mm (41 mm), and 473 (26) for individuals caught in 2009, 2011, 2012, 2013, 2014, and 2015, respectively. The mean length of age x.3 individuals (513 mm, SD=24 mm) sampled in 2016 was similar to those sampled in previous years, with mean lengths observed of 548 mm (40 mm), 540 mm (53 mm), 503 mm (35 mm), and 526 mm (49 mm), 480 mm (28 mm) and 548 (40mm), in 2009, 2011, 2012, 2013, 2014, and 2015, respectively.

Similar to the results observed for mean fork length, mean weight of age x.1 Sockeye Salmon was larger in 2016 (714 g, SD = 157 g) than observed in six previous years of sampling. For example, mean weight of age x.1 individuals was 490 g (104 g), 510 g (114 g), 491 g (143 g), 496 g (111 g), 576 g (180 g), and 631 g (136 g) for individuals caught in 2009, 2011, 2012, 2013, 2014, and 2015, respectively (Beacham et al. 2016). Mean weight of age x.2 individuals sampled during 2016 (1,461 g, SD = 271 g) was slightly heavier but similar to those sampled in previous years, with mean weights observed of 1300 g (221 g), 1270 g (230 g), 1251 g (251 g), 1272 g (367 g), 1371 g (330 g), and 1311 g (230 g) for individuals caught in 2009, 2011, 2012, 2013, 2014, and 2015, respectively. The mean weight of age x.3 individuals sampled during 2016 (1,798 g, SD = 330 g) was similar to those sampled in previous years, with mean weights observed of 2140 g (573 g), 2060 g (730 g), 1530 g (303 g), 1933 g (589 g), 1462 g (309 g), and 1,311 g (680 g) in 2009, 2011, 2012, 2013, 2014, and 2015, respectively.

Analysis of stock composition indicated that the catch was dominated by stocks of Bristol Bay origin, similar to results of tagging experiments (Myers et al. 1996), scale pattern analysis (Bugaev and Myers 2009), and previous analyses of genetic stock composition (Habicht et al. 2010; Beacham et al. 2011; Beacham et al. 2016). Juvenile Sockeye Salmon catches in the eastern Bering Sea were also reported to be dominated by salmon of Bristol Bay origin (Seeb et al. 2011). Based upon geography and relative abundance, Sockeye Salmon of Bristol Bay origin should be expected to dominate catches of immature Sockeye Salmon rearing in the Bering Sea, with Sockeye Salmon originating from Russia the next most abundant stock. These were precisely the results observed from our analysis of immature Sockeye Salmon rearing in the central Bering Sea in July and August of 2009, 2011, 2012, 2013, and 2014 (Beacham et al. 2016).

However, in 2015, Russian-origin Sockeye Salmon were noticeably absent from the immature Sockeye Salmon sampled, comprising only 1% of the sample. It is uncertain why the Russian-origin component of the immature Sockeye Salmon sampled in the southern central Bering Sea during 2015 declined to the lowest relative proportion since comparative studies began with the 2009 research survey. However, in the 2016 sampling, the Russian-origin component of the catch was estimated at 11.3%, in line with results observed prior to 2015.

Previous analyses reported by Beacham et al. (2016), along with the results of Habicht et al. (2010), indicated that some small portion of Canadian Sockeye Salmon rear in the Bering Sea during summer, with perhaps some trace contribution by Sockeye Salmon from Washington State. In 2016, Canadian-origin Sockeye Salmon were the third most abundant stock in the sample of immature Sockeye Salmon sampled during the research cruise, similar to previous surveys, comprising about 3% of the sample, similar to values observed prior to the 2015 survey. Recent genetic and otolith mark analyses indicated that Canadian Chum Salmon (*O. keta*) were also distributed in the Bering Sea (Urawa et al. 2009). As outlined by Habicht et al. (2010), it is uncertain whether rearing of Canadian-origin salmon in the Bering Sea is something new, perhaps brought on by changes in climate, or is in fact typical of a normal rearing pattern of summer movement in the Bering Sea and winter rearing in the Gulf of Alaska.

Improved information of location and timing of specific stocks of Sockeye Salmon in the Bering Sea and North Pacific Ocean can be obtained through the application of DNA technology to salmon stock identification problems. The major limitation at the present time to refine knowledge on stock-specific areas of ocean residence and timing of migration movement is the difficulty and cost associated with obtaining the appropriate samples from ocean rearing areas and migration routes.

