

**Japanese Research Plan in the Bering Sea and the Gulf of Alaska  
during the Summer of 2002 for BASIS**

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# Japanese Research Plan in the Bering Sea and the Gulf of Alaska during the Summer of 2002 for BASIS

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## Introduction

Unanticipated changes in the ocean productivity of Bering Sea ecosystem are affecting Asian and North American societies and economies through reduction and possible elimination of important commercial and subsistence fisheries. An international effort is required to detect and monitor changes in salmon and their ecosystem because stocks from all major salmon producing nations are distributed in the Bering Sea, intermingle in international waters, and migrate across the national economic zones. At the 2001 annual meeting of the North Pacific Anadromous Fish Commission (NPAFC), Canada, Japan, Russia, and the United States agreed to plan and coordinate a new international program that will form the basis for long-term, large-scale ecosystem research on salmon in the Bering Sea (NPAFC, 2001).

The somatic growth of Japanese chum salmon is affected by offshore environment in the North Pacific Ocean (Ishida et al. 1993). Environment in the Bering Sea may be a key to determine the somatic growth of Japanese chum salmon, because Japanese chum salmon are distributed in the Bering Sea during the summer growth period (Urawa 2000). Japan continues to monitor summer salmon stocks and environments in the Bering Sea using research gillnets since 1992. However, data of the monitoring research is not sufficient to estimate abundance of salmon, because of the limited survey area in the central Bering Sea. Thus, we need intensive surveys in the whole areas of the Bering Sea using trawl nets to determine salmon abundance and their ecosystem structures.

## Objectives of Research

Our short term (5 years) purpose is to estimate abundance and spatial distribution of salmon by stocks, and basic ecosystem structures in the Bering Sea. In the first year 2002, we will focus on spatial distribution of salmon, fishing efficiencies of trawl and gillnet, and develop proper research plan for abundance estimation of salmon. The research will be conducted in cooperation with the Bering-Aleutian Salmon International Survey (BASIS) plan (NPAFC, 2001).

## Participants

? Fisheries Research Agency :

- Takashi Ynagimoto, Hokkaido National Fisheries Research Institute (Calibration of echo soundering) (Tokyo – Kushiro before Leg 1)
- Tomonori Azumaya, Hokkaido National Fisheries Research Institute (Leg 1)
- Msa-aki Fukuwaka, Hokkaido National Fisheries Research Institute (Leg 1)
- Akira Kusaka, Hokkaido National Fisheries Research Institute (Leg 1)
- Atsushi Tsuda, Hokkaido National Fisheries Research Institute (Legs 2, 3)
- Tsuneo Ono, Hokkaido National Fisheries Research Institute (Leg 2)
- Hiroaki Saito, Tohoku National Fisheries Research Institute (Leg 2)
- Orio Yamamura, Hokkaido National Fisheries Research Institute (Legs 3, 4)
- ?National Salmon Resources Center (Sapporo):
- Shigehiko Urawa (Leg 4)
- ?Hokkaido University:
- Isao Kudo (Leg 2)
- Yoshifumi Noiri (Leg 2)
- Yosuke Taira (Leg 2)
- Akihisa Urano (Leg 4)
- Syunpei Sato (Leg 4)
- ?National Institute for Environmental Studies:
- Yukihiro Nojiri (Leg 2)
- Shigenobu Takeda (Leg 2)
- ?Marine Biological Research Institute of Japan:
- Hiroshi Kiyosawa (Leg 2)
- ?Central Research Institute of Electric Power Industry:
- Jun Nishioka (Leg 2)
- Takeshi Yoshimura (Leg 2)
- ?Nisshinbo Laboratory (Chiba), Nisshinbo Industry Inc.:
- Syougo Mroiya (Leg 4)
- ?Scientists from NPAFC contracting Parties (Canada, U.S.A. and Russia):
- Davydenko V.A., Kamchatcka Fishery & Oceanography Research Institute, Russia (Legs 1-3 )
- Ellen Martinson, NOAA, U.S.A. (Leg 4)
- ?Assistant researchers (whole legs):
- Eiko Tada, Tokyo University of Fisheries
- Haruyasu Higuchi, Hokkaido University
- Osamu Sakai, Hokkaido University
- Naoki Tanimata, Hokkaido University

### **Research Vessel**

*Kaiyo maru* (Fisheries Agency of Japan) 2,630 tonne, 3,500 horse power × 2

## Tentative Schedule

June 20 –September 30, 2002 for 103 days (Table 1)

## Survey Area

The sampling stations are the BASIS's and *Wakatake maru* fixed locations in the Bering Sea (Figure 1 and Table 2). In the Gulf of Alaska Iron, chlorophyll and phytoplankton production at the sea surface will be monitored. In the continental shelf of eastern Bering Sea, only hydrographic samplings will be conducted, because the depth in this area are less than 100 m.

