Microsatellite Identification of Sockeye Salmon Rearing in the South Central Bering Sea During Summer 2018

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 2018 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 71.5% of all sockeye salmon caught, with the catch dominated by sockeye salmon of Bristol Bay origin (67.5%). Russian-origin sockeye salmon accounted for 26.3% of the catch, while Canadian-origin salmon accounted for an average of 2.1% of the catch, with 388 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

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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 2018 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 cruise conducted with the Japanese research vessel *Hokko-maru* in the central and southern Bering Sea during 2018 (Honda et al. 2019).   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 (e.g., 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, British Columbia. 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, with the majority along the 175°W transect (Figure 1). However, higher catches were observed along the 175°E transect in 2018 compared with 2017. 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 430 mm. Sockeye salmon sampled in 2018 were smaller than those sampled during the 2017 survey (Figures 2 and 3). For example, mean fork length of x.1 individuals was 350 mm (n=241, SD=24 mm) for individuals caught in 2018, whereas mean fork length for age x.1 sockeye salmon caught in 2017 was 360 mm. Mean length of age x.2 individuals was 461 mm (n=142, SD=28 mm) for individuals caught in 2018 (476 mm for age x.2 sockeye salmon sampled in 2017), and mean fork length of x.3 individuals was 421 mm (n=8, SD=77 mm), with mean length of age x.3 sockeye salmon estimated as 521 mm in 2017.

Body weight distributions were similarly well defined between age x.1 and x.2 individuals, with x.1 individuals typically less than 900 g in weight, reflective of the distribution of body weight (Figure 3). Mean weight of age x.1 individuals was 516 g (SD=141 g) for individuals caught in 2018 compared with 575 g for age x.1 individuals sampled in 2017.   Similarly, mean weight of age x.2 individuals was 1,249 g (SD=277 g) for individuals caught in 2018 compared with 1,366 g for age x.2 sockeye salmon sampled in 2017, and mean weight of age x.3 individuals was 1,032 g (SD=560 g) compared with a mean weight of 1,778 g for age x.3 sockeye salmon sampled during 2017.

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 67.5% of all sockeye salmon caught during the 2018 survey, with Alaskan-origin salmon comprising 71.5% of the catch (Table 1). Russian-origin salmon accounted for 26.3% of the catch during 2018. Canadian-origin sockeye salmon accounted for 2.1% of the catch during 2018. 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 28.4% of individuals estimated to be of Egegik River origin (Table 1). Sockeye salmon from the Naknek River drainage were estimated to be the next most important contributor to the catch, comprising 13.5% of the salmon sampled. Sockeye salmon from the Nushagak River drainage were estimated to comprise 8.7% of the sample. Sockeye salmon from

the Wood River drainage were estimated to comprise 8.1% of immature sockeye salmon sampled during the cruise, and those from the Kvichak River 7.4% (Table 1).

Russian-origin Sockeye salmon caught in the central Bering Sea during 2018 comprised 26.3 % of the immature Sockeye salmon sampled (Table 1). The most abundant stocks contributing from Russia originated from Kuril Lake (14.1%), Bolshaya River (4.1%), Karaginsky Bay (3.8%), and the Palana River (2.3%). Canadian-origin salmon were estimated to originate primarily from the Fraser River (1.0%) and the Nass River (0.4%) and comprised 2.1% of the research catch (Table 1).

Discussion

Mean fork length of the age x.1 immature sockeye salmon sampled in the south central Bering Sea during 2018 was very similar to those observed in previous years of sampling. For example, the mean fork length of age x.1 individuals was 350 mm (SD=24 mm) in 2018 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), 372 mm (26 mm), and 385 mm (27 mm) for individuals caught in 2009, 2011, 2012, 2013, 2014, 2015, and 2016, respectively (Beacham et al. 2016). The smaller mean length of age x.1 individuals observed in 2018 relative to 2017 was very similar to that observed during 2009-2013. Although the mean length of age x.2 individuals sampled during 2018 (461 mm, SD = 28 mm) was smaller than that observed during 2017, it was very 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), 473 mm (26 mm), and 481 mm (28 mm) for individuals caught in 2009, 2011, 2012, 2013, 2014, 2015, and 2016, respectively. The mean length of age x.3 individuals (421 mm, SD=77 mm) sampled in 2018 was less than that observed in 2017, and also smaller than 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), 548 mm (40 mm), and 513 mm (24 mm) in 2009, 2011, 2012, 2013, 2014, 2015, and 2016, respectively. Given the small sample size (8 individuals), ageing errors likely contributed to this lower estimate, as three individuals designated as x.3 recorded fork lengths of < 345 mm.

