Geographic Origin of High-Seas Chum Salmon Determined by Genetic and Thermal Otolith Markers

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Abstract: Genetic stock identification (GSI) and thermal otolith marking techniques were used for determining the stock origin of chum salmon (*Oncorhynchus keta*) caught in two offshore transects (165°W and 145°W) in the Gulf of Alaska during June and July 1998. In the central Gulf of Alaska (49–56°N, 145°W), 49 thermally-marked chum salmon were found (14.5%, n = 339 fish). Most of these marked salmon were immature fish originated from four hatcheries in southeast Alaska (SEAK) and Prince William Sound (PWS), while two immature fish were from Nitinat Hatchery on southwest Vancouver Island, British Columbia (BC). In the western Gulf of Alaska (45–50°N, 165°W), however, we found only two marked chum salmon (1.1%, n = 188 fish) that had been released from the Hidden Falls and Gastineau hatcheries in SEAK. The GSI results indicated that North American chum salmon were common in the central Gulf of Alaska (15% west Alaska, 25% Alaska Peninsula/Kodiak, 28% SEAK/PWS, and 18% BC stocks), and Asian chum salmon were predominant in the western Gulf of Alaska (25% Japan, 53% Russia, and 13% west Alaska stocks). The GSI and thermal mark results suggest that in the central Gulf of Alaska, the contribution of SEAK/PWS hatchery stocks was high among the ocean age .1 group, but decreased with increasing ocean age mainly because other stocks such as west Alaska stocks penetrated these waters after the second year of their ocean life.

INTRODUCTION

Thermal marking of salmonid otoliths has been well developed as an effective tool to determine the hatchery origin of salmon. This technology has been primarily used for stock management in near-shore interception fisheries (Hagen et al. 1995). Now, large numbers of thermally-marked chum (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*) are annually released from hatcheries in Alaska (U.S.A), British Columbia (Canada), Washington State (U.S.A.), and Russia. Japan has also started thermal marking, beginning with the 1998 brood year stocks. Many thermally-marked chum and pink salmon are collected in the eastern Bering Sea (Ignell et al. 1997) and coastal waters of the Gulf of Alaska (Farley and Munk 1997, 1998). In the meantime, a genetic stock identification (GSI) technique has been developed for estimating stock compositions of high-seas chum salmon (Seeb et al. 1995; Urawa et al. 1997, 1998; Wilmot et al. 1998; Winans et al. 1998; Seeb and Crane 1999a, 1999b). By combining both identification techniques, we expect to be able to estimate the stock origins and components of wild and hatchery salmon in the ocean.

In the Gulf of Alaska, chum salmon occur all the

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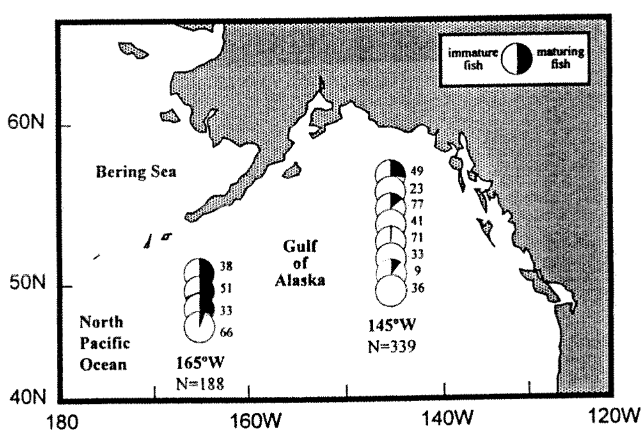
year around. North American juveniles migrate westward along the coast of the Gulf of Alaska during summer and fall (Hartt and Dell 1986), but it is unclear where they disperse in the following winter and spring. Tagging recoveries have indicated that Asian chum salmon migrate into the Gulf of Alaska in addition to North American stocks (Neave et al. 1976; Ogura 1994). However, quantitative analyses have not been applied to the stock components of immature and maturing chum salmon in these waters. The present study was conducted to determine the stock structure of chum salmon in the offshore waters of the Gulf of Alaska during summer by using genetic and thermal marks.

