

# **BIOLOGICAL RESEARCH**

## **General**



# **Climate Change and Halibut Biology – Year Two Progress Report**

by

Steven R. Hare

## **BACKGROUND**

In 1997, the IPCH embarked upon a three year fisheries oceanography project to examine the influence of climate variability on Pacific halibut biology, particularly growth and recruitment. In the first year of the project, most of our work was focused on climate change in the North Pacific (Hare 1998a). The principal results were description and publication of a paper on the Pacific Decadal Oscillation (PDO, Mantua et al. 1997) and compilation of an Ocean Bottom Properties (OBP) database. The PDO (Figure 1) is a large-scale climate signal, reminiscent of the El Niño Southern Oscillation, that varies on an interdecadal time scale and has been linked to strong fluctuations in many biological populations of the North Pacific (Francis et al. 1998). The OBP database is a compilation of all available information on oceanic conditions within 15 meters of the bottom over the Alaska continental shelf. The purpose of compiling the OBP database was to obtain environmental data more relevant to halibut than the commonly used ocean surface data. The OBP database was further upgraded in 1998 with the addition of 30 years of Russian bottom trawl data and now totals 145,000 near bottom observations.

In the second year of this project, several research projects were conducted, attaining varying levels of completion. We developed a conceptual model mechanistically linking changes in atmospheric forcing to fluctuations in marine biological populations. We used the compiled climate data to begin looking at potential density-independent impacts on halibut biology. Observed variations in halibut growth and recruitment were also examined for evidence of density dependent response. Finally, to consider halibut in an ecosystem context, we examined recruitment patterns for halibut, groundfish, pelagic and salmonid species from the U. S. West Coast to the Bering Sea. Each of these projects is briefly summarized below. A summary of collaborative activities is also provided as one of the major operational components of the IPHC Fisheries Oceanography initiative was coordination and integration into other North Pacific Ecosystem research activities.

## **BOTTOM UP MODEL OF POPULATION VARIABILITY**

This project was a collaboration with Robert Francis and Warren Wooster from the University of Washington and Anne Hollowed from NMFS and resulted in a primary publication this year (Francis et al. 1998). The goal of this research was to develop a conceptual model linking decadal-scale climate variability and widely observed NE Pacific marine population fluctuations (e.g., Clark et al. in press). The work followed logically on work conducted and published on interdecadal climate variability (Mantua et al. 1997) the previous year as part of the IPHC Fisheries Oceanography initiative. The conceptual model we developed is illustrated in Figure 2. Following description of the model, we reviewed evidence of interdecadal variability at all trophic levels of the

Northeast Pacific, from primary production to apex predators. For reasons of space and availability, only the abstract of the paper is included here.

### **Abstract**

A major reorganization of the Northeast Pacific biota transpired following a climatic 'regime shift' in the mid 1970s. In this paper, we characterize the effects of interdecadal climate forcing on the oceanic ecosystems of the NE Pacific Ocean. We consider the concept of scale in terms of both time and space within the North Pacific ecosystem and develop a conceptual model to illustrate how climate variability is linked to ecosystem change. Next we describe a number of recent studies relating climate to marine ecosystem dynamics in the NE Pacific Ocean. These studies have focused on most major components of marine ecosystems - primary and secondary producers, forage species, and several levels of predators. They have been undertaken at different time and space scales. However, taken together they reveal a more coherent picture of how decadal scale climate forcing may affect the large oceanic ecosystems of the NE Pacific. Finally, we synthesize the insight gained from interpreting these studies. Several general conclusions can be drawn:

- 1) There are large-scale, low-frequency, and sometimes very rapid changes in the distribution of atmospheric pressure over the North Pacific which are, in turn, reflected in ocean properties and circulation.
- 2) Oceanic ecosystems respond on similar time and space scales to variations in physical conditions.
- 3) Linkages between the atmosphere/ocean physics and biological responses are often different across time and space scales.
- 4) While the cases presented here demonstrate oceanic ecosystem response to climate forcing, they provide only hints of the mechanisms of interaction.
- 5) A model whereby ecosystem response to specified climate variation can be successfully predicted will be difficult to achieve because of scale mismatches and non-linearities in the atmosphere-ocean-biosphere system.

### **PACIFIC BASIN CLIMATE VARIABILITY AND PATTERNS OF NORTHEAST PACIFIC MARINE FISH PRODUCTION**

This work was in collaboration with Anne Hollowed of NOAA and Warren Wooster of UW and resulted in a primary publication (Hollowed et al., in press). In this analysis we examined recruitment trends for the major salmon, pelagic and groundfish species and their relationship to the major climate signals in the Northeast Pacific. The two climate signals we used were the Pacific Decadal Oscillation (Mantua et al. 1997) and "Nino North". Nino North was defined from an analysis of sea surface temperature data and yielded an index that differed from traditional El Niño indices in that it indexed events based on the strength of their northern (as opposed to equatorial) impacts. We found that recruitment in a large fraction of the Northeast Pacific marine fish stocks appears to be related to either PDO or Niño North forcing. For reasons of space and availability, only the abstract is included here

## **Abstract**

Review of oceanographic and climate data from the North Pacific and Bering Sea revealed climate events that occur on two principal time scales: a) 2-7 years (i.e., El Niño Southern Oscillation (ENSO) events), and b) interdecadal (i.e., Pacific Decadal Oscillation). The timing of ENSO events and of related oceanic changes at higher latitudes was examined. The frequency of ENSO events was high in the 1980s. Evidence of ENSO forcing on ocean conditions in the North Pacific (Niño North conditions) was more frequently observed along the West Coast than in the western Gulf of Alaska and the eastern Bering Sea. Recruitment data for 23 groundfish and 5 non-salmonid pelagic species from three large geographic regions were examined for evidence of Pure Temporal Variability (PTV) caused by large scale forcing at one or more of the time scales noted in oceanographic and climate data. Most salmonids and some flatfish exhibited high autocorrelation in recruitment coupled with a significant step in recruitment in 1977, suggesting a relationship between PDO forcing and recruitment success. Six of the dominant groundfish stocks (Atka mackerel, Pacific cod, Pacific hake and walleye pollock) exhibited low autocorrelation in recruitment. Pacific hake and Gulf of Alaska walleye pollock exhibited a higher incidence of strong year classes in years associated with Niño North conditions. These findings suggest the PTV may play an important role in governing year-class strength of Northeast Pacific marine fish stocks.

## **INVERSE PRODUCTION REGIMES: ALASKA AND WEST COAST SALMON**

This study was in collaboration with Nate Mantua and Robert Francis of the UW and resulted in a primary publication (Hare et al. 1999). In this study, we focused on the response of coastwide salmon populations to climatic forcing. The main result of the analysis is illustrated in Figure 3, showing how Alaska catches of salmon are inversely related to West Coast catches over the past 70 years. Only the abstract is included here.

## **Abstract**

A principal component analysis reveals that Pacific salmon catches in Alaska have varied inversely with catches from the United States West Coast during the past 70 years. If variations in catch reflect variations in salmon production, then results of our analysis suggest that the spatial and temporal characteristics of this "inverse" catch/production pattern are related to climate forcing associated with the Pacific Decadal Oscillation, a recurring pattern of pan-Pacific atmosphere-ocean variability. Temporally, both the physical and biological variability are best characterized as alternating 20- to 30-year-long regimes punctuated by abrupt reversals. From 1977 to the early 1990's, ocean conditions have generally favored Alaska stocks and disfavored West Coast stocks. Unfavorable ocean conditions are likely confounding recent management efforts focused on increasing West Coast Pacific salmon production. Recovery of at-risk (threatened and endangered) stocks may await the next reversal of the Pacific Decadal Oscillation. Managers should continue to limit harvests, improve hatchery practices and restore freshwater and estuarine habitats to protect these populations during periods of poor ocean productivity.

## **DENSITY DEPENDENT AND INDEPENDENT CONTROL OF HALIBUT GROWTH AND RECRUITMENT**

This topic is the focal point of the Fisheries Oceanography project and research aimed at assessing the relative influence of density dependent and independent processes continues. One primary publication (Clark et al. in press) was completed this year and a presentation was given at the American Geophysical Union Ocean Sciences Annual Meeting in February. The dominant feature of halibut growth and recruitment is the decadal-scale nature of the observed variation. Understanding the mechanisms behind this long-term temporal variability is expected to improve out management of the halibut resource. While it is premature to report on findings of this work, it is worth noting the similarity among the PDO and salmon catch indices described above and recruitment of halibut 8 year olds as illustrated in Figure 4.