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Table 1. Mean catch per unit effort (CPUE 13 stations (Fig. 1)) and estimated stock compositions (% , 95% confidence limits in parentheses) of mixed-stock samples of Sockeye Salmon from the central Bering Sea, 2016. Estimated stock compositions were derived from applying a 415-population baseline for each sample, with a Pacific Rim distribution of the baseline ranging from Japan, Russia, Alaska, British Columbia, and Washington State as outlined by Beacham et al. (2016). Reporting regions with no estimated stock composition are not listed. N is sample size.

Major region	Stock	Estimate			
Age		All	Age x.1	Age x.2	Age x.3
CPUE		30.0	16.5	12.5	0.9
N		382	210	159	12
British Columbia	Fraser River	0.2 (0.3)	0.3 (0.4)	0.0 (0.1)	0.3 (3.4)
	Central Coast	0.6 (0.5)	0.1 (0.3)	0.5 (0.8)	3.4 (6.3)
	Skeena River	0.8 (0.5)	0.0 (0.1)	1.3 (0.9)	14.8 (10.0)
	Stikine River	0.8 (0.9)	1.6 (1.0)	0.6 (1.2)	1.3 (4.9)
	Taku River	0.3 (0.3)	0.5 (0.5)	0.2 (0.7)	0.7 (3.7)
	Alsek River	0.6 (0.6)	0.4 (0.9)	2.0 (1.6)	0.0 (2.0)
Southeast Alaska	SE Alaska	0.1 (0.3)	0.3 (0.5)	0.3 (0.7)	0.3 (1.5)
Central Alaska	Cook Inlet	1.4 (0.8)	1.6 (0.9)	2.5 (1.8)	1.2 (4.7)
	Copper River	0.3 (0.4)	0.6 (0.6)	0.0 (0.1)	0.0 (0.7)
	Kodiak Island	1.1 (0.9)	1.2 (1.2)	2.5 (2.2)	0.0 (1.5)
	Chignik River	2.4 (1.5)	1.2 (1.6)	2.0 (2.7)	10.7 (11.5)
Western Alaska	Kuskowim	0.0 (0.1)	0.1 (0.5)	0.0 (0.1)	0.1 (1.0)
	Norton Sound	0.0 (0.1)	0.0 (0.1)	0.0 (0.2)	0.0 (0.5)
Bristol Bay	Ugashik River	9.3 (2.6)	3.7 (4.7)	17.3 (4.5)	0.0 (0.8)
	Egegik River	26.3 (4.0)	35.7 (5.4)	16.5 (5.6)	3.6 (8.7)
	Naknek River	6.7 (2.8)	3.6 (3.1)	12.2 (4.8)	37.6 (22.3)
	Alagnak River	4.8 (1.3)	6.1 (1.9)	2.8 (2.2)	0.0 (0.8)
	Kvichak River	7.4 (3.2)	7.9 (3.7)	3.5 (4.3)	0.1 (1.4)
	Nushagak River	15.3 (2.9)	18.1 (4.4)	16.2 (4.4)	1.0 (4.8)

	Wood River	6.2 (2.0)	8.2 (2.9)	3.7 (2.6)	0.5 (3.2)
	Igushik River	0.1 (0.4)	0.0 (0.2)	0.0 (0.3)	0.0 (0.1)
	King Salmon River	3.9 (1.6)	0.1 (0.5)	5.6 (2.5)	4.8 (11.5)
Russia	Karaginsky Bay	2.2 (2.8)	1.0 (2.2)	0.1 (0.6)	0.6 (3.1)
	Olutorsky Bay	0.4 (0.7)	0.9 (1.3)	0.1 (0.3)	2.5 (5.4)
	Kamchatka River	0.5 (0.7)	1.0 (1.5)	0.4 (1.0)	0.1 (1.0)
	Northwestern Kamchatka	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.1 (1.0)
	Southeastern Kamchatka	1.9 (1.5)	1.0 (1.5)	2.2 (1.7)	16.1 (15.3)
	Kuril Lake	5.8 (2.0)	4.1 (1.9)	7.4 (3.0)	0.1 (1.7)
	Palana River	0.4 (0.4)	0.7 (0.7)	0.0 (0.1)	0.0 (0.2)
	Bolshaya River	0.1 (0.5)	0.0 (0.2)	0.0 (0.1)	0.0 (0.3)