MATERIALS AND METHODS

Fish Samples

Chum salmon (n = 527 fish) were captured on two transects (165°W and 145°W) in the Gulf of Alaska by research gillnet operations of T/V *Oshoro maru* during June and July 1998 (Fig. 1). The fork length, body weight and gonad weight of each fish were recorded, and scales were removed for age determination. Maturity was determined from gonad weights (Takagi 1961). The sagittal otoliths, muscle, heart, and liver were collected from each fish. The sagittal otoliths were dried and kept in cell well plates until used for detection of otolith marks. The other tissues (muscle, heart, and liver) were immediately frozen at -80°C, and shipped to the Genetics Section of the National Salmon Resources Center for genetic analysis. The catch per unit effort (CPUE) was calculated: CPUE = total catch (number)/set of research gillnets (30 tans).

Fig. 1. Map showing sampling locations with the proportion of maturing and immature chum salmon caught in 145°W and 165°W transects of the Gulf of Alaska during June and July 1998. Numerals indicate the number of fish samples at each sampling location.



Detection of Thermal Otolith Marks

The left sagittal otoliths were mounted on glass slides using thermoplastic cement (Buehler Co.), and then ground to expose the primordia. If the left sagittal otoliths were not available, the right sagittal otoliths were used. Otolith microstructures were observed under a light microscope, and the microstructure patterns were compared to the thermal mark patterns of voucher specimens collected from hatcheries before releases. All otoliths were read independently by two readers.

GSI Analysis

Samples were examined for protein electrophoretic variation on horizontal starch gels using standard procedures described by Aebersold et al. (1987). Standard nomenclature for loci and alleles was used as outlined in Shaklee et al. (1990). Alleles were compared and standardized for 20 polymorphic loci (Seeb et al. 1995). We used the simplified baseline data set formulated in Seeb et al. (1995) for 69 stock groups that was augmented by Wilmot et al. (1998) to a 77 stock group/20 locus data set. Our analyses are based on a 19-locus baseline (Table 1). We dropped dipeptidase (PEPA) from our analyses because it was frequently missed in the electrophoretic screening of mixture samples (Winans et al. 1998). Stock contributions were estimated with a conditional maximum likelihood algorithm (Pella and Milner 1987) using the GIRLS program of Masuda et al. (1991). Standard deviations of estimates were estimated by 500 bootstrap resamplings of the baseline and mixture samples. Stock contribution estimates were made of individual stocks and then pooled to regional stock groups used by Seeb et al. (1995) and Wilmot et al. (1998). The regional stock groups are Japan, Russia, west Alaska (summer run), Yukon River (fall run), Alaska Peninsula/Kodiak, southeast Alaska (SEAK) and Prince William Sound (PWS), British Columbia (BC), and Washington. Simulation studies indicated that average stock contribution estimates were greater than 80% accurate when true group contributions were 100% (Wilmot et al. 1998).

RESULTS

Thermal Otolith Mark

Thermal otolith marks were detected in 51 chum salmon (9.7% of fish examined; Table 2). All of these marked chum salmon were immature except for one maturing fish caught in the central Gulf of Alaska (56°N, 145°W).

In the central Gulf of Alaska (49–56°N, 145°W), 49 chum salmon with thermally-marked otoliths were found (14.5%, n = 339 fish). Most of these marked

Table 1. Protein coding loci of enzymes used for stock identification of high-seas chum salmon and the tissues and buffers in which they were resolved. Enzyme nomenclature and EC numbers follow IUBMBNC (1992). Locus nomenclature follows Shaklee et al. (1990).

Enzyme	EC No.	Locus	Tissue[*1]	Buffer[*2]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	H, M	ACE7.0, TBE
		*mAAT-1**	H	ACE7.0
Aconitae hydratase	4.2.1.3	*mAH-3**	H	ACE7.0
Alanine aminotransferase	2.6.1.2	*ALAT**	M	TBE
Esterase-D	3.1.-.-	*ESTD**	H, M	TBCLE, TBE
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*G3PDH-2**	H	ACE7.0
Glucose-6-phosphate isomerase	5.3.19	*GPI-B1,2**	M	TBCLE
		*GPI-A**	M	TBCLE
Isocitrate dehydrogenase (NADP$^+$)	1.1.1.42	*mIDHP-1**	H, M	ACE7.0
		*sIDHP-2**	L	ACE7.0
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1**	M	ACE7.0, TBCLE
		*LDH-B2**	M	TBCLE
Malate dehydrogenase	1.1.1.37	*sMDH-A1**	H, L	ACE7.0, TC4
		*sMDH-B1,2**	H, M	ACE7.0
Malic enzyme (NADP$^+$)	1.1.1.40	*mMEP-2**	M	ACE7.0
		*sMEP-1**	M	ACE7.0
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	H	TBE
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1**	H, L, M	ACE7.0, TC4, TBE
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	H, L, M	ACE7.0