Similar to the results observed for mean fork length, mean weight of age x.1 sockeye salmon was observed in 2018 (516 g, SD = 141 g) was less than that observed in 2017, but similar to that observed during 2009-2013. 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), 631 g (136 g), and 714 g (157 g) for individuals caught in 2009, 2011, 2012, 2013, 2014, 2015, and 2016, respectively (Beacham et al. 2016). Mean weight of age x.2 individuals sampled during 2018 (1,249 g, SD = 477 g) was less than observed during 2017, but similar to those sampled in 2009-2013, 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), 1311 g (230 g), and 1,461 g (271 g) for individuals caught in 2009, 2011, 2012, 2013, 2014, 2015, and 2016, respectively. The mean weight of age x.3 individuals sampled during 2018 (1,032 g, SD = 560 g) was less than observed in 2017, and also less than observed 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), 1,311 g (680 g), and 1,798 g (330 g) in 2009, 2011, 2012, 2013, 2014, 2015, and 2016, respectively. Likely, the previously noted possible ageing errors may account for this finding.

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 and again in 2017, Russian-origin sockeye salmon were noticeably absent from the immature sockeye salmon sampled, comprising only 2% of the sample. In 2017, catches were quite low along the 175°W transect, and this likely contributed to the low Russian-origin component of the immature Sockeye salmon sampled in the southern central Bering Sea during 2017. In 2018, catches along the 175°W transect increased relative to 2017, and Russian-origin sockeye salmon comprised 26.3% of the 2018 research catch.

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. The majority of the research catch was concentrated along the 175°W transect, where salmon of Canadian origin could conceivably be expected to be in higher relative proportions than in more western regions of the Bering Sea, but similar results have been observed in other years (Beacham et al. 2016). 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.

variation for the Sockeye salmon sampled from the Bering Sea. Funding for the study was provided by Fisheries, Oceans, and the Coast Guard Canada.

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Table 1. Mean catch per unit effort (CPUE 13 stations (Fig. 1)) and estimated stock compositions (%, standard deviations in parentheses) of mixed-stock samples of sockeye salmon from the central Bering Sea, 2018. 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		Age x.1	Age x.2	Age x.3	All
CPUE					
N		222	140	8	388
Columbia River	Okanagan River	0.1 (0.1)	0.0 (0.1)	0.0 (0.7)	0.0 (0.0)
British Columbia	Fraser River	0.4 (0.5)	2.1 (1.2)	0.0 (2.4)	1.0 (0.5)
	Vancouver Is.	0.5 (0.4)	0.0 (0.1)	0.0 (0.8)	0.3 (0.3)
	Central Coast	0.2 (0.4)	0.0 (0.2)	1.6 (6.6)	0.0 (0.1)
	Skeena River	0.1 (0.3)	0.0 (0.1)	0.5 (2.6)	0.0 (0.1)
	Nass River	0.6 (0.6)	0.0 (0.2)	0.0 (1.5)	0.4 (0.4)
	Stikine River	0.3 (0.4)	2.4 (2.0)	0.1 (2.6)	0.2 (0.3)
	Taku River	0.1 (0.4)	0.1 (0.4)	0.0 (2.1)	0.0 (0.2)
	Alsek River	0.0 (0.2)	1.0 (1.2)	0.1 (2.8)	0.1 (0.3)
Southeast Alaska	SE Alaska	0.0 (0.2)	0.0 (0.7)	0.0 (2.1)	0.1 (0.3)
Central Alaska	Cook Inlet	0.2 (0.4)	1.8 (1.6)	0.0 (2.5)	0.3 (0.4)
	Copper River	0.0 (0.1)	0.3 (0.6)	0.1 (1.5)	0.3 (0.4)
	Kodiak Island	0.7 (0.8)	10.5 (3.2)	0.1 (2.7)	2.9 (1.3)
	Chignik River	0.0 (0.1)	2.0 (2.6)	0.1 (1.5)	0.1 (0.3)
Western Alaska	Kuskowim	0.0 (0.0)	2.6 (2.2)	0.1 (1.4)	0.3 (0.7)
	Norton Sound	0.0 (0.1)	0.0 (0.2)	0.0 (0.6)	0.0 (0.2)
Bristol Bay	Ugashik River	0.6 (2.1)	1.8 (3.3)	0.0 (0.7)	0.2 (0.8)
	Egegik River	34.2 (5.1)	14.0 (5.5)	58.9 (21.8)	28.4 (3.8)
	Naknek River	15.9 (4.1)	16.1 (4.2)	0.4 (3.2)	13.5 (3.0)