## **THE OCEAN BOTTOM PROPERTIES DATABASE**

This project involves the compilation of oceanographic data to assist our ongoing analysis of factors influencing halibut recruitment and growth. At year's end, the database consisted of nearly 145,000 observations of eight hydrographic variables (Table 1) measured within 15 m of the ocean bottom along the continental shelf from the Bering Strait to southern California. All of these records have been carefully verified for accuracy. The principal additions this year were 35 years of Russian bottom trawl temperature data and an update (through 1994) of National Oceanic Data Center hydrocasts. Several dozen data requests have been filled from outside agencies as word of this apparently unique resource has spread. A two year proposal to expand this database with collected but undigitized data is presently being considered by NOAA's data rescue program, ESDIM (Environmental Data Service and Information Management).

## **IPHC COLLABORATIONS IN NORTH PACIFIC ECOSYSTEM RESEARCH**

The IPHC does not have the wherewithal to fund an independent full-scale fisheries oceanography research program. It does stand to benefit, however, from the significant amount of ongoing ecosystem/climate research currently being conducted in the North Pacific (Table 2). The IPHC funded one research scientist and began establishing collaborative relationships with several of the agencies, organizations and initiatives engaged in research relevant to Pacific halibut biology. The most significant of these collaborations are summarized.

### **IPHC-organized Regime Shift Impacts group**

This informal group of 25 Northwest scientists convened at the IPHC for four meetings this year. The formation of this group was motivated by the highly unusual climate and biological anomalies of 1997 which were followed by equally unusual anomalies in 1998. In these meetings, we discussed these events, heard presentations and considered evidence for a possible regime shift. IPHC staff gave two presentations:

- 1) Density dependent and independent control of halibut growth and recruitment
- 2) Has there been a large-scale change in the climate of the North Pacific since the regime shift of 1976-77?

## **Bering Sea Ecosystem Research Plan**

A comprehensive, coordinated research plan for the Bering Sea has been drafted, led by a joint effort between NOAA, the U. S. Department of Interior and ADF&G. Two workshops were held in Anchorage (December 1997 and June 1998) to draft and then finalize the plan. The IPHC contributed to both meetings, made two presentations and assisted in the final editing of the plan. The presentations were on 1) The biological and physical databases of the IPHC and 2) Past and Ongoing Bering Sea research activities of the IPHC.

## **North Pacific Marine Science Organization (PICES)**

The IPHC joined the steering committees for two International Symposia, partly sponsored by PICES and scheduled for 1999 and 2000. The first is the Science Board Symposium for the 1999 PICES Annual Meeting being held in Vladivostok, Russia. The Symposium is titled The nature and impacts of North Pacific climate regime shifts. The second is a joint effort between the IPHC, IATTC, PICES and Scripps Institute of Oceanography and is titled Beyond El Niño: A Conference on Pacific climate variability and marine ecosystem impacts, from the Tropics to the Arctic. This will be held in April 2000 in La Jolla, California. Both of these symposia are expected to bring together a diverse group of scientists working on aspects of climate-fisheries interactions in the North Pacific.

## **MISCELLANEOUS ACTIVITIES AND PROJECTS**

In addition to the work cited above, a number of other activities were conducted in 1998. Two other refereed publications were produced: Hare et al. (1998) was a comment and response discussing factors hypothesized as having an influence on the variability of salmon populations in Bristol Bay, Alaska. Hare (1998b) was a short humorous article written on the media coverage of the 1997/98 El Niño event and a plea for more funding directed at ecosystem type variability. The IPHC participated in evaluating and selecting proposals submitted to the Arctic Research Initiative, a NOAA-funded initiative to examine climate impacts on the resources of the Arctic. The IPHC also maintained its involvement in the Pacific Northwest Climate Impacts Group, a UW project looking at how climate affects society in the Pacific Northwest region. Finally, all activities conducted in the Fisheries Oceanography project are summarized and updated regularly on the web site established last year:

<http://www.iphc.washington.edu:80/PAGES/IPHC/Staff/hare/html/decadal/decadal.html>  
<http://www.iphc.washington.edu:80/PAGES/IPHC/Staff/hare/html/papers/OBT/obt.html>  
<http://www.iphc.washington.edu:80/PAGES/IPHC/Staff/hare/html/decadal/post1977/post1977.html>  
<http://www.iphc.washington.edu:80/PAGES/IPHC/Staff/hare/html/1997ENSO/1997ENSO.html>

Collectively, these sites contain information on all aspects of climate and ecosystem variability in the North Pacific and a contemporary assessment of our efforts to understand the linkages between the two.

## **FUTURE RESEARCH AND DIRECTION**

Plans for the next year include pursuing several lines of research.

- Continue research on nature of climate variability and, in particular, examine how ocean climate at depth differs from surface conditions
- Analyze historical variations in halibut growth increment. We will update and extend an earlier study (Hagen and Quinn 1991) and look at temporal variation in halibut growth as recorded in their otoliths. This project was rescheduled from last year due to funding problems.
- Construct an environmental-based recruitment index for halibut. This is in collaboration with scientists at NOAA who are engaged in similar work for other groundfish species.
- Compare growth changes across different species of halibut and attempt to construct a growth model for halibut.
- Examine Pacific basin-wide patterns of zooplankton biomass variability. This is in collaboration with Japanese, Canadian and US scientists.

## **REFERENCES**

- Francis, R. C., S. R. Hare, A. B. Hollowed, and W. S. Wooster. 1998. Effects of interdecadal climate variability on the oceanic ecosystems of the NE Pacific. *Fish. Oceanogr.* 7: 1-21.
- Hagen, P. T. and T. J. Quinn II. 1991. Long-term growth dynamics of young Pacific halibut: evidence of temperature-induced variation. *Fish. Res.* 11: 283-306.
- Clark, W. G., S. R. Hare, A. M. Parma, P. J. Sullivan and R. J. Trumble. In press. Decadal changes in growth and recruitment of Pacific halibut (*Hippoglossus stenolepis*). Accepted *Can. J. Fish Aquat. Sci.*, 27 August 98.
- Hare, S. R. 1998a[Unpub]. Climate change and halibut biology – Year one progress report. IPHC Report of Assessment and Research Activities 1997.
- Hare, S. R. 1998b. Recent El Niño brought downpour of media coverage. *EOS, Trans. AGU* 79: 481.
- Hare, S. R., N. J. Mantua and R. C. Francis. 1999. Inverse production regimes: Alaskan and West Coast Salmon. *Fisheries* 24(1): 6-14
- Hare, S. R., R. C. Francis, E. V. Farley, Jr, J. M. Murphy. 1998. A comment and response on time series outlier analysis. *Alaska Fish. Res. Bull.* 5(1):67-73.
- Hollowed, A. B., S. R. Hare, and W. S. Wooster. In Press. Pacific - Basin Climate Variability and Patterns of Northeast Pacific Marine Fish Production. Proceedings of the 10th 'Aha Huliko'a Hawaiian Winter Workshop on Biotic Impacts of Extratropical Climate Variability in the Pacific, January 26-29, 1998.