[*1]H, heart; L, liver; M, muscle.
[*2]Buffers and electrophoretic protocol are from Aebersold et al. (1987).

Table 2. Thermally-marked chum salmon caught in the Gulf of Alaska in the summer of 1998. FL, fork length; BW, Body weight; Mat, maturity according to the criteria of Takagi (1961); RBr, Regional band rings showing coding structure of thermal marks (Munk and Geiger 1998); F, female; M, male; IM, immature; MT, maturing; TM ID, thermal mark identification: AFK, Armin F. Koernig Hatchery; DIPAC, Gastineau Hatchery; HF, Hidden Falls Hatchery; NIT, Nitinat Hatchery; PC, Wally H. Noerenberg Hatchery (Port Chalmers); WHN, Wally H. Noerenberg Hatchery.

No	Date	Lat (N)	Long (W)	Sex	FL (mm)	BW (g)	Gonad (g)	Mat	Age	RBr	TM ID
1	08-Jul-98	52°00	145°00	M	400	780	1	IM	0.1	1:1.4	AFK96
2	11-Jul-98	49°00	145°00	M	401	620	1	IM	0.1	1:1.4	AFK96
3	06-Jul-98	54°00	145°00	M	611	2420	3	IM	0.4	2:1.3	DIPAC93
4	06-Jul-98	54°00	145°00	M	525	1680	1	IM	0.3	1:1.4	DIPAC94
5	30-Jun-98	45°30	165°00	F	456	1120	7	IM	0.2	1:1.5	DIPAC95
6	05-Jul-98	55°00	145°00	M	479	1220	1	IM	0.2	1:1.5	DIPAC95
7	06-Jul-98	54°00	145°00	F	506	1520	12	IM	0.2	1:1.5	DIPAC95
8	06-Jul-98	54°00	145°00	F	506	1440	11	IM	0.2	1:1.5	DIPAC95
9	07-Jul-98	53°00	145°00	F	491	1480	12	IM	0.2	1:1.5	DIPAC95
10	07-Jul-98	53°00	145°00	M	514	1540	1	IM	0.2	1:1.5	DIPAC95
11	07-Jul-98	53°00	145°00	F	497	1560	10	IM	0.2	1:1.5	DIPAC95
12	07-Jul-98	53°00	145°00	F	467	1220	13	IM	0.2	1:1.5	DIPAC95
13	08-Jul-98	52°00	145°00	M	506	1460	1	IM	0.2	1:1.5	DIPAC95
14	08-Jul-98	52°00	145°00	F	512	1600	14	IM	0.2	1:1.5	DIPAC95
15	08-Jul-98	52°00	145°00	F	488	1340	11	IM	0.2	1:1.5	DIPAC95
16	06-Jul-98	54°00	145°00	M	378	560	1	IM	0.1	1:1.6	DIPAC96
17	08-Jul-98	52°00	145°00	M	405	740	1	IM	0.1	1:1.6	DIPAC96
18	09-Jul-98	51°00	145°00	M	358	520	1	IM	0.1	1:1.6	DIPAC96
19	09-Jul-98	51°00	145°00	M	384	680	1	IM	0.1	1:1.6	DIPAC96
20	11-Jul-98	49°00	145°00	F	369	540	5	IM	0.1	1:1.6	DIPAC96
21	11-Jul-98	49°00	145°00	M	395	620	2	IM	0.1	1:1.6	DIPAC96
22	29-Jun-98	47°00	165°00	F	467	1300	11	IM	0.2	1:1.3,2.3	HF95
23	04-Jul-98	56°00	145°00	F	529	1820	110	MT	0.3	1:1.3,2.3	HF95
24	06-Jul-98	54°00	145°00	F	477	1180	14	IM	0.2	1:1.3,2.3	HF95

continue...

