FINAL REPORT ON ANALYSES OF SALMON COLLECTED IN TAIWAN R.O.C., 31 AUGUST-5 SEPTEMBER 1989

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EXECUTIVE SUMMARY

Background

A team of five NMFS scientists traveled to Kaohsiung, R.O.C., to sample salmon and steelhead confiscated by Taiwanese authorities from two Taiwanese boats (Sung Ching No. 1 and Ta Feng No. 11) suspected of involvement in illegal high-seas drift net fisheries. The objectives were to 1) determine the species composition of the catch, 2) determine the continent of origin for the fish, and 3) look for fin clips, indicative of hatchery-reared fish from North America, possibly carrying coded-wire tags. Laboratory analyses of scale patterns and genetic and parasite characters were performed in Seattle at the Northwest Fisheries Science Center.

Results and Conclusions

Based on a sample of 1,059 fish (about 2% of the total), almost 90% of the catch consisted of two species, coho salmon (50.3%) and chum salmon (38.6%), with the balance comprised of pink salmon (4.9%), sockeye salmon (2.6%), chinook salmon (2.5%), and steelhead (1.0%).

Thirteen fin-clipped steelhead were found after sampling a total of about 5,500 fish (approximately 10% of the total catch). No coded-wire tags were detected.

Strong inferences about continent or region of origin can be made for some species, but such determinations are hindered by a general lack of adequate baseline information, primarily for Asian stocks and to a lesser extent for those from Alaska and northern British Columbia. Age data, morphological examination, and genetic and parasitic analyses as appropriate indicate that an Asian, probably a Soviet, origin appears likely for at least a substantial portion of the chum and chinook salmon analyzed, and perhaps the coho salmon as well. The presence of fin-clipped fish and the prevalence of steelhead with a single freshwater growth ring on their scales indicate that a substantial proportion of the steelhead came from hatcheries in North America, and genetic data confirm that a Kamchatkan origin is unlikely. The data for pink salmon are inconclusive regarding place of origin, and for sockeye salmon there is sufficient information from age and genetic data only to rule out southern British Columbia and Washington as major contributors.

BACKGROUND

In July 1989, a sting operation carried out by NOAA Enforcement and U.S. Coast Guard personnel resulted in the arrest of several foreign nationals thought to be involved in the illegal capture and sale of Pacific salmon. Two Taiwanese boats, the Sung Ching No. 1 and the Ta Feng No. 11, fled the scene of the sting operation (lat. 39°N, long. 171°E) and returned to their home port, Kaohsiung, R.O.C., where they were held, and the catch confiscated, by the Council of Agriculture for violation of Taiwanese law. On 27 August, a team of five scientists from the Coastal Zone and Estuarine Studies Division (CZES) of the Northwest Fisheries Science Center was sent to Kaohsiung to examine the confiscated catch. The team returned on 7 September.

OBJECTIVES

The sampling trip had three major objectives: 1) determine the species composition of the catch, 2) determine the continent of origin for the fish, and 3) look for fin clips, indicative of hatchery-reared fish from North America, possibly carrying coded-wire tags.

METHODS

The Taiwanese government arranged for transfer of the fish from the boats to a secured cold storage room. The fish, which were flash frozen individually and (according to videotapes taken on board) piled loosely and apparently at random in the holds, were placed in nylon bags (about 13 fish per bag) aboard the boats and transferred to four bins at the cold storage facility. Bins 1, 2, and 3 held fish from the Sung Ching No. 1 (137.6 tons), and Bin 4 held fish from the Ta Feng No. 11 (19.3 tons). Based on an estimated average weight of 5 lbs per fish, the number of fish in the four bins was estimated to be about 62,000. To meet the above objectives, samples were taken as follows:

1) Species composition. To approximate a representative sample of the entire catch, bags were selected randomly from the four bins in proportion to the total contents of each bin. This sampling process yielded 1,059 usable fish, estimated to be about 2% of the overall catch. A preliminary species identification was made in the field by visual observation and scale examination. Scales were also taken to Seattle for further laboratory analysis, and a piece of muscle tissue was taken from each fish for electrophoretic analysis of genetic characters unique to each species.

2) Continent of origin. The scales and muscle tissues taken for species identification were also examined for evidence relating to continent or region of origin. To check for the presence of parasites diagnostic for certain regions, tissues were also collected as follows: the epaxial muscle anterior to the dorsal fin from sockeye salmon, the brain from chinook salmon, and the kidney and lower intestine from steelhead. For individuals tentatively identified as chum salmon, portions of liver, heart, and eye tissue were also collected for use in a genetic stock identification (GSI) analysis. To meet the goal of a 500-fish sample of chum salmon for the GSI analysis, 17 additional bags (about 220 fish) were sorted beyond those used for the species composition analysis. Tissue samples were taken from the chum salmon and steelhead (five) in the bags, and all the fish were examined for fin clips.