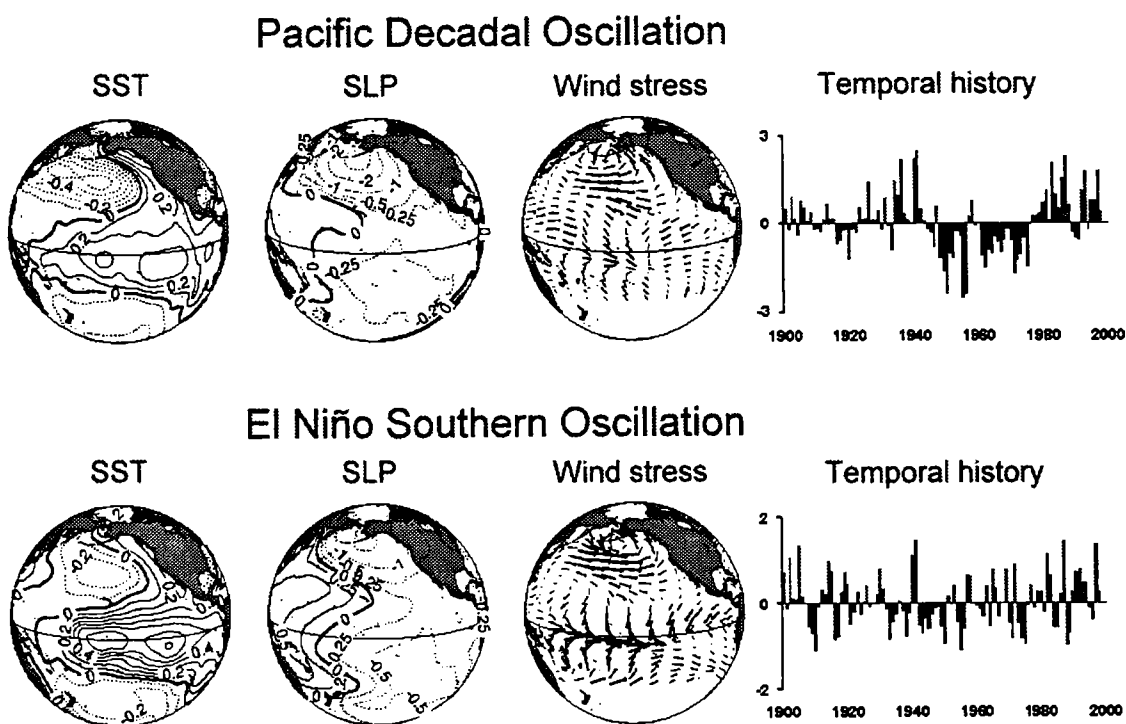
Mantua, N. J., S. R. Hare, Y. Zhang, J.M. Wallace, and R.C. Francis. 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. *Bull. Amer. Meteor. Soc.* 78: 1069-1079.

**Table 1. Parameters and number of observations contained in the Ocean Bottom Properties Database at the end of 1998.**

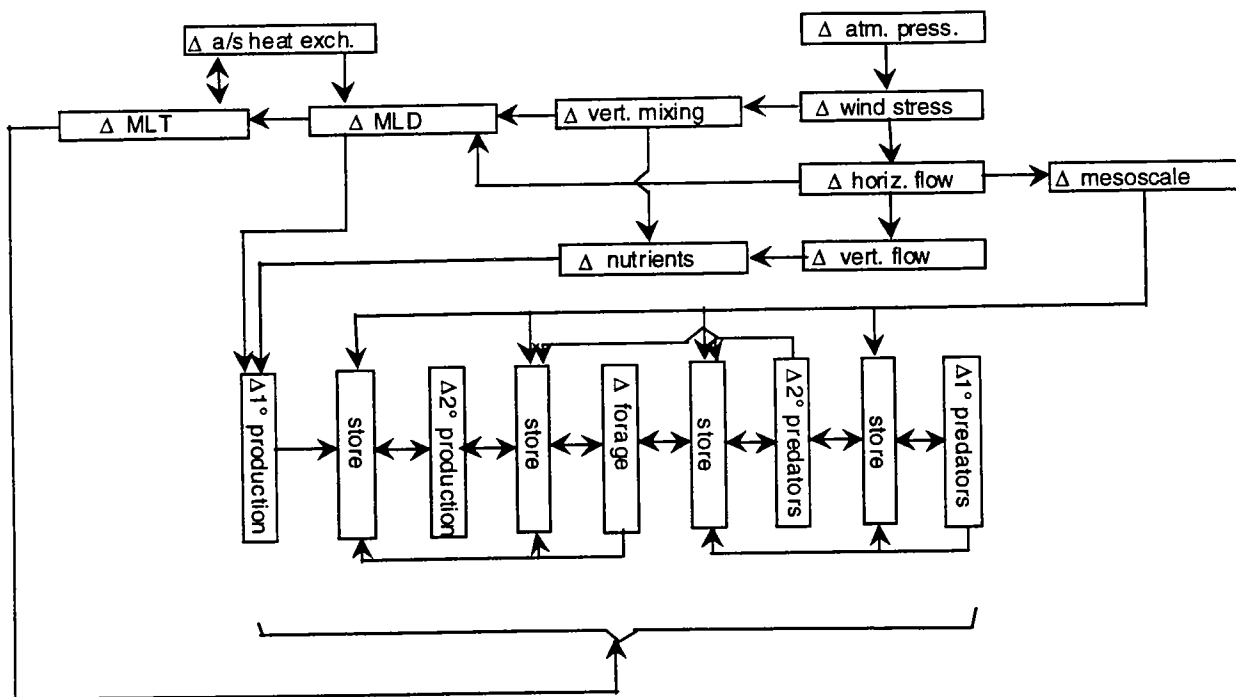
Parameter	No. of records
Dissolved oxygen	11296
Nitrate	2260
Nitrite	1807
Phosphates	4675
PH	419
Salinity	43095
Silicates	2578
Temperature	78672

**Table 2. Partial list of organizations, agencies and funded initiatives conducting Ecosystem/Climate research in the North Pacific potentially relevant to Pacific halibut biology.**

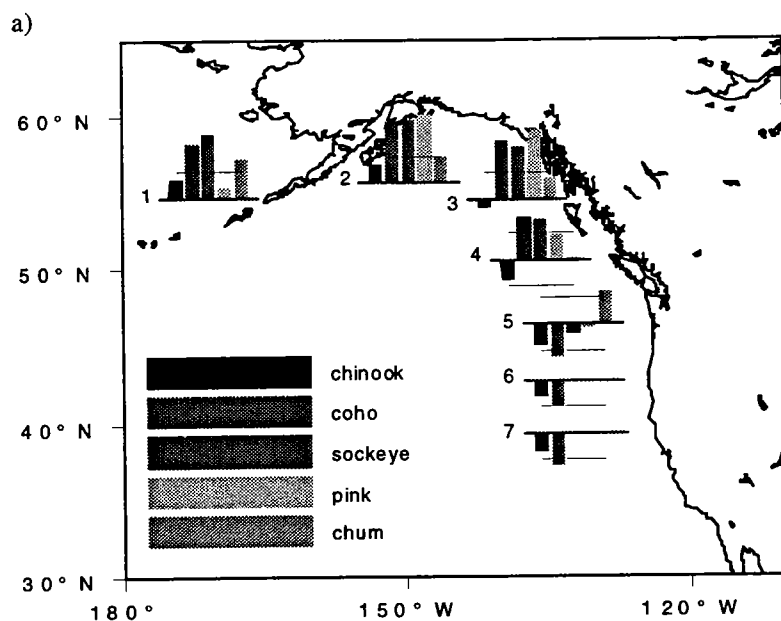
Acronym	Organization
NOAA	National Oceanic and Atmospheric Association
FOCI	Fisheries Oceanography Coordinated Investigations
PMEL	Pacific Marine Environmental Laboratory
SEBSCC	Southeast Bering Sea Carrying Capacity
OCC	Ocean Carrying Capacity
BESIS	Bering Sea Impacts Study
PNCERS	Pacific Northwest Coastal Ecosystems Regional Study
PICES	North Pacific Marine Science Organization
CIFAR	Cooperative Institute for Arctic Research
CalCOFI	California Cooperative Oceanic Fisheries Investigations
CriSP	Columbia River Salmon Passage
NPAFC	North Pacific Anadromous Fish Commission
JISAO	Joint Institute for the Study of the Atmosphere and Oceans
GLOBEC	Global Ocean Ecosystem Dynamics



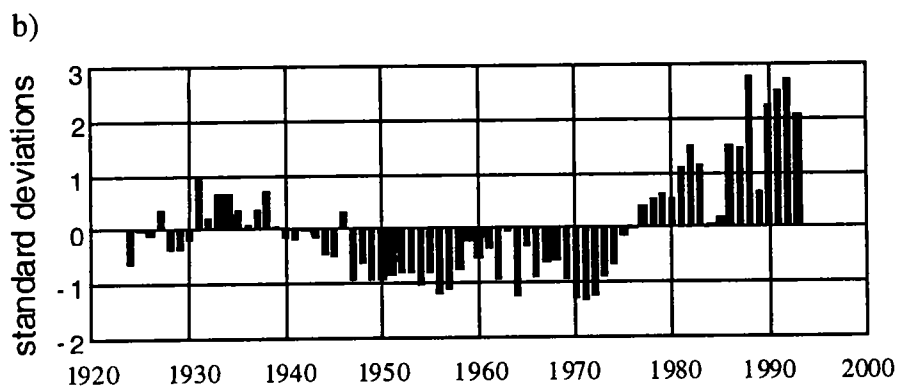
**Figure 1.** A comparison of anomalous climate conditions associated with the positive phases of the Pacific Decadal Oscillation (PDO) and El Niño Southern Oscillation (ENSO). The values show °C for sea surface temperature (SST), millibars for sea level pressure (SLP) and direction and intensity of surface wind stress. The longest wind vectors represent a stress of 10 m<sup>2</sup>/s<sup>2</sup>. Actual anomaly values for a given year associated with the PDO and ENSO are computed by multiplying the climate anomaly with the associated temporal index. Adapted from Mantua et al. (1997).