## Field Survey

### ?? Fish Sampling

#### *Trawl operation*

To catch salmon and other nektonic species, one-hour trawl operation will be made in the surface layer (from the surface to 60 m in depth) with 5 knots towing speed. **The net size is 208 m long, 63.2 m head rope, 400 m warp and the cod-end made of 11 mm knotless mesh.**

#### *Salmon treatments*

All salmon in the catches will be counted by species. The principal biological characters that will be measured include fork length, body weight, sex, and gonad weight. Gonad weight will be used as an index of maturity. Juvenile (ocean age-.0) salmon will be frozen in the round for laboratory collection of length, weight, stomach contents, scales, otoliths, and tissues for genetic analysis. Immature and adult salmon will be sampled aboard the vessel for scales, otoliths, tissues (muscle, heart, liver and brain), and stomach contents for feeding, growth, stock identification, parasite, and neuroendocrine analyses (Table 3). Tissue samples for genetic analyses will be kept frozen at -80°C. Some chum salmon will be frozen in the round for parasite and lipid analyses. Heads will be collected from all salmon with missing adipose fins for laboratory examination for coded-wire tags.

#### *Salmon Abundance estimation*

The abundance of salmonids in the whole areas of the Bering Sea using trawl nets will be estimated, and compared to those in the Central Bering Sea using gillnet by the R/V *Wakatake maru* in the same time around same time. These surveys will provide that it is adequate to conduct stock assessment of Japanese-origin salmon in the Bering Sea.

#### *By-catch organisms*

By-catch organisms will be sorted according to species and measured and its body weights and body lengths will be recorded. Pollack will be kept frozen and squids will be kept in a 10% formalin seawater solution.

### ?? Zooplankton Sampling

NORPAC net (vertical tow) with attached flow meter will be hauled vertically from 150 m. Carry

out surface will tow of the ORI NET (0.67 mm mesh)(Legs 1, 3, and 4). RMT will tow the depth of 200 m. Zooplankton will be preserved in a 10% formalin seawater solution (Leg 4). Multiple Opening/Closing net will be made in the malt layers with 2 knots towing speed. The sampling will be carried out at each depth layers from 400 m to surface with changing nets (Leg 4).

### ?? **Oceanography, DO and Nutrient**

Oceanographic observations will be made with CTD after fishing operations. CTD observations will be changed to XCTD observations based on conditions at trawl locations. Several sensors on the CTD “octopus” will collect data (temperature, salinity, depth, and dissolved oxygen (DO)) from 0-3000 (Leg 1) meters or 0-1500 m (Legs 3 and 4). CTD Rosette sampling using the 2.5 liter Niskin bottle x 13 depth will be made for collection of the water at the depth of 0 (bucket sample), 10, 20, 30, 50, 75, 100, 125, 150, 200, 250, 300, 400 and 500 m for salinity, DO and the nutrient,  $\text{NO}_2+\text{NO}_3$ ,  $\text{PO}_4$ , and  $\text{SiO}_3$ . Investigations of vertical thermal and saline structure (0-1000 m) using XCTD. Salinity will be confirmed by auto-salinometer analysis. Dissolved oxygen will be measured by titration method.

### ?? **Acoustic Survey**

Make echo sounding research during daytime at the station of the trawl observation by Simrad EK500. Reduce the cruising speed (8-10 knots) from the site that is 8-10 nautical miles before the fixed station in order to make echo sounding research when we have enough time to survey.

### ?? **ADCP Observation**

Observe the vertical distribution of sea currents using the ADCP system. Currents direction and speed will be measured at 3 layers.

### ?? **Solar Radiation**

Solar radiation studies using meteorological radiometer, Print out every 5 min.

### ?? **Other Information on Sea Weather**

Measure and record continuously other meteorological elements on the sea weather using the automated meteorological monitoring equipment during the entire cruise.

### ?? **Tag Survey**

Sampling of salmon will be done by Hook and line fishing for tag survey.

### ?? **Iron Fertilization Experiment (Leg 2)**

Iron, SF6, chlorophyll, phytoplankton production at the sea surface will be monitored. Vertical profiles of biological and chemical parameter at the center of iron patch and reference site will be measured. Incubations for primary production and microzooplankton grazing will be analyzed. Light attenuation will be measured. HPLC for phytoplankton pigments, POC/PON opal,  $\text{TCO}_2$ , Alkalinity, iron, iron solubility capacity, trace metals, bacterial abundance, nano-flagellates abundance,

microzooplankton abundance, phytoplankton species composition, mesozooplankton abundance and species composition will be measured.