3) Fin-clipped fish. Visual checks for fin-clipped fish were made throughout the study. On the last day in Taiwan, all sampling efforts were devoted to searching an additional 320 bags for fin-clipped fish.

RESULTS

General

The appearance of growth at the edge of all the scales examined was similar to that of salmon or steelhead caught in the ocean in June or July (cf Bilton and Ludwig 1966).

Species Composition

Based on the laboratory analyses of scale and genetic characters performed in Seattle, positive species identification was made for 1,059 fish: 533 coho salmon (50.3%), 409 chum salmon (38.6%), 52 pink salmon (4.9%), 28 sockeye salmon (2.6%), 26 chinook salmon (2.5%), and 11 steelhead (1.0%). Assuming these fish were representative of the entire catch, the standard deviations of the species composition estimates were 1.5% for coho and chum salmon and less than 1% for the remaining species.

Fin-clipped Fish

Including those checked on the final day, about 5,500 fish (estimated to be approximately 10% of the total catch) were examined for fin clips. Thirteen fin-clipped fish were found, all of which were steelhead. A coded-wire tag detector was used at the sampling site to determine if any of the fin-clipped steelhead carried a tag, but no tags were found. This was not an unexpected result because, in the most recent 3 years for which data are available, only 11% of fin-clipped steelhead from North America were tagged (Light et al. 1988). Nevertheless, heads from 11 fin-clipped steelhead were brought back to Seattle for further analysis to ensure that tags were not missed due to inadequate power of detection. This work has not yet been completed. If any tags are found, the data will be furnished as a supplement to this report.

Continent of Origin

Chum salmon

Based on scale analysis, the majority of the chum salmon had spent 3 years in the ocean and 0+ years in fresh water (Table 1), which would have made them a minimum of 4 years old at spawning. Since age-4 fish are common in all chum-producing regions in North America and Asia (Shepard et al. 1968), the age data are not very informative regarding origin of the fish. One scale was also examined from each of 409 fish for holes due to resorption near the focus (center). Preliminary data suggest that this character is common in many Asian populations but rare in North America (Bigler 1989). Sixty-six scales (16%) were found to have holes, suggesting a substantial contribution of Asian fish.

No known diagnostic freshwater parasites occur in chum salmon.

In the GSI analysis, genotypic frequencies for 507 fish from the catch were compared with archived (baseline) frequencies previously obtained for over 150 different spawning populations. Estimates of stock composition are developed based on the assumption that the fish in question actually come only from source populations represented in the baseline dataset. In the present case, this assumption may not be realistic. Extensive genetic data are available for most chum-producing areas of North America, but information from Asia is much less complete, both in terms of geographic coverage and the number of genetic characters (gene loci) surveyed (Winans et al. in press). In particular, there are large areas of northern U.S.S.R. for which we have no data, and the Soviet populations that are included have data for only a few gene loci.

Present GSI stock composition analysis requires data for each genetic character for each stock included in the baseline. However, stocks in the baseline were surveyed for varying numbers of genetic characters. Therefore, we tried several GSI analyses, each differing in the number of genetic characters used and the number of potential

Table 1. Age composition (%) for salmon and steelhead in the species composition analysis (N = 1,059) as determined from scale growth increments.

	Species					
Age[a]	Coho N=533	Chum N=409	Pink N=52	Sockeye N=28	Chinook N=26	Steelhead N=11
0.1		1	100			
0.2		16				
0.3		78				
0.4		4				
1.1	18					9
1.2				18	97	9
1.3						27
2.1	72					
2.2				69		
2.3				7		
3.1	1					
3.2				3		
4.2						9
5.2						9
Undet.	9	1		3	3	36

[a]Number before decimal is freshwater age; number following is seawater age.

source populations that could be included. Estimated contributions for the major regions differed considerably among the analyses. For example, estimates for the Asian contribution ranged from 13 to 57%, and that for Washington from less than 1 to 47%. Such instability in the results usually indicates that the baseline dataset is not adequate to satisfactorily describe the catch. We also examined frequencies in the catch at a number of other gene loci for which there were data from Washington and parts of British Columbia and Alaska, and collectively these data indicated that it is unlikely that more than a small portion of the catch came from these areas. The contribution from Japanese stocks also appeared to have been small (10% or less). There were no genetic data that exclude the possibility that most or all of the chum salmon came from the U.S.S.R., but this observation is based on data for relatively few gene loci.

Summary: The data on focal scale resorption and the genetic data were both consistent with a Soviet origin for a substantial portion of the chum salmon catch, although some contribution from other areas cannot be excluded.