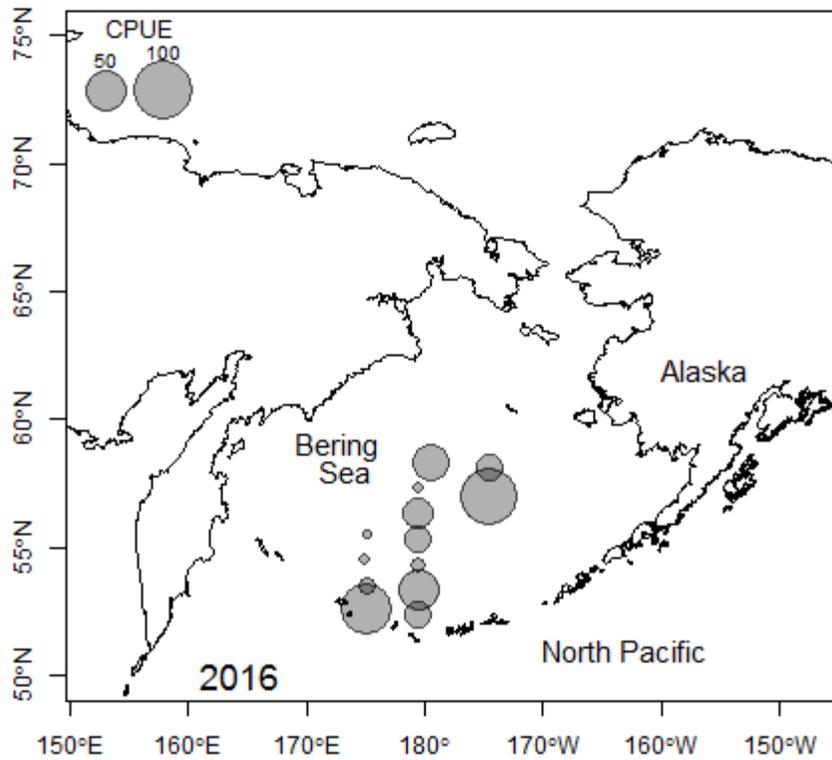


Figure 1. Catch locations of immature Sockeye Salmon during the 2016 research cruise of the *Hokko-maru* in the central Bering Sea, with catch abundance outlined by size of the symbol.