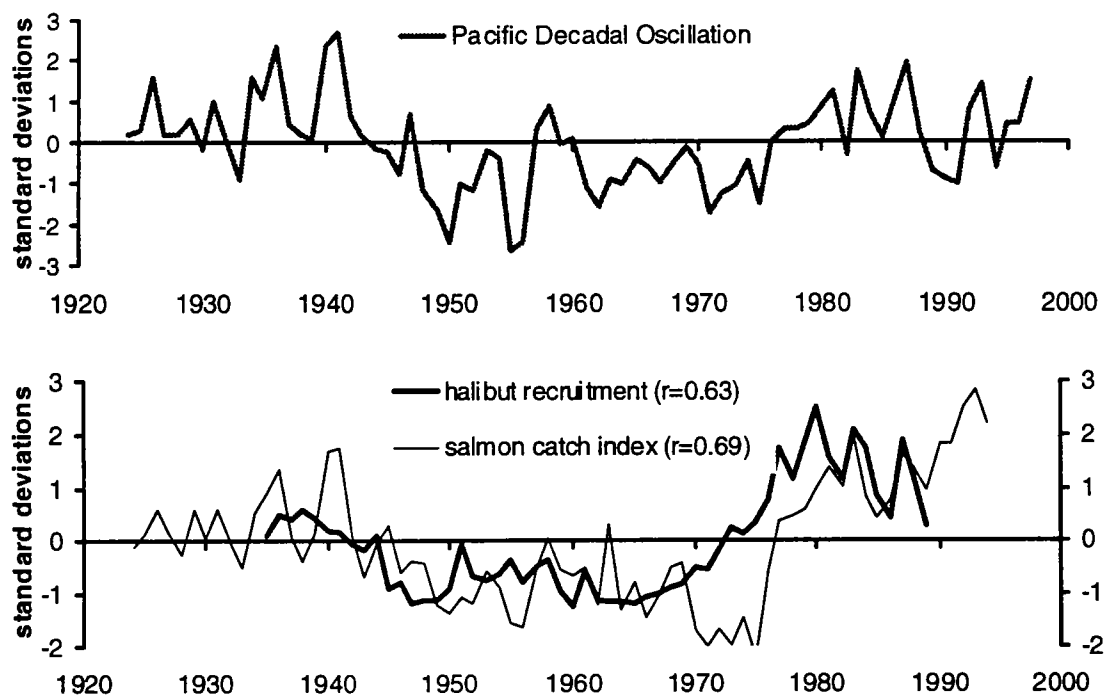
**Figure 2.** A conceptual model illustrating potential pathways by which effects of climate can be mechanistically transmitted to marine biota.



**Figure 3a.** The principal loading vector from a principal component analysis of Pacific salmon catch records. Colored bars are correlation coefficients between principal component score (Fig. 3b) and salmon catch time series. Digits indicate catch region: 1 – western Alaska, 2 – central Alaska, 3 – southeast Alaska, 4 – British Columbia, 5 – Washington, 6 – Oregon, 7 – California. Horizontal bars are 95% confidence intervals modified for autocorrelation (using an average lag-1 coefficient of 0.47) in the catch time series.



**Figure 3b.** The leading principal component score . Values are standard deviations and give the temporal strength of the associated loading vector (Fig. 3a).



**Figure 4.**

# **Completion of Halibut Early life History Project**

by

Robert J. Trumble and Hanwu Liu

Over the course of the Halibut Early Life History (ELH) Project (originally Halibut Culture Project until redirected and renamed), many research results accrued (Liu et al. 1998, Stickney and Liu 1993). The results were related to brood stock maintenance, spawning, fertilization, and development through first feeding. The project never achieved metamorphosis, the primary goal. As a result of budget problems and failure to achieve metamorphosis, the Early Life History Project was terminated in August 1998. A list of references from the project are presented in Appendix 1.

At the January, 1997 Annual Meeting of the International Pacific Halibut Commission (IPHC), the Commissioners limited funding for the project, and specified that attaining metamorphosis of halibut larvae during 1997 was a requirement for funding continuation in 1998. A water quality failure at the laboratory in 1997 killed all the halibut larvae about two weeks before the anticipated beginning of metamorphosis. Salmon and Pacific herring larvae also died during the water quality failure. As a result of the near miss of attaining metamorphosis in 1997, the Commissioners agreed to continue funding for the project in 1998, with the same requirement for metamorphosis in 1998.

In 1998, the ELH Project conducted an experiment to evaluate survival and growth of halibut larvae incubated at different temperatures. One day after hatching, 20 larvae were placed into 24 4-l glass jars. Six jars each were placed into four water baths at temperatures of 5°, 7°, 9°, and 11° C. Two jars from each water bath were sampled for survival and length at 10, 20, and 30 days after beginning the experiment. While larvae survived and grew at about the same rate when held at 5° and 7° C, all larvae at 9° and 11° C died within the first 10 days (Table 1).

## **REFERENCES**

- Liu, H., R. J. Trumble, and D. A. McCaughran. 1998. Halibut early life history study: Project status. Int. Pac. Halibut Comm. Rep. of Assessment and Research Activities 1997. pp. 327-332.
- Stickney, R. R. and H. Liu. 1993. Culture of Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*Hippoglossus stenolepis*). Rev. Fish. Sci. 1(3), pp. 285-309

**Table 1. Survival (numbers of larvae) and length (mm) of Pacific halibut incubated at different temperatures.**

Day	Temperature							
	5° C		7° C		9° C		11° C	
	Survival	Length	Survival	Length	Survival	Length	Survival	Length
0	20	5.9	20	5.9	20	5.9	20	5.9
10	20	7.1	18	7.3	0	N/A	0	N/A
20	15	9.1	16	9.5	0	N/A	0	N/A
30	12	12.4	13	12.9	0	N/A	0	N/A



## APPENDIX 1.

### List of References from the Halibut Early Life History Project

This is a listing of publications stemming directly from operations supported by IPHC. They are presented in chronological order.

1. Liu, H.W., Stickney, R.R., Dickhoff, W.W., Smith S.D., 1989, Preliminary results of spawning Pacific Halibut (*Hippoglossus stenolepis*). Northwest Environmental Journal (5)1, pp. 180.
2. Liu, H.W., 1991. Laboratory studies on the spawning and the early life history of Pacific halibut (*Hippoglossus stenolepis*). Thesis (Ph. D.)--University of Washington, 1991.
3. Liu, H.W., Stickney, R.R., Smith, S.D. 1991. A note on the artificial spawning of Pacific halibut. Prog. Fish Cult. 53(3), pp. 189-192.
4. Liu, H.W., Stickney, R.R., Dickhoff, W.W., 1991. Changes in plasma concentration of sex steroids in adult Pacific halibut, *Hippoglossus stenolepis*. J. World Aquacult. Soc. (22)1, pp. 30-35.
5. Stickney, R.R., Liu, H.W., 1991. Spawning and egg incubation of Pacific halibut. World Aquacult. 22(4), pp. 46-48.
6. Stickney, R.R., Liu, H.W., Smith, S.D., 1991. Recent advances in halibut (*Hippoglossus* spp.) culture. [In] Marine ranching: proceedings of the seventeenth U.S.-Japan meeting on aquaculture. October 16, 17, and 18, 1988. Japan. Ralph Svrjcek, editor. NOAA Technical Report NMFS 120, pp. 9-13.
7. McFarlane, G.A., Jensen, J.O.T., Andrews, W.T., Groot, E.P., 1991. Egg and yolk sac larval development of Pacific halibut (*Hippoglossus stenolepis*). Int. Pac. Halibut Comm. Tech. Rep. No. 24, 22 pp.
8. Liu, H.W., Stickney, R.R., Dickhoff, W.W., McCaughran, D.A., 1993. Early larval growth of Pacific halibut *Hippoglossus stenolepis*. J. World Aquacult. Soc. (24)4, pp. 482-485.
9. Liu, H.W., Stickney, R.R., Dickhoff, W.W., McCaughran, D.A., 1993. Neutral buoyancy salinity of Pacific halibut *Hippoglossus stenolepis* eggs and larvae. J. World Aquacult. Soc. (24)4, pp. 486-492.
10. Stickney, R.R., Liu, H.W., 1993. Culture of Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*Hippoglossus stenolepis*). Rev. Fish. Sci.1(3), pp. 285-309.