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	Alagnak River	0.0 (0.1)	0.2 (0.5)	0.0 (0.8)	0.3 (0.4)
	Kvichak River	10.4 (3.7)	0.2 (0.9)	0.3 (2.6)	7.4 (2.9)
	Nushagak River	2.4 (1.8)	17.1 (4.3)	0.2 (2.1)	8.7 (2.2)
	Wood River	8.6 (2.2)	10.1 (3.7)	5.2 (8.9)	8.1 (2.0)
	Igushik River	0.0 (0.2)	0.1 (0.7)	18.5 (17.8)	0.5 (1.2)
	King Salmon River	0.1 (0.4)	0.1 (0.7)	0.0 (0.3)	0.4 (1.1)
	Togiak River	0.0 (0.0)	0.0 (0.3)	0.0 (0.5)	0.0 (0.0)
Russia	Karaginsky Bay	1.3 (2.1)	3.6 (4.7)	0.0 (1.1)	3.8 (2.7)
	Chukotka	0.0 (0.0)	0.0 (0.1)	0.0 (0.7)	0.0 (0.0)
	Navarinsky	0.0 (0.0)	0.0 (0.1)	0.0 (0.5)	0.0 (0.1)
	Olutorsky Bay	0.2 (0.6)	0.1 (0.5)	0.0 (0.8)	0.8 (1.0)
	Kamchatka River	0.2 (0.6)	0.2 (0.6)	3.4 (9.5)	0.3 (0.6)
	Northwestern Kamchatka	0.0 (0.1)	0.0 (0.1)	0.0 (1.0)	0.0 (0.1)
	Southeastern Kamchatka	0.0 (0.2)	0.7 (1.9)	0.0 (0.9)	0.9 (1.2)
	Kuril Lake	15.4 (3.2)	9.8 (4.5)	1.4 (7.4)	14.1 (3.2)