Table 2. continued.

No	Date	Lat (N)	Long (W)	Sex	FL (mm)	BW (g)	Gonad (g)	Mat	Age	RBr	TM ID
25	08-Jul-98	52°00	145°00	M	504	1640	1	IM	0.2	1:1.3,2.3	HF95
26	08-Jul-98	52°00	145°00	M	482	1280	1	IM	0.2	1:1.3,2.3	HF95
27	05-Jul-98	55°00	145°00	M	368	520	1	IM	0.1	1:1.3,2.4	HF96
28	06-Jul-98	54°00	145°00	F	382	640	5	IM	0.1	1:1.3,2.4	HF96
29	07-Jul-98	53°00	145°00	M	336	460	1	IM	0.1	1:1.3,2.4	HF96
30	08-Jul-98	52°00	145°00	F	388	680	12	IM	0.1	1:1.3,2.4	HF96
31	09-Jul-98	51°00	145°00	M	418	740	1	IM	0.1	1:1.3,2.4	HF96
32	09-Jul-98	51°00	145°00	M	376	600	1	IM	0.1	1:1.3,2.4	HF96
33	10-Jul-98	50°00	145°00	M	392	660	1	IM	0.1	1:1.3,2.4	HF96
34	11-Jul-98	49°00	145°00	F	333	390	3	IM	0.1	1:1.3,2.4	HF96
35	05-Jul-98	55°00	145°00	F	631	3000	12	IM	0.2	1:1.3+2.3	NIT95
36	08-Jul-98	52°00	145°00	F	405	780	3	IM	0.1	1:1.4	NIT96
37	09-Jul-98	51°00	145°00	F	378	620	7	IM	0.1	1:1.6	PC96E
38	09-Jul-98	51°00	145°00	M	368	560	1	IM	0.1	1:1.6	PC96E
39	11-Jul-98	49°00	145°00	F	418	860	8	IM	0.1	1:1.6	PC96E
40	06-Jul-98	54°00	145°00	M	429	820	1	IM	0.1	1:1.3,2.2	WHN96L
41	08-Jul-98	52°00	145°00	M	428	960	1	IM	0.1	1:1.3,2.2	WHN96L
42	08-Jul-98	52°00	145°00	F	407	740	5	IM	0.1	1:1.3,2.2	WHN96L
43	09-Jul-98	51°00	145°00	M	394	600	1	IM	0.1	1:1.3,2.2	WHN96L
44	09-Jul-98	51°00	145°00	F	379	640	7	IM	0.1	1:1.3,2.2	WHN96L
45	10-Jul-98	50°00	145°00	F	354	460	7	IM	0.1	1:1.3,2.2	WHN96L
46	11-Jul-98	49°00	145°00	F	404	780	10	IM	0.1	1:1.3,2.2	WHN96L
47	11-Jul-98	49°00	145°00	M	423	820	1	IM	0.1	1:1.3,2.2	WHN96L
48	11-Jul-98	49°00	145°00	M	395	640	1	IM	0.1	1:1.3,2.2	WHN96L
49	11-Jul-98	49°00	145°00	F	385	660	9	IM	0.1	1:1.3,2.2	WHN96L
50	11-Jul-98	49°00	145°00	M	390	640	1	IM	0.1	1:1.3,2.2	WHN96L
51	11-Jul-98	49°00	145°00	M	375	620	1	IM	0.1	1:1.3,2.2	WHN96L

fish were released from four hatcheries in SEAK and PWS, while two fish were from the Nitinat Hatchery on southwest Vancouver Island, BC. The percentage of thermally-marked fish was higher in southern waters (21.5%, 49–52°N) than in northern waters (8.9%, 53–56°N) (Fig. 2). The CPUE of thermally-marked fish showed a similar trend (Fig. 3). The number of thermally-marked immature chum salmon decreased with increase in ocean age: 31 fish (36.9%) in age 0.1, 15 fish (7.9%) in age 0.2, and one fish (3.6%) in age 0.3 groups (Fig. 4).