11. Stickney, R.R., Seawright, D., 1993. A bibliography on Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*Hippoglossus stenolepis*) culture, with abstracts. Int. Pac. Halibut Comm. Tech. Rep. No. 30, pp. 25-57.
12. Liu, H.W., Stickney, R.R., Dickhoff, W.W., McCaughran, D.A., 1994. Effects of environmental factors on egg development and hatching of Pacific halibut *Hippoglossus stenolepis*. J. World Aquacult. Soc. (25)2, pp. 317-321.
13. Stickney, R.R., 1994. A review of the research efforts on Pacific halibut, *Hippoglossus stenolepis*, with emphasis on research and development needs. [In] Marine Fish Culture and Enhancement. Nosh, T., Freeman, K.(eds.), Washington Sea Grant Program, Univ. of Washington, Seattle, 59 pp.
14. Stickney, R.R., Liu, H.W., 1995. Status and potential for Pacific halibut culture. Bull. Aquacult. Assoc. Can. 95(4), pp. 5-9.

# **Continuing the Investigation into the Occurrence and Causes of the Chalky Condition in Pacific halibut**

by

Stephen M. Kaimmer

## **INTRODUCTION**

During 1998, Commission staff continued a project begun in 1996 to investigate the incidence of chalky halibut. Once again, the industry was asked to file reports on the incidence of chalky halibut in the commercial landings, and a survey was sent out after the close of the season which asked for a recap of 1998 chalky halibut experiences.

## **MATERIALS AND METHODS**

Our efforts during 1998 focused on a series of surveys to more fully document the occurrence of chalky halibut, both over time and area. The IPHC staff used mailings and our port samplers to provide the industry a form intended to document all occurrences of chalky halibut during 1998. Respondents were asked to duplicate this form, fill out one for each observed occurrence of chalky halibut during 1998, and either mail or fax them to the IPHC office before the end of the year. After the close of the 1998 fishing season, IPHC address files were used to send a questionnaire to media, fisher's groups, and all individuals who had bought halibut during 1997. This survey was very similar to the one mailed out at the end of the 1997 fishing season and requested halibut buyers to summarize 1998 experiences with chalky halibut. Due to poor response from Area 2A, a phone survey was conducted early in December to a number of Area 2A buyers, soliciting summary information on pounds handled and pounds of chalky halibut observed during 1998.

## **RESULTS**

During 1998, we received only 9 chalky halibut incident reports from 5 different respondents. This compares to 53 individual reports during 1997. We received 27 responses to our end-of-season survey, summarizing experiences with chalky halibut during 1998, about 2/3 more than we received in 1997. Only two surveys were returned for fish bought in Area 2A. As a special focus, a series of phone interviews were conducted to gather additional information about this area.

### **1998 chalky halibut incidents**

Chalky incident reports were filed by five companies, for a total of 9 reports (5 by one company, and 1 each by four companies). Just over 12,000 pounds of chalky fish were reported on incident forms. Three of the reports were not able to identify the landing vessel, so approximately 1000 pounds (identified by buyer feedback) and 245 pounds (identified in fillet lines) cannot be used in calculating percent occurrence in these landings. Of the remaining 6 reports, the percent

chalky ranged from less than .25 percent to over 20 percent. The latter report was also the largest single chalky load reported, with 8,000 pounds in one delivery. In all cases fish was chilled at delivery. One processor observed that their chalky halibut is most often associated with fish which have "begun to gray on the white side, generally starting at the fin area and working its way to the middle of the fish".

In all cases, fish had been dressed when caught and were well chilled when delivered. In four cases, fish had been bled, and in two of these, the fish had been stunned and bled. Processors reported no occurrences from Area 2A, three occurrences from Area 2B, four occurrences were identified as from Area 2C, and two occurrences from Area 3A.

In seven occurrences detailed by the incident reports in 1998 the chalky fish was identified at the plant during later processing following the fish purchase, and in two instances the chalky halibut was identified by a customer complaint or return. All cases resulted in a downgrading or discarding of the chalky fish.

### **1998 Year-end Survey**

Responses are as follows:

There were 27 responses to the year-end survey. Twenty-two respondents identified themselves as buyers or processors, 1 as a broker, 2 as a cold storage, and 4 as retail sellers. Respondents indicated handling almost 58 million pounds of halibut during 1998, about 2/3 of the overall 1998 production.

Twenty-four of the respondents indicated IPHC area. Two buy halibut from Area 2A, eight from Area 2B, twelve from Area 2C, sixteen from Area 3A, fifteen from Area 3B, and nine from Areas 4 (totals more than 27, since many respondents have activity in more than one area).

Twenty-two respondents reported seeing chalky halibut both in 1998 and in earlier years. Four additional respondents indicated that they had not seen chalky halibut either during 1998 or in the past. Chalky halibut is an "issue" for eighteen of the respondents. It is "not an issue" for eight of the respondents.

All respondents indicated an amount of chalky fish seen in 1998, ranging from 0.0 to 4.1 percent of fish handled. Respondents reported about 324,000 pounds of chalky halibut from almost 58 million pounds handled, about 0.56 percent. This is about 50% lower than the overall rate of chalky halibut reported in the 1997 survey. The 1998 coverage has about the same poundage from Canada but about 2 to 3 times the poundage from Alaska, where chalky rates seem to be lower. The overall decrease is probably an artifact of greater sampling in areas with lower chalky incidence.

It is difficult to estimate chalky halibut by area, since many respondents identified more than one IPHC area of operation, but did not identify chalky fish by area. This is particularly true for estimating chalky fish during 1998 between Canadian and Alaskan waters. Although eight buyers indicated activity in Area 2B, only one indicated buying only in Area 2B, one indicated buying in both Areas 2B and 2C, and the remaining six bought from more than two areas. The first two buyers were major buyers in Area 2B, and represent most of the Area 2B landings. To make some kind of comparison between Alaskan and Canadian landings possible, we can assume that the buyer which operated in both Area 2B and 2C experiences similar rates of chalky halibut in both

areas. Using this assumption, the estimated rate of chalkiness would be 0.4 percent in the U.S. and 0.9 percent in Canada. These percentages are very similar to those found in the 1997 survey.

Three respondents indicated that they had identified chalky halibut at time of delivery, thirteen during later processing in the plant, and fifteen by later claims from buyers.

All respondents who experienced chalky fish indicated the result of the occurrence as a price reduction to the buyer. Five respondents indicated that in some cases the fishermen were also given a lower price.

The surveys included space for comments on topics not covered directly by the survey questions. Three respondents included opinions that "chalky halibut is a very big problem in the industry." This problem was further explained as one of compensation, as the chalky condition is usually not noticed until after the fisherman has been paid for the fish. This translates into dollar losses for buyers.

### **Special Area 2A phone survey**

We were able to identify and contact nine companies which each handled more than 5,000 pounds of Area 2A halibut during 1998. Six of these provided information on total pounds handled and pounds of chalky halibut. Overall, the six companies handled almost 130,000 pounds of halibut during 1998, both treaty and non-treaty commercial sales (about 1/3 of the total Area 2A commercial quota), and reported 3,100 pounds of chalky halibut. No chalky halibut was observed from sales in the northern portion of Area 2A. About 40 percent of the respondents from southern 2A saw no chalky fish during 1998. The remaining respondents had incidences ranging from 2 to over 16 percent. For those respondents who indicated some chalky halibut during 1998, the average percentage of chalkiness reported was 12.7 percent. One other respondent indicated only that his purchases were "chalky," and another curtailed their buying in Area 2A during 1998 due to dollar losses from chalky fish during 1997.

## **DISCUSSION AND RECOMMENDATIONS**

The general trend suggested by the 1996 and 1997 surveys appears to continue. Previous surveys indicated an incidence of about 0.5 percent in the U.S. and 1.0 percent in Canada, with some amount of chalky halibut reported for all IPHC areas, and for all months of the fishery. The survey of the 1998 fishery indicated similar incidences, 0.4 and 0.9 percent, respectively, in the U.S. and Canada. There is again a trend which suggests higher percentages of chalkiness in Areas 2, and much lesser percentages in the westward areas 3 and 4.