## **Laboratory Survey**

### **?? Nutrients Measurements**

Nutrient,  $\text{NO}_2+\text{NO}_3$ ,  $\text{PO}_4$ , and  $\text{SiO}_3$  will be analyzed .

### **?? Scale Analyses**

Ages will be determined by visual examination of scale patterns for all salmon. Scales will be collected from the INPFC preferred area of the fish body. For juvenile salmon, two scales per fish will be collected, placed on gummed cards with the sculptured surface up and impressed in transparent acetate. Procedures for immature and adult salmon will be similar, except that scales will be mounted on gummed cards during shipboard processing. Scale impressions will be provided to scientists in the member nations by request.

### **?? Stomach Content Analyses**

The salmon stomachs will be removed and frozen individually. After thawing, the stomach samples will be weighted on a balance before and after removal of stomach contents. The weight of the contents will be obtained by subtraction. A stomach content index (SCI) will be calculated as the ratio of measured prey weight to salmon body weight times 100.

### **?? Genetic Stock Identification**

Origin of chum salmon will be estimated by allozyme and mitochondrial (mt) DNA analysis. The muscle, heart, and liver are collected from all chum salmon, and immediately frozen at  $-80^\circ\text{C}$  for laboratory analysis. The tissues are examined for 20 allozyme loci on horizontal starch gels at the National Salmon Resources Center, Sapporo. At Hokkaido University (Sapporo), DNA is isolated from the liver, and the nucleotide sequences of 500 bp variable portion from the 5' end of mtDNA are examined as described in Sato et al. (2001). We will also test a newly developed microarray system to determine mtDNA haplotypes by using blood samples on board. Stock contributions will be estimated with a conditional maximum likelihood algorithm using SPAM.

### **?? Otolith Mark Detection**

The left and right sagittal otoliths will be removed from all pink and chum salmon to detect thermal marks. Otolith samples will be examined at the National Salmon Resources Center, Sapporo. The left sagittal otoliths will be mounted sulcus-side up, using thermal resin, on petrographic slides, and then ground to expose primordia. If left sagittal otoliths are not available or are overground, then right sagittal otoliths will be used. Otolith microstructure will be examined under a compound microscope, and the microstructure patterns will be compared to mark patterns from Asian and North American hatchery voucher specimens. All otoliths will be read independently by a second reader to minimize reader error and provide confidence in readings.

### ?? **Lipid Content Analyses**

Total lipid content (TL) of chum salmon will be determined to estimate their trophic condition. The muscle and liver are collected from frozen round samples of chum salmon caught at four stations (n=60 each) in north, south, central and western waters. At the National Salmon Resources Center, Sapporo, TL will be extracted from the muscle and liver by Folch's method using chloroform/methanol and measured gravimetrically. Lipids were extracted by homogenizing the white muscle (10 g) or liver (10 g) with 50 ml of methanol and 120 ml of chloroform. The homogenate is filtered through a lipid free paper into glass vessel. The crude extract and water are mixed in a separately funnel in the proportions 8:4:3 by volume. The lower phase is collected, and solvent is evaporated with rotary evaporator. Water and protein contents will be also analyzed for several chum samples.

### ?? **Parasite Survey**

The whole body of chum salmon (n=100) will be frozen for the parasite survey in a laboratory. The muscle, body cavity and internal organs will be examined to determine the prevalence and intensity of the parasitic nematode *Anisakis simplex* larvae.

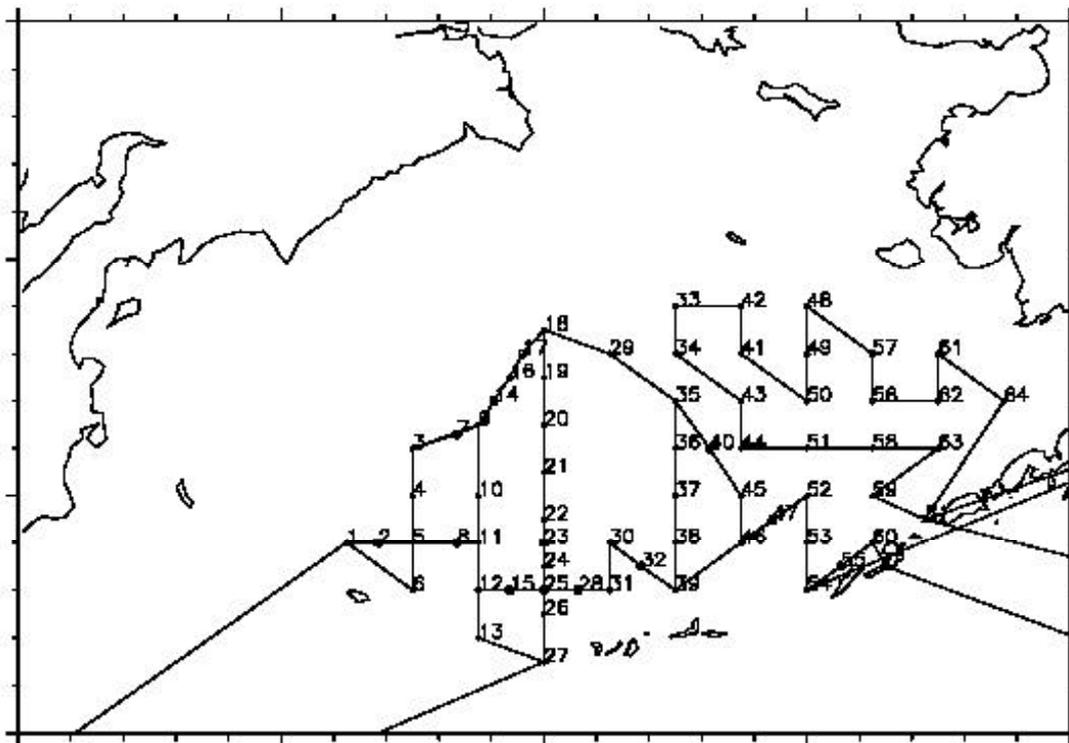
### ?? **Molecular Neuroendocrine Basis Analyses**