	Palana River	2.2 (1.2)	0.1 (0.4)	8.4 (10.0)	2.3 (0.9)
	Bolshaya River	5.1 (2.1)	2.5 (2.9)	0.0 (0.6)	4.1 (1.5)
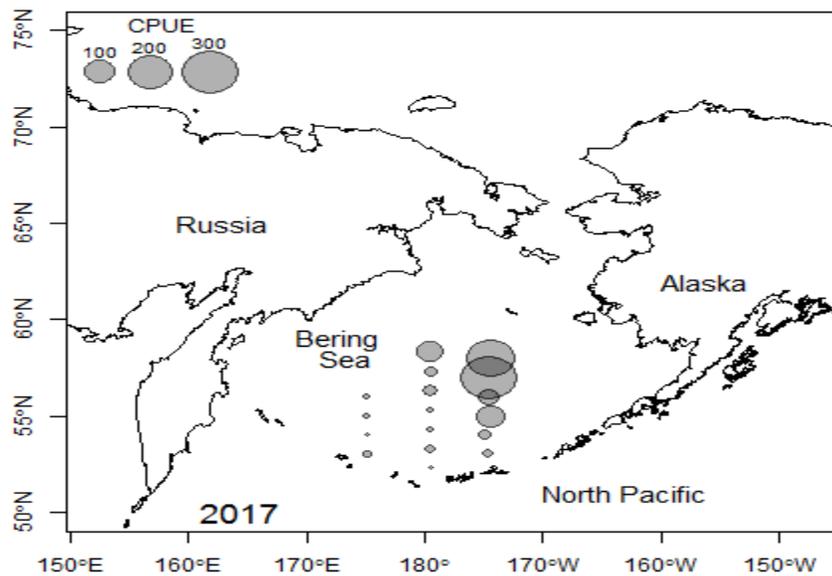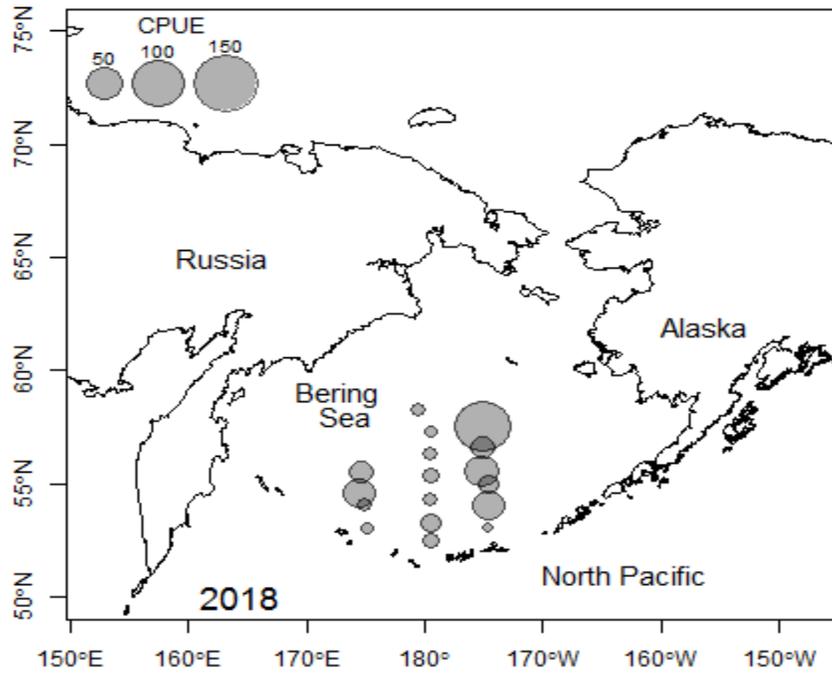
Fig. 1. Catch locations of immature sockeye salmon during the 2018 and 2017 research cruises of the *Hokko-maru* in the central Bering Sea, with catch abundance outlined by size of the symbol.
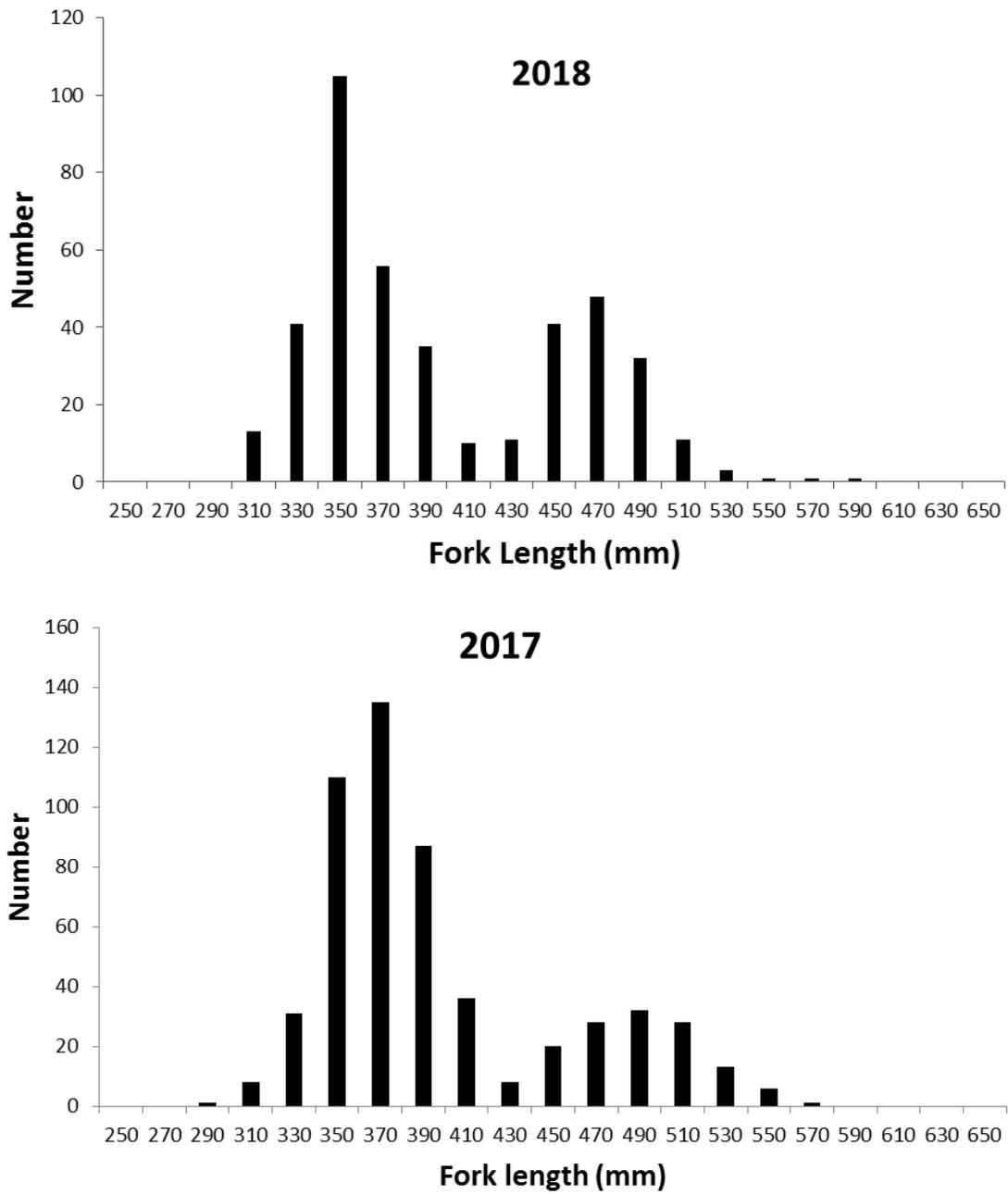