One caution should be considered when interpreting the survey and incident data. Many fish, probably more than half of the entire production, are not cut at the buying plant beyond removal of the head. The whole fish are either shipped fresh to wholesale buyers or frozen for the same market. In many cases, this makes it difficult or even impossible to determine whether the fish is chalky until after it is sold. While most of the chalky fish reported was recognized through claims by subsequent buyers, it is possible that a large proportion of chalky fish goes unreported. It is also possible the chalky fish goes purposely unreported, in an effort to downplay the problem. We have no way of determining whether either of these biases in fact exist, and, if so, the degree to which they might effect our results.

Chalky halibut has occurred in the directed halibut fishery for at least 30 years. Research directed at this problem dates back to the late 1960's, when a series of field projects established a link between acidity buildup caused by capture stress and the post mortem development of the chalky condition. Various reasons have been suggested for the onset of chalkiness. In general, it appears that a fish which dies from, or in a state of, exhaustion, will have a high degree of lactic acid, a byproduct of exhaustion, in the muscle tissue. This exhaustion could be caused by intense exercise prior to or during the capture process, or possibly by temperature or air exposure while lying on deck prior to dressing.

While a reading of the literature suggests that proper handling of setline halibut can reduce the development of chalkiness, in no case is there any indication that fish handling can stop or reverse the development of chalkiness once the fish dies in an exhausted state. Video observations of halibut hooking indicate that halibut swim vigorously once hooked and then go through a series of resting and darting behaviors prior to longline retrieval. A freshly hooked fish may be in a higher state of exhaustion than one which has been captured for a longer period of time. IPHC research from the 1960's indicates that trawl caught fish have a much higher rate of chalkiness than those caught on longline.

Overall, the incidence of chalkiness appears to be on the order of about a half to one percent, with some trend to higher chalkiness in the extreme southern areas. It is possible that either higher water temperatures or higher air temperatures during capture either increase capture stress or in some way accelerate the chalky process. Halibut are not chalky when they are killed. The early studies demonstrated that chalkiness developed in iced product 3 to 7 days after death, and the condition could develop after thawing at a much later date in fish which were frozen. While it may not be possible to eliminate chalky halibut from our fishery, a method to determine the tendency for chalkiness at dock delivery would be most advantageous.

When applied to total landing figures, an overall incidence of one-half to one percent could represent three to six hundred thousand pounds of chalky fish being sent to market. It is possible that increased diligence by fishers can reduce the occurrence of chalky fish. For the most part, fishers now are very aware of procedures to maximize quality of landed product (stunning, bleeding, and rapid chilling), and our studies have not yet suggested any additions to these procedures which would minimize chalkiness. From the current and previous years incidence reports, and from anecdotal data, there is only the weakest pattern in area and time of chalky fish occurrence, the suggestion that chalkiness is more common during hot months, or from the more eastern areas. It is possible that chalkiness is a fact of the fishery, even when fish are handled as well as possible.

Unfortunately, the IPHC does not have resources available for further investigation of the chalky halibut problem. However, two avenues suggest themselves. First, a more detailed description of the process by which a fish becomes chalky. This would discuss the biochemical changes in the flesh which 'trigger' the onset of the chalky condition. While this information is known in general, it is possible that a more detailed investigation, probably a combination of literature review and laboratory research, could help determine points at which the process could be stopped or reversed. Second, a procedure to simply and cheaply identify tissue pH when fish is sold would allow chalky fish to be identified during the initial selling process between the fisherman and the buyer. This could reduce dollar loss to buyers, as well as keep chalky halibut from getting into the marketplace. Either of these avenues of investigation could be pursued in cooperation with a university or fishery technology center.

# Genetic Variation in Pacific Halibut (*Hippoglossus stenolepis*) Detected with Novel Microsatellite Markers

by

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## INTRODUCTION

The study of molecular genetic variation within and among populations is one approach to examining population structure. In the case of marine fishes, genetic differences among spawning aggregations that are both statistically significant and stable over time, if detected, are strong evidence that these spawning aggregations represent reproductively isolated populations, although the absence of such differences does not prove the converse. Biochemical methods have been used to examine this issue in numerous species of marine fishes (reviewed in Shaklee and Bentzen 1998). The outcomes of such studies have ranged from genetic homogeneity (panmixia) on ocean basin scales to highly significant genetic subdivision (suggestive of subdivided populations) on much smaller scales. Generally, species with potential for extensive dispersal either as pelagic larvae or highly mobile adults show less population subdivision than species that lack these attributes. However, exceptions exist where genetic data have revealed population structure that has been unexpected on the basis of distributional data or apparent dispersal tendencies (Shaklee and Bentzen 1998).

This report describes a genetic study of Pacific halibut (*Hippoglossus stenolepis*) carried out with the ultimate goal of examining population structure by surveying genetic variation in this species across its range. The population survey was preceded by a preliminary phase in which we developed the microsatellite genetic markers needed for the population analysis.

Microsatellites, also known as simple sequence repeats, are repetitive DNA sequences composed of short motifs repeated in tandem arrays (e.g., CACACACA...) (reviewed in O'Reilly and Wright 1995, O'Connell and Wright 1997). The genomes of all eukaryotic organisms contain thousands of microsatellite arrays distributed across all chromosomes. Microsatellites often exhibit extensive allelic polymorphism in the number of repeats that occur within particular arrays. The allelic variation in any given microsatellite array can be assayed by first amplifying it from a sample of genomic DNA via the polymerase chain reaction (PCR) in conjunction with oligonucleotide primers that anneal to non-repetitive DNA sequences that bracket the array. Alleles are resolved by high-resolution electrophoresis of the amplified microsatellite array on polyacrylamide gels.

Microsatellites have several desirable attributes as genetic markers: 1) They are often highly variable, exhibiting many alleles. 2) They can be analyzed relatively easily from small tissue samples such as fin clips. 3) They are diploid, co-dominant markers—meaning that they are biparentally inherited (unlike mitochondrial DNA which is inherited only from mothers and is effectively haploid) and that both alleles are detected in heterozygotes. 4) The number of microsatellite loci that are potentially available for analysis is effectively unlimited. Analysis of microsatellite variation

has been used to resolve population genetic structure in many species, including some marine fishes (O'Connell et al. 1998; Bentzen et al. 1996; see also reviews by O'Connell and Wright 1997; O'Reilly and Wright 1995).

Microsatellites also present some disadvantages as genetic markers. Chief among these is that the DNA primers needed to analyze specific microsatellites can only be designed after microsatellites have been cloned and sequenced from the species of interest, or a reasonably close relative, such as another member of the same genus or family. For many heavily studied taxa (such as salmonids, or even gadid fishes) numerous primers for microsatellite loci are available. However, for Pacific halibut at the outset of this study, no microsatellite primers were available. Therefore, a critical first step in this study was to clone and develop novel microsatellite genetic markers for Pacific halibut. A second potential disadvantage of microsatellites is the degree of polymorphism exhibited by some of them is actually too high to be optimal for use in population studies. This is a problem we experienced with the microsatellites we developed in this study.

## METHODS

### Microsatellite cloning

Microsatellites were isolated from two halibut genomic DNA libraries. In each case, halibut genomic DNA was first digested with a restriction enzyme, then size-fractionated on a 1% agarose gel. The 300-600 base pair (bp) DNA fraction was excised from the gel and purified by phenol/chloroform extraction, then ligated into an appropriate plasmid vector and finally, used to transform competent *E. coli* cells via the heat-shock method (Sambrook et al. 1989). For the first library halibut DNA was cleaved with *Hae*III and ligated into dephosphorylated *Sma*I-digested pGEM (Promega) which was then used to transform DH5a cells (Gibco BRL); for the other library the restriction enzyme, vector and cells were *Mbo*I, *Bam*HI-cut pZero plasmid (Invitrogen) and Top10F' cells (Invitrogen), respectively. Colonies were transferred to nylon membranes (Nytran: Schleicher and Schuell) and probed with fluorescein-labeled (GT)<sub>15</sub> and (GA)<sub>15</sub> oligonucleotides (Amersham). Following stringency washes the membranes were treated using the Vistra signal amplification kit (Amersham), then scanned on a Molecular Dynamics FluorImager 575. Clones that yielded strong hybridization signals were grown overnight, then extracted using the Qiaprep plasmid DNA isolation kit (Qiagen Inc.) and sequenced using Applied Biosystems Inc. (ABI) *Taq* DyeDeoxy™ terminator chemistry and an ABI 373A automated sequencer.