Coho salmon

Ages read from scale patterns (Table 1) indicated that all fish were maturing this year (i.e., had spent 1 year at sea), and most had spent 2 years in fresh water, which is typical of fish from U.S.S.R. and Alaska (Godfrey 1965). Many of the fish showed obvious changes in external jaw morphology (kyping) indicative of sexual maturity.

Muscle tissues from a subsample of 80 coho salmon were electrophoretically surveyed for 35 gene loci, and allele frequencies were compared with the only available baseline data (19 samples taken from spawning populations in Washington and southern British Columbia; NMFS, Seattle, unpublished data). The allele frequencies in the confiscated fish were similar to those observed in North America. The genetic data thus do not provide evidence against a North American origin, but they must be regarded as largely inconclusive because no Asian or Alaskan data are available at present.

The coho salmon appeared unusual in lacking the irregular black spots on the dorsal surface and upper caudal fin that most North American literature accounts describe as characteristic of the species.

Summary: **The coho salmon showed signs of early sexual maturity, suggesting they may have been captured close to land, and freshwater age data suggested an Alaskan or Soviet origin. Physical appearance of the fish differed from that typically found in coho salmon from North America.**

Sockeye salmon

Over 90% of the sockeye salmon aged from scale patterns had spent 2 years in fresh water (Table 1), suggesting an origin in U.S.S.R. or Alaska (Mosher 1963).

The dorsal musculatures of 27 fish were examined for the presence of plerocercoids of the cestode *Triaenophorus crassus*. This parasite is rare elsewhere, but Bristol Bay sockeye salmon are often infected with this tapeworm, at frequencies ranging from 2 to 56%, depending on the stock and year (Margolis 1963). All samples were negative.

In 26 fish surveyed for genetic variation at the LDH-4 locus, the fast (125) allele occurred at a frequency of 13.5%. This allele is rare or missing in stocks from Washington and southern British Columbia (Withler 1985), but it is more common in many stocks from northern British Columbia and Alaska and the three Soviet stocks (Kirpichnikov 1981) for which data are available.

Summary: **Southern British Columbia and Washington were unlikely sources for the sockeye salmon based on age and genetic data. The fish did not carry a parasite largely restricted to the Bristol Bay area, but its absence does not preclude some Bristol Bay contribution.**

Chinook salmon

All chinook salmon aged by scale analysis had spent 1 year in fresh water and 2 years at sea (Table 1). This would appear to exclude most fall-run chinook salmon from North America, which typically migrate to sea at age-0. Fish aged 1.2 make up the majority of the chinook salmon caught in the North Pacific by Japanese fishing boats (Myers et al. 1987).

The confiscated chinook salmon were unusual in having black pigment covering the mandibles almost to the isthmus, a condition not described in literature for North American stocks.

Brains dissected from 26 fish were examined microscopically for the presence of the myxozoans Myxobolus arcticus and M. neurobius. Chinook salmon taken in waters west of 170°E are usually heavily infested with M. arcticus, but this parasite is not seen in fish caught in the Bering Sea, and only a single infected fish was found in a survey of 11 North America rivers from the Yukon River in Alaska to the Klamath River in California (Urawa and Nagasawa 1988). In the same survey, M. neurobius occurred only in samples from the Columbia River, affecting from 11 to 48% of the fish. Of the 26 confiscated chinook salmon examined, 17 (65%) were positive for M. arcticus, and M. neurobius was not found in any specimens.

Extensive genetic data are available for chinook salmon populations from California to southeastern Alaska, but none from Asia. The few gene loci resolved from muscle tissue for the 26 chinook salmon included in the species identification analysis provided little information about possible origin.

Summary: **Freshwater age data exclude most fall-run stocks from North America, and the high incidence of a myxozoan parasite is consistent with a Siberian origin for much of the catch. Jaw coloration was atypical for North American fish.**

Pink Salmon

Scale analysis indicated that the pink salmon were all maturing fish (having spent 1 year at sea) (Table 1), but this would be expected for pink salmon from every geographic region.

Because they spend such a short time in fresh water, pink salmon have no known diagnostic parasites.

In the 52 fish for which muscle samples were analyzed electrophoretically, the frequencies of rare alleles at the SOD-1 and GPI-3 loci were higher than are found in most North American populations (T. Gharrett, NMFS Auke Bay, personal communication), but it is difficult to infer much from these data due to the small sample.

<u>Summary</u>: Little can be said about the origin of the pink salmon.

Steelhead

In contrast to the other species discussed previously, all of which are broadly distributed in North America and Asia, the center of abundance for steelhead is the Columbia River Basin. Over 80% of North American steelhead are produced in Washington, Oregon, Idaho, and California (Light, 1987), and Asian populations are restricted to the U.S.S.R., primarily along the west coast of Kamchatka (Light et al. 1989).