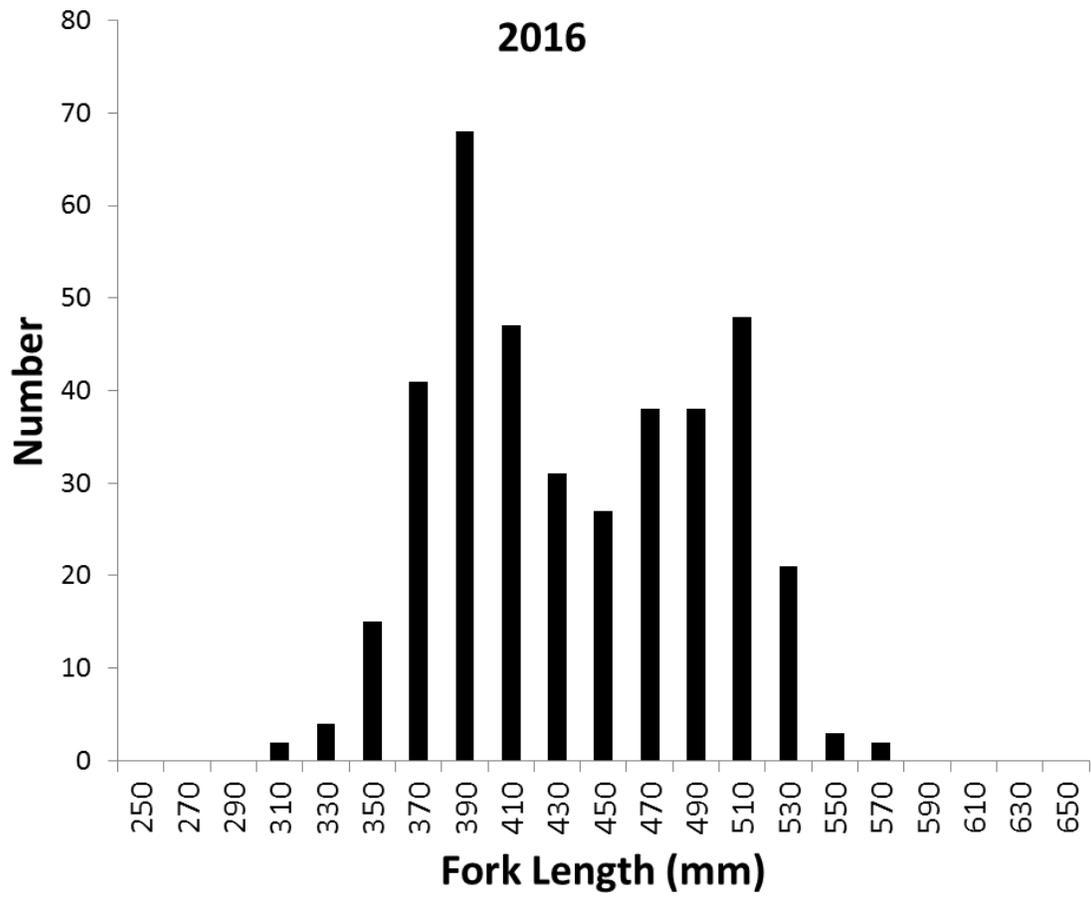


Figure 2. Frequency histogram based upon fork length of immature Sockeye Salmon caught during the 2016 research cruise of the *Hokko-maru* in the central Bering Sea.

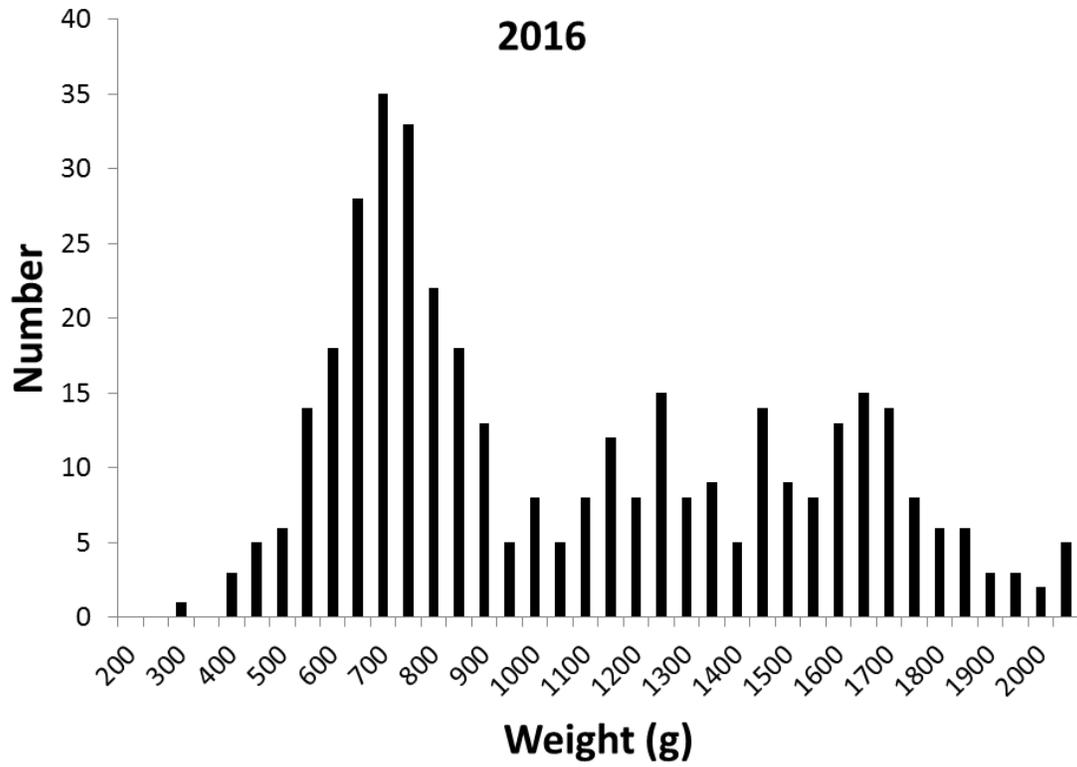


Figure 3. Frequency histogram based upon weight (grams) of immature Sockeye Salmon caught during the 2016 research cruise of the *Hokko-maru* in the central Bering Sea.