Amplification primers were designed for clones that contained microsatellites with at least 10 repeats, and sufficient unique sequence on either side of the microsatellite array to facilitate primer placement. Primers were designed with the aid of the programs Primer3 (Rozen and Skaletsky 1996) and Cprimer (Bristol and Andersen 1995). Primers were synthesized and tested under varying conditions of stringency for their ability to facilitate selective PCR amplification of single, polymorphic microsatellite loci in a panel of six halibut DNA samples. Three primer sets that yielded highly specific amplifications of polymorphic microsatellites were selected for use in a population survey.



## Population survey

Halibut were collected from five geographic regions over a two year period (Table 1). Fin tissue was preserved either in DMSO/NaCl solution or in EtOH. Genomic DNA was isolated from 50-100 mg fin tissue using the phenol/chloroform method (Hoelzel and Green, 1992). The success of the DNA extractions varied with the preservation method and source of the samples; generally, DNA from samples preserved in EtOH was sufficient in quality and yield to permit assay of microsatellites, whereas samples preserved in the NaCl/DMSO solution usually yielded no DNA or DNA that was severely degraded and not suitable for further analysis (Table 1). Due to the degraded condition of all samples from the Bering Sea, genetic analyses were only carried out on halibut samples from three areas in the Pacific region (Figure 1) and on nine Atlantic halibut samples that yielded DNA of adequate quality.

A 'triplex' PCR was used to amplify three halibut microsatellites, Hst5, Hst15 and Hst16 in a single tube. PCRs were carried out in 10 mL volumes comprised of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.8 mM dNTPs, 0.4 units *Taq* (Promega), 0.24 mM each primer and 50 ng DNA template in a Perkin Elmer 9600 thermocycler. The amplification profile comprised the following: one cycle of 92°C (2 min) and 24 cycles of 94°C (30 s) + 55°C (30 s) + 72°C (30 s).

Amplified microsatellites were detected using an ABI 373A sequencer operated in GeneScan mode (ABI 1996). For each sample, 0.5 mL of the multiplex PCR product was combined with 2.5 mL formamide, 0.50 mL 25 mM EDTA and 0.5 mL (1.0 fmol) Perkin-Elmer GS350 internal size standard. Allele sizes were determined using the internal lane sizing standard and a local Southern sizing algorithm in the GeneScan analysis software (ver. 2.0; ABI, 1994). Genotypes for each locus were determined using Genotyper software (ver. 1.1; ABI 1994) and then exported into other statistical software for further analysis.

The number of alleles, allelic range and both observed and expected heterozygosity ( $H_o$  and  $H_e$ ) were calculated for each population. Tests for departures from Hardy-Weinberg equilibrium (HWE) and heterogeneity in allele frequencies were performed using probability tests available in version 3.1b of the GENEPOP software package (Raymond and Rousset, 1995). Estimates of subpopulation structure were obtained using two measures:  $q$ , Weir and Cockerham's (1984) unbiased estimate of  $F_{ST}$ , and  $r_{ST}$ , Goodman's (1997) unbiased estimate of the  $R_{ST}$  measure originally proposed by Slatkin (1995). Unlike  $q$ ,  $R_{ST}$  incorporates information about allele size, and is thought to give a less biased estimate of population structure for microsatellites when migration rates are low and coalescence times are relatively large (Slatkin, 1995). Estimates of  $q$  and  $r_{ST}$ , and bootstrap estimates of their significance levels, were calculated using FSTAT (Goudet, 1997) and RST CALC (Goodman, 1997), respectively. Significance levels ( $\alpha$ ) for all statistical tests were determined using sequential Bonferroni adjustments for simultaneous tests (Rice, 1989).

## RESULTS

### Microsatellite Cloning

A screen of ~2,000 clones in the two libraries led to the identification of 30 'positives' selected for further analysis. Sequence analysis of these clones revealed that at least 28 contained distinct microsatellite arrays (App. 1). Primers were designed for 17 clones (App. 2) and tested on a panel of six halibut samples; of these, amplifications with 12 primer sets produced products that

were either monomorphic or else consisted of complex or poorly resolved band patterns. Of the remaining six primer sets, three sets (Hst5, 15, 16) that facilitated amplification of well-resolved polymorphic microsatellite loci were chosen for further population screening.

## Population Survey

Analysis of 358 Pacific halibut revealed high levels of polymorphism in each of the three microsatellite loci in all three sampling regions (Table 2, Figure 2). The number of alleles per locus was 33-58 (mean = 47) and observed heterozygosities ( $H_o$ ) were 93-100% (mean = 96%). The genetic diversity of the Atlantic halibut sample was lower, with 6-12 alleles per locus (mean = 9.3) and  $H_o$  = 38-89% (mean = 72%); however, the lesser extent of genetic variation observed in the Atlantic halibut was undoubtedly due at least in part to the small sample size. None of the three Pacific halibut samples deviated significantly from Hardy-Weinberg equilibrium at any of the three loci (Table 2).

Probability tests of homogeneity of allele frequencies in pairwise comparisons of the three Pacific halibut samples revealed significant differences between the Russia and Washington samples at two loci, Hst5 and Hst15 (Table 3). The Russia and Gulf of Alaska samples also differed significantly at one locus, Hst15; whereas, the Gulf of Alaska and Washington samples did not differ significantly at any of the three loci. Measures of genetic subdivision ( $F_{ST}$  and  $R_{ST}$ ) among populations were extremely low ( $F_{ST}$  = 0.001 - 0.003 and  $R_{ST}$  = -0.0026 - 0.0083 in pairwise comparisons, respectively; Table 4). The overall  $r_{ST}$  for the three samples was negative, and hence not significantly different from zero; whereas, the overall  $F_{ST}$ , although extremely low (0.001), was significant ( $P$  = 0.034). The significance of the overall  $F_{ST}$  was driven entirely by Hst15 (single locus  $F_{ST}$  = 0.0033,  $P$  = 0.02); single locus  $F_{ST}$  estimates for Hst5 and Hst15 were not significantly different from zero ( $P$  > 0.30).

## DISCUSSION

The three microsatellite loci that we studied in halibut all exhibited extremely high levels of polymorphism, with 33-50 alleles per locus and heterozygosities >93% in all cases for Pacific halibut, and reaching 100% for locus Hst5 in all three population samples. This extreme variability contrasts with much lower genetic variability seen in halibut with mtDNA. Bickham and Patton (1992) sequenced 1,117 bp of mtDNA in each of eight Pacific halibut sampled off Oregon and in the Bering Sea, but observed only two haplotypes defined by a single variable nucleotide. Haplotype diversity ( $h$ , a measure analogous to heterozygosity for nuclear loci) was ~0.5, and nucleotide diversity ( $p$ ) was 0.0005. However, the extensive variability of the three halibut microsatellites is not unusual among dinucleotide microsatellite loci that have been studied in other marine fishes and invertebrates (e.g., Bentzen et al., 1996; Bentzen, unpub. data).

Comparisons of allele frequencies and analyses of population substructure using  $F_{ST}$  and  $R_{ST}$  statistics suggested that genetic differentiation among the three regions sampled is of very low magnitude (Figure 2, Table 4). The overall  $F_{ST}$ , although statistically significant ( $P$  = 0.029) was 0.001, which is equivalent to stating that 99.9% of the genetic variation in halibut occurs within populations, and only 0.1% is distributed among populations. However, allele frequencies did differ significantly between Russia and Washington at two loci, and between Russia and Gulf of Alaska at one locus. These results suggest that the halibut populations, although at or verging on

genetic panmixia may nonetheless be structured in distinct reproductive groups. Further, the two most geographically distant samples, Washington and Russia, were more different from each other than either was from the geographically intermediate Gulf of Alaska sample. This suggests that halibut populations are structured by distance on oceanic scales, such that populations that are closer to each other are more likely to exchange migrants. We observed no differences between the Washington and Gulf of Alaska samples, which is consistent with the possibility that these are part of a single northeast Pacific stock of halibut.