The brain, pituitary, gonad and blood will be collected from individual chum salmon to analyze molecular neuroendocrine basis of initiation of homing migration. The brain and pituitary are immersed in cold RNAlater immediately after removal, and the levels of mRNAs for hormone precursors are determined by a real-time PCR method. The gonad is histologically examined to see sexual maturity. The blood is centrifuged, separated into plasma and blood cells, and frozen in a deep freezer. The plasma is later used to analyze the levels of various hormones, while blood cells to determine haplotype for genetic stock identification.

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**Figure 1.** Sampling locations in the Bering Sea and the Gulf of Alaska.

**Table 1.** Tentative cruise plan for *Kaiyo maru*, June-September 2002.

Date	(JST)	Items	Days accumulated
<b>LEG 1</b>			
June	20	leave Tokyo	
June	23	arrive Kushiro	
June	25	leave Kushiro	
June	29	start of survey in the Bering Sea	
July	14	end of survey	
July	17	arrive Dutch Harbor	
<b>LEG 2</b>			
July	20	leave Dutch Harbor	
July	23	start of survey in the Gulf of Alaska	
August	4	end of survey	
August	7	arrive Vancouver	
<b>LEG 3</b>			
August	11	leave Vancouver	
August	17	start of survey in the eastern Bering Sea	
August	26	end of survey in the eastern Bering Sea	
August	30	arrive Kodiak Island	
<b>LEG 4</b>			
September	2	leave Kodiak Island	
September	4	start of survey in the Bering Sea	
September	20	end of survey in the Bering Sea	
September	24	arrive Kushiro	
September	27	leave Kushiro	
September	30	arrive Tokyo; end of cruise	103





**Table 3.** Sampling plan during *Kaiyo maru* cruise (legs 1-4) in 2002.

Cruise #	Oceanography	Primary production	Zooplankton sampling	No. of Trawls	Salmon measurement	Chum salmon							Pink salmon		By-catch samples (pollack, squid)
						Otolith mark	GSI (muscle, liver, heart)	Stomach contents	Lipid analysis	Parasite	Blood	Brain tissues	Otolith	Stomach contents	
Leg 1	CTD (3000 m), XCTD	Nutrient at 0-500 m in depth	NORPAC, ORI	24	Count # of each species, and record folk length, weight, sex, gonad weight and scales for max 60 fish in each species at each station	60 fish at each station. Tissues are frozen at -80C.		20 fish at each station	250 frozen round samples in total	None	None	None	60 fish at each station	20 fish at each station	Pollack (kept frozen) Squid (kept formalin)
Leg 2	CTD			1		None		20 fish at each station	None	None	None	None	None	20 fish at each station	Pollack (kept frozen) Squid (kept formalin)
Leg 3	CTD (bottom or 1500m)	Nutrient from surface to bottom or 500 m in depth	NORPAC, ORI	10		60 frozen round samples at each station		20 fish at each station	None	None	None	None	60 frozen head or round samples at each station	20 fish at each station	Pollack (kept frozen) Squid (kept formalin)
Leg 4	CTD (1500 m)	Nutrient at 0-500 m in depth	NORPAC, ORI, RMT, ION	28		60 fish samples plus frozen round samples at each station. Tissues are frozen at -80C.		20 fish at each station	250 frozen round samples in total	100 frozen round samples in total	60 fish at each station	about 100 fish in total	60 otolith samples plus frozen head samples at each station	20 fish at each station	Pollack (kept frozen) Squid (kept formalin)