In the western Gulf of Alaska (45–50°N, 165°W), thermal marks were found in only two chum salmon (1.1%, n = 188 fish; Fig. 2). These marked fish were released from the Hidden Falls and Gastineau hatcheries in SEAK.

Genetic Stock Identification

The GSI results indicated that North American chum salmon were common in the central Gulf of Alaska, and Asian chum salmon were predominant in the western Gulf of Alaska (Fig. 5).

In the central Gulf of Alaska, Alaskan stocks comprised 72% of immature chum salmon, while the proportion of southern North American stocks (BC and Washington) was high (58%) among maturing fish (Fig. 6). A geographical comparison of stock composition in immature chum salmon (Fig. 7) indicated that the percentage of SEAK/PWS stocks was higher in the southern waters (49–52°N) than in northern waters (53–56°N). In the age 0.1 group, SEAK/PWS stocks were dominant (41%), and west Alaskan stocks were rarely present (Fig. 8). However, the proportion of SEAK/PWS stocks decreased with an increase in ocean age, and west Alaskan stocks accounted for 21% and 17% of age 0.2 and 0.3 groups, respectively.

In the western Gulf of Alaska, the proportion of Russian and Japanese stocks was 56% and 18% among immature fish, and 41% and 34% among maturing fish, respectively (Fig. 6). North American stocks were rare except for the west Alaskan stock whose proportion was 15% and 12% in immature and maturing groups, respectively.

Fig. 2. Proportion (%) of chum salmon with thermally-marked otoliths caught on transects at 145°W and 165°W in the Gulf of Alaska during June and July 1998. Numerals following hatchery identification initials indicate number of thermally-marked fish. All marked fish were immature except for one maturing fish (56°N, 145°W) indicated by an underline.

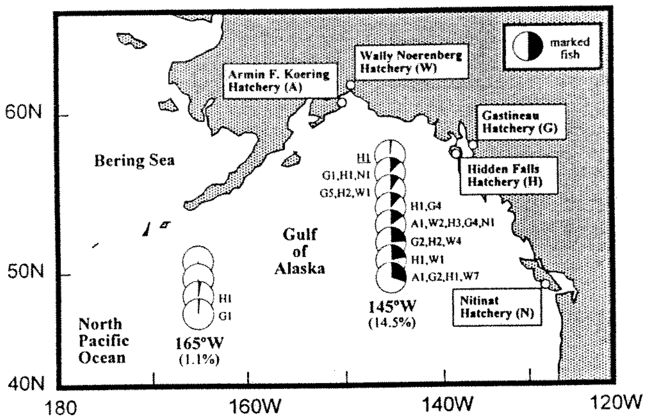


Fig. 3. CPUE (fish catch per set of research gillnets) of thermally-marked and unmarked chum salmon on transects at 145°W and 165°W in the Gulf of Alaska in the summer of 1998.

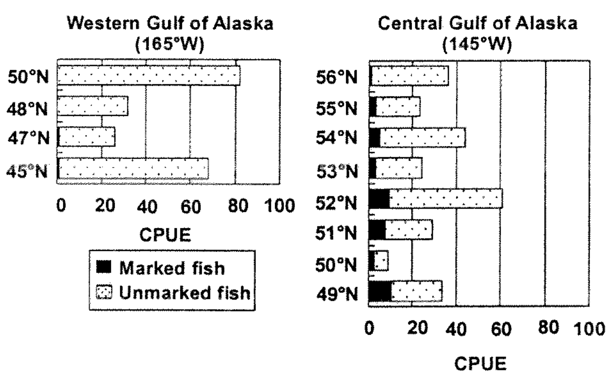


Fig. 4. Age composition of thermally-marked fish among immature chum salmon caught at 145°W transect in the Gulf of Alaska in the summer of 1998.

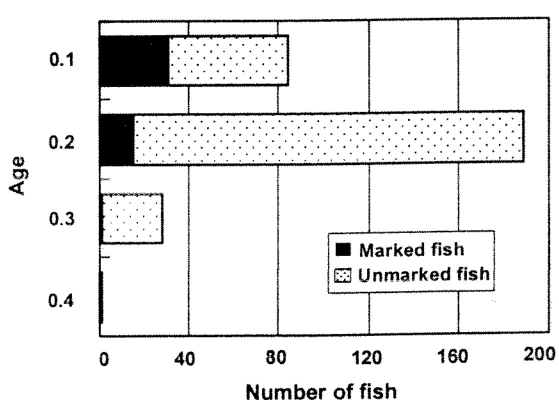


Fig. 5. GSI-estimated stock composition (%, A) and CPUE (number of fish per set of research gillnets, B) of chum salmon caught on transects at 145°W and 165°W in the Gulf of Alaska in the summer of 1998. Bars indicate SD of estimates. West AK, West Alaska; Pen/Kodiak, Alaska Peninsula and Kodiak; SEAK/PWS, Southeast Alaska and Prince William Sound; BC, British Columbia, WA, Washington.

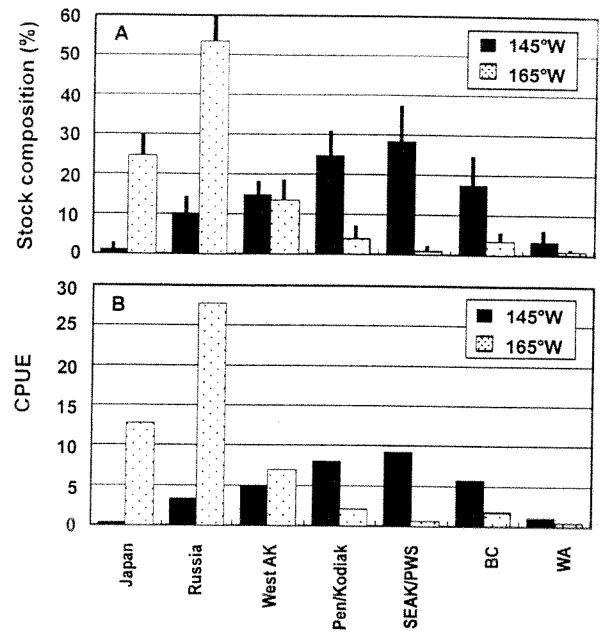
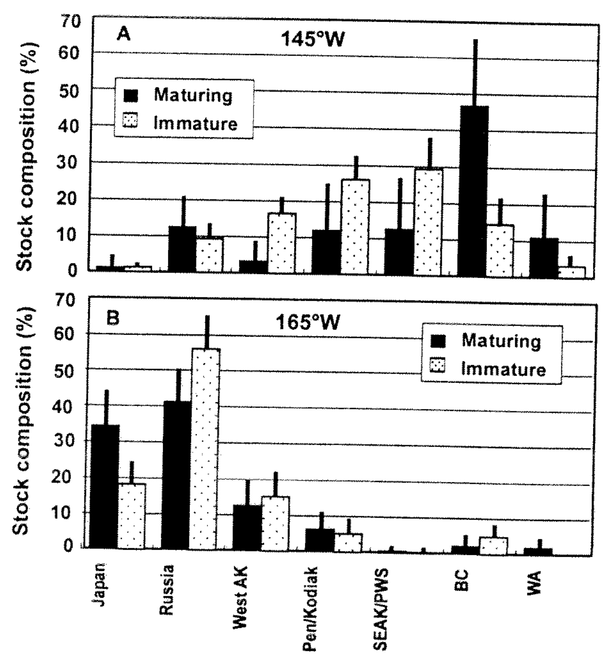


Fig. 6. GSI-estimated stock composition (%) of immature and maturing chum salmon caught on transects at 145°W (A) and 165°W (B) in the Gulf of Alaska in the summer of 1998. Bars indicate SD of estimates. West AK, West Alaska; Pen/Kodiak, Alaska Peninsula and Kodiak; SEAK/PWS, Southeast Alaska and Prince William Sound; BC, British Columbia, WA, Washington.



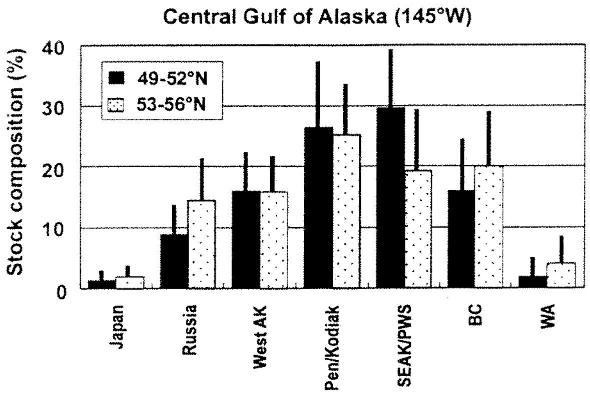


Fig. 7. A comparison of GSI-estimated stock composition (%) of immature chum salmon caught in the southern (49–52°N) and northern (53–56°N) waters at 145°W transect in the Gulf of Alaska in the summer of 1998. Bars indicate SD of estimates. West AK, West Alaska; Pen/Kodiak, Alaska Peninsula and Kodiak; SEAK/PWS, Southeast Alaska and Prince William Sound; BC, British Columbia; WA, Washington.

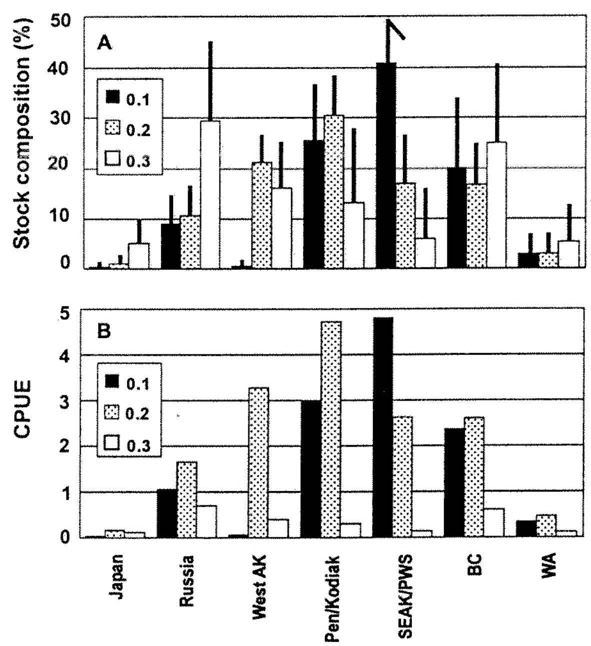


Fig. 8. By age stock composition (%, A) and CPUE (number of fish per set of research gillnets, B) of immature chum salmon caught at 145°W transect in the Gulf of Alaska in July 1998. The compositions were estimated by genetic stock identification. Bars indicate SD of estimates.

DISCUSSION

The 1996 winter GSI results showed that various regional stocks of North American and Asian chum salmon intermingled in the central Gulf of Alaska, but Asian stocks were dominant in the western Gulf of Alaska (Urawa and Ueno 1997; Urawa et al. 1997). The present summer GSI results indicated similar stock composition estimates in the western Gulf of Alaska. In the central Gulf of Alaska, however, North American stocks (central and southeast Alaska, and BC) were common in the summer of 1998.

The GSI results were supported by data of thermal otolith marks showing frequent occurrence of marked chum salmon in the central Gulf of Alaska and rare detection in the western waters. Among these marked fish (n = 51), 49 originated from four hatcheries in PWS and SEAK, where about 200 million chum fry were released annually after thermal marking (Fig. 9; Geiger and Munk 1998). Two other marked chum salmon were from Nitinat Hatchery in BC, where about 30 million chum fry were thermally marked annually (Hargreaves et al. 1999). In the Gulf of Alaska, we found no marked chum salmon that were released from Washington (Volk and Hagen 1998) and Russia (Akinitcheva and Rogatnykh 1999).

Ninety-eight percent of thermally-marked chum salmon were immature when they were caught in the Gulf of Alaska during late June and early July. This may reflect their spawning season: by this time maturing fish have already moved to coastal waters for spawning in PWS and SEAK. The GSI results suggested that maturing chum salmon originating from southern North America (BC and Washington) were still distributed in the northern waters of the central Gulf of Alaska in early July. This is possible because the main spawning season of southern stocks is usually between October and December. Thermal mark and GSI results indicated that this biased distribution may be caused partly by entry of northern populations such as Alaska Peninsula and west Alaska stocks.

Contributions of SEAK/PWS stocks to immature chum salmon in the central Gulf of Alaska were estimated by both GSI and thermal marks (Fig. 10). This comparison suggested that most of SEAK/PWS chum salmon in the Gulf of Alaska may be hatchery origin if the survival rate is similar among thermally-marked and unmarked hatchery fish. The number of thermally-marked chum salmon of PWS/SEAK ori-

Fig. 9. Annual changes in number of thermally otolith marked chum salmon juveniles released from hatcheries in southeast Alaska and Prince William Sound.

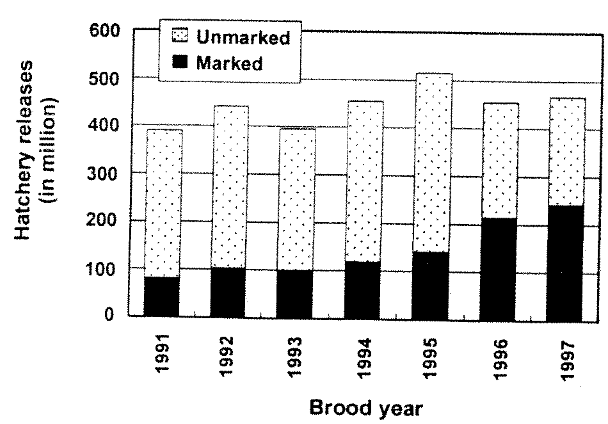
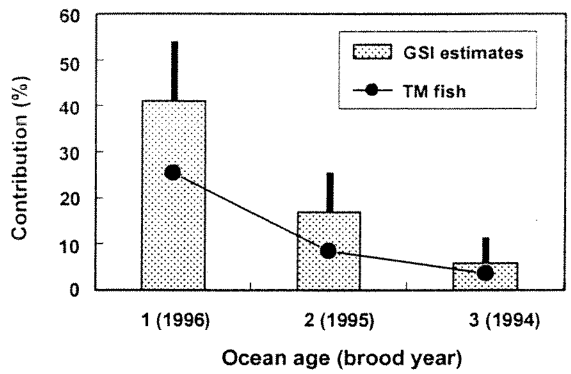


Fig. 10. Contributions of Southeast Alaska (SEAK)/Prince William Sound (PWS) stocks to immature chum salmon caught in the central Gulf of Alaska (145°W) in July 1998. TM fish, thermally-marked fish originating from SEAK/PWS. Bars indicate SD of GSI estimates.



gins decreased as ocean age increased among immature chum salmon in the central Gulf of Alaska (Fig. 4). This decrease may partly reflect annual changes in the number of thermally marked fish released from hatcheries (Fig. 9). However, the GSI analysis also showed a similar trend toward a high proportion of SEAK/PWS stocks (41%) among age 0.1 fish, which decreased to 17% and 6% among age 0.2 and 0.3 fish, respectively (Fig. 10). In contrast, west Alaskan chum salmon rarely appeared in the age 0.1 group, but comprised 17–21% of older age groups. The proportion of Russian chum salmon also increased to 29% in the age 0.3 group. These results suggest that chum salmon stocks along the Gulf of Alaska coasts (SEAK/PWS, Alaska Peninsula and BC) are dominant in early ocean life in the central Gulf of Alaska, but their predominance may decrease because other stocks (such as west Alaska and Russia) enter these waters in after the second year of their ocean life. This influx of other stocks may bias the distribution towards an apparently lower proportion of SEAK/PWS stocks in northern waters and a higher proportion in southern waters where younger fish were more abundant.

Thermal otolith marking is an effective tool for determining hatchery origins of individual salmon in high seas as well as in coastal waters. However, some duplication of codes occurs within or between Alaska, British Columbia and Russia, because of the limited number of thermal marks (Hagen 1999) and poor coordination among agencies. In the present study, these duplications could be resolved by secondary characters. However, duplicated thermal marks are a challenge even for the experienced observer (Munk 1999). Thermal marking has been primarily used for coastal fishery management in Alaska, and coordination of thermal mark coding has not occurred among countries or states. Now there are large numbers of thermally-marked salmon released from hatcheries in North Pacific Rim countries. This provides a good opportunity to study life histories and population dynamics of hatchery salmon and their relations to wild stocks in the ocean. To reduce duplications of thermal mark patterns, we should consider coordination of marking strategies.

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