All of the inferences made above are tentative. The power of this genetic analysis was weakened by the small number of loci employed, and possibly by the extreme variability of the microsatellite loci. The level of polymorphism exhibited by the three microsatellites employed in this study was well above the optimum for broad-scale studies of population structure. Heterozygosities at each of the three loci reached or approached the limiting value of 100%, which directly limits their utility in detecting population subdivision using the  $F_{ST}$  measure. Moreover, the extreme variability of these loci implies very high mutation rates, which could lead to convergent mutations in different populations, which in turn would lead to underestimates of the true level of reproductive isolation among populations. In a microsatellite-based study of the population genetics of Atlantic cod Bentzen et al. (1996) found that the least variable microsatellite locus showed the strongest geographic structuring on ocean basin scales, whereas three extremely variable loci (similar in variability to the three halibut microsatellite loci) revealed showed weaker differentiation on regional and transoceanic scales.

The biggest weakness of this study was likely the use of samples collected from summer feeding grounds rather than winter spawning sites. Halibut are known to migrate long distances between summer feeding grounds and winter spawning areas; hence, if halibut are philopatric and 'home' to their natal regions to spawn, relative genetic homogeneity of halibut sampled over broad areas during the summer may not be indicative of genetic differentiation of halibut on the spawning grounds.

### **Recommendations for further work**

Analysis of additional samples will be needed to test the validity of the apparent differences between Russia and the North American populations. It would also be of interest to test for potential differentiation between Pacific Ocean and Bering Sea populations. Some marine fishes, such as herring (*Clupea pallasii*) (Grant and Utter 1984; Bentzen unpublished data) and walleye pollock (*Theragra chalcogramma*) (reviewed in Bailey et al. 1999) show marked differentiation between the Bering Sea and adjacent areas in the north Pacific, despite the fact that these species have continuous distributions spanning the border of the two sea basins.

It would also be helpful to study more genetic loci, preferably ones exhibiting more moderate levels of variability than those assayed in the present study. For these reasons, it would be helpful to survey halibut with less variable microsatellite loci, or other moderately variable DNA markers. We experienced unusually poor success in developing usable microsatellite markers based on dinucleotide (GT)<sub>n</sub> or (GA)<sub>n</sub> repeats in halibut. Despite an apparent abundance of these loci in Pacific halibut, the majority of the microsatellites we cloned did not prove suitable for use as genetic markers. Although the reason(s) for this result are not clear, our experience suggests that it would be preferable to develop microsatellite markers based on tri- or tetranucleotide motifs (e.g.,

AAT, or GACA). Such tri- and tetranucleotide microsatellites are typically easier to resolve in population studies, and are often somewhat less variable than the hypervariable dinucleotide loci we employed in this study.

## REFERENCES

- Bailey, K.M., T.J. Quinn, P. Bentzen and W.S. Grant. 1999. Population structure and dynamics of walleye pollock, *Theragra chalcogramma*. *Advances in Marine Biology* (in press).
- Bentzen, P., C.T. Taggart, D.E. Ruzzante, and D. Cook. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Can. J. Fish. Aquat. Sci.* 53:2706-2721.
- Bickham, J.W., and J.C. Patton. 1992. Preliminary analysis of molecular techniques and search for variation in mitochondrial DNA of halibut. Report to the International Pacific Halibut Commission.
- Bristol G., and R. Andersen. 1995 Cprimer Manual. Dept of Medicine Hem.-Onc., School of Medicine, University of California, Los Angeles 90095 U.S.A.
- Goudet, J. 1995. FSTAT version 1.2: a computer program to calculate F-statistics. *Journal of Heredity* 86:485-486.
- Grant, W.S., D.J. Teel, T. Kobayashi, and C. Schmitt. 1984. Biochemical population genetics of Pacific halibut (*Hippoglossus stenolepis*) and comparison with Atlantic halibut (*H. hippoglossus*). *Can. J. Fish. Aquat. Sci.* 41:1083-1088.
- Grant, W.S., and F.M. Utter. 1984. Biochemical population genetics of Pacific herring (*Clupea pallasi*). *Can. J. Fish. Aquat. Sci.* 41:856-864.
- Hoelzel, A.R. and Green, A. (1992). Analysis of population-level variation by sequencing PCR-amplified DNA. In: *Molecular Genetic Analysis of Populations. A Practical Approach*, Hoelzel, A.R. [ed.]. New York: Oxford University Press, 159-186.
- O'Connell, M., and J.M. Wright. 1997. Microsatellite DNA in fishes. *Rev. Fish Biol. Fish.* 7:331-363.
- O'Connell, M., M.C. Dillon, J.M. Wright, P. Bentzen, S. Merkouris and J. Seeb. 1998. Genetic structuring among Alaskan Pacific herring (*Clupea harengus pallasi*) populations identified using microsatellite variability. *J. Fish Biol.* 53:150-163.
- Olsen, J.B., J.K. Wenburg, and P. Bentzen. 1996. Semi-automated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellites. *Molecular Marine Biology and Biotechnology* 5:259-272.

- O'Connell, M., and J.M. Wright. 1997. Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries* 7:331-353.
- O'Reilly, P.T., and J.M. Wright. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *J. Fish Biol.* 47 (Suppl. A):29-55.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetic software for exact tests and ecumenicism. *Journal of Heredity* 86:248-249.
- Rozen, S., and H. Skaletsky. 1996. Primer3 design program. Available at [http://www-genome.wi.mit.edu/genome\\_software/other/primer3.html](http://www-genome.wi.mit.edu/genome_software/other/primer3.html)
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: a laboratory manual*, 2<sup>nd</sup> edition. Cold Spring Harbor Laboratory Press. New York.
- Shaklee, J.B. and P. Bentzen. 1998. Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science* 62:589-621.
- Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457-462.
- Weir, B.S., and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.
- Wright, J.M., and P. Bentzen. 1994. Microsatellites: genetic markers for the future. *Rev. Fish. Biol. Fish.* 4:384-388.

**Table 1. Source of halibut samples, method of preservation and results of DNA extractions.**

Source	Preservative	N	Yield	Comments
<b>First collection</b>				
Russia	EtOH	207	200	4 samples contaminated by other samples
Bering Sea	NaCl/DMSO	173	0	70 extractions attempted; all samples severely degraded
Atlantic	NaCl/DMSO	45	9	most samples degraded
<b>Second collection</b>				
Gulf of Alaska	EtOH	139		extractions attempted on all samples, some degraded
Washington	EtOH	41	41	samples in good condition

**Note:** Yield is the number of samples that yielded DNA suitable for microsatellite analysis.

**Table 2. Summary statistics for microsatellite polymorphism in halibut.**

	Washington	Gulf of Alaska	Russia	Total Pacific halibut	Atlantic
<b>Hst5</b>					
<b>N</b>	41	117	198	356	8
<i>n</i>	32	43	49	58	6
<i>range</i>	111-223	111-237	109-245	109-245	107-155
$H_O$	1.000	1.000	1.000	1.000	0.375
$H_E$	0.946	0.944	0.941	0.945	0.508
	0.057	0.060	0.063	0.058	-0.262
<b>D</b>					
$p(D)$	0.549	0.813	0.810	0.9982	----
<b>Hst15</b>					
<b>N</b>	41	117	199	357	9
<i>n</i>	23	28	28	33	10
<i>range</i>	131-203	129-193	129-203	129-203	139-173
$H_O$	0.927	0.966	0.935	0.944	0.889
$H_E$	0.924	0.936	0.926	0.933	0.852
	0.003	0.032	0.010	0.012	0.043
<b>D</b>					
$p(D)$	0.8781	0.0908	0.1175	0.2958	----
<b>Hst16</b>					
<b>N</b>	41	117	200	358	9
<i>n</i>	27	38	44	50	12
<i>range</i>	80-174	80-170	74-190	74-190	90-184
$H_O$	0.951	0.940	0.935	0.939	0.889
$H_E$	0.937	0.948	0.945	0.948	0.895
	0.015	-0.009	-0.011	-0.010	-0.007
<b>D</b>					
$p(D)$	0.4562	0.3745	0.2780	0.1416	----

**Note:** *N*, number of fish; *n*, number of alleles; *range*, smallest and largest alleles observed;  $H_O$ , observed heterozygosity;  $H_E$ , expected heterozygosity; *D*, measure of heterozygote excess or deficiency  $((H_O - H_E) / H_E)$  and  $p(D)$ , estimated probability of observed heterozygote deficiency or excess, calculated using appropriate one-tailed test (see Methods for details).

**Table 3. Probability of homogeneity of allele frequencies among halibut samples estimated from pair-wise probability tests.**

Comparison		Hst5	Hst15	Hst16
Washington	Gulf of