Most (over 70%) of the steelhead that could be aged from scales had spent only 1 year in fresh water (Table 1), which is typical of hatchery fish (Wahle and Smith 1979) but rare for wild fish. Hatcheries, primarily in the four states mentioned previously, account for about 51% of North American steelhead (Light 1987), but no artificial production is known to occur in Asia (Light 1989).

Because there is no deliberate fin-clipping of steelhead in Asia (Light et al. 1988), the occurrence of thirteen fin-clipped fish is strong evidence for a North American origin. However, little can be inferred about their place of capture because North American steelhead are reported to range through an extensive area from the North American continent west to nearly 162°E longitude (Light et al. 1985).

Of the 11 fish electrophoretically examined for variation at the LDH-4 gene locus, 3 carried the slow (76) allele. Published reports (Okazaki, 1985) indicate this allele is not found in Kamchatkan steelhead, but it is common in populations from Washington and British Columbia.

Sixteen samples (includes the five steelhead recovered from the extra fish examined for continent of origin) of posterior kidney tissue were examined under low-power light microscopy for the detection of encysted metacercaria of the trematode Nanophyetus salmincola. Heavy infestations of Nanophyetus have been found in steelhead from many coastal rivers in California, Oregon, and Washington (Margolis 1985). All samples were negative. Scrapings from lower intestines from the same 16 fish were examined for the presence of the trematode Plagioporus shawi. This intestinal fluke is limited to steelhead trout from certain areas in California, Oregon, Idaho, and Washington (Margolis 1985; Dalton 1987). All samples were negative.

Summary: A majority of the steelhead were apparently from North American hatcheries.

CONCLUSIONS

Almost 90% of the catch consisted of two species, coho salmon (50.3%) and chum salmon (38.6%). Because a large number of fish were analyzed for species composition (N = 1,059) and efforts were taken to ensure that they approximated a random sample from the entire catch, considerable confidence can be placed on the estimated contributions of each species.

Strong inferences about continent or region of origin can be made in some cases, but such determinations are hindered by a general lack of adequate information about Asian stocks. An Asian, probably Soviet, origin appears likely for at least a substantial portion of the chum and chinook salmon analyzed. The data for coho salmon indicate an Alaskan or Soviet origin. The data for pink salmon are inconclusive regarding place of origin, and for sockeye salmon they are sufficient only to rule out southern British Columbia and Washington as major contributors. Fin-clip and age data indicate that a substantial portion of the steelhead are from North American hatcheries, and this conclusion is supported by genetic data.

FUTURE PROSPECTS

This report shows that a variety of methods can provide important information regarding the freshwater origins of salmon and steelhead captured in the North Pacific. Each method, however, has limitations, either intrinsically or in the context of currently available information. Coded-wire tags are completely diagnostic if found, but they occur rarely in most oceanic fisheries and their absence provides little useful information. Freshwater parasites can be very informative, but diagnostic ones are currently restricted to certain regions for sockeye and chinook salmon and steelhead. Age structure is often correlated with latitude and may provide useful information regarding origins, albeit for fairly broad geographic regions. Scale pattern analysis has been used successfully to resolve some mixtures; however, a major potential limitation to this approach is that temporal changes within stocks may be so large that baseline samples are necessary from the same brood years found in the mixture. In North America, genetic stock identification (GSI) techniques are currently being used to provide information about stock composition to managers concerned with mixed-stock fishery problems for chinook, chum, pink, and sockeye salmon. Typically, the contributions from different river drainages can be accurately estimated, and the power of resolution may extend to different rivers within drainages. Extension of GSI to the problem of continent-of-origin is straightforward, but at present implementation is seriously limited by the lack of adequate baseline information, principally from Asia but also from Alaska and northern British Columbia as well.

Currently, a lack of baseline data limits the effective use of all intrinsic biological markers in forensic analyses. Such deficiencies make it difficult to rebut a potential claim that the fish in question came from a region not represented in the baseline. It is our opinion that with adequate baseline coverage, freshwater sources can be accurately identified for most (perhaps all) species of anadromous salmonids in the North Pacific. An integrated approach that takes advantage of all available information is the best strategy for achieving this forensic objective. We feel that a high priority should be given to efforts to obtain new baseline samples, and that all sampling efforts should be designed to provide adequate material for parasite, scale, and genetic analyses. Concurrently, work should begin to apply rapidly-evolving DNA technology to the high seas problem. DNA analysis holds great promise for providing unequivocal identification to place of origin for anadromous salmonids captured at sea.

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coho salmon. Tony Gharrett of the NMFS Auke Bay Laboratory supplied allele frequency data for pink salmon. The Washington Department of Fisheries, Olympia; the NMFS laboratory at Auke Bay; and the U. S. Fish and Wildlife Service, Anchorage, provided unpublished genetic data used in the chum salmon GSI analyses.

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