Fig. 2. Frequency histogram based upon fork length of immature sockeye salmon caught during the 2018 and 2017 research cruises of the *Hokko-maru* in the central Bering Sea.
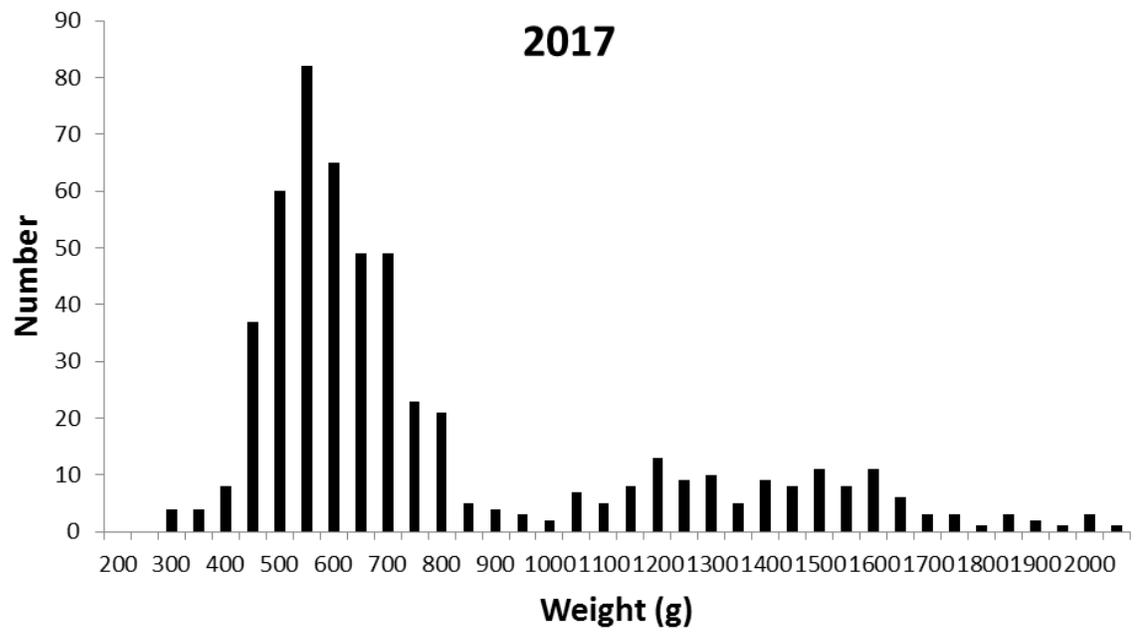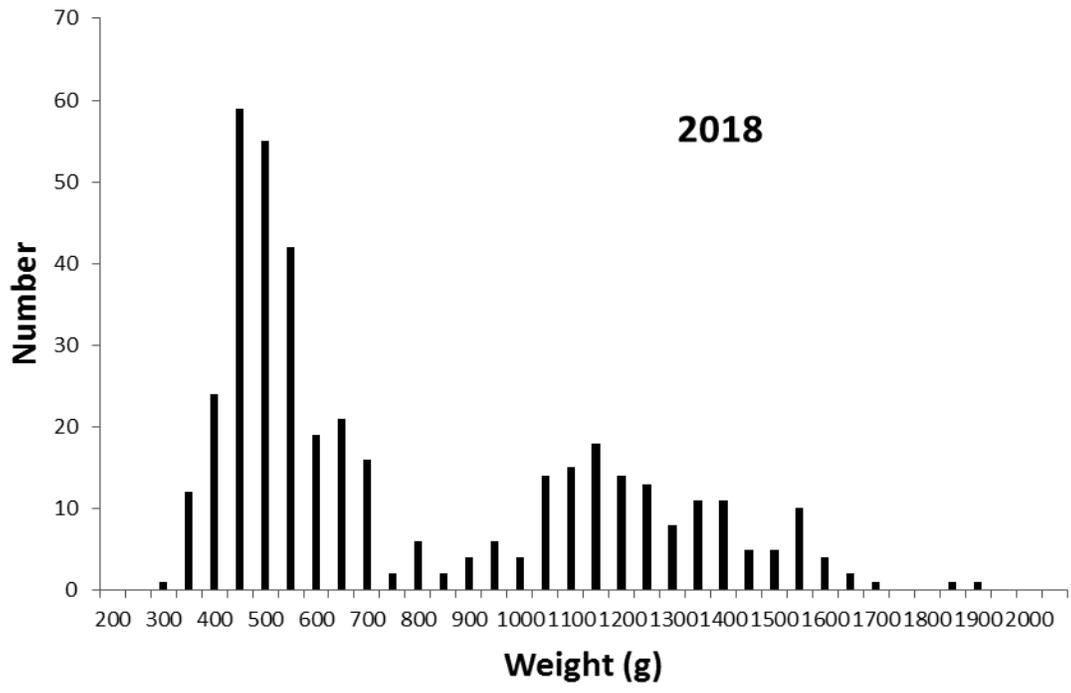
Fig. 3. Frequency histogram based upon weight (grams) of immature sockeye salmon caught during the 2018 and 2017 research cruise of the *Hokko-maru* in the central Bering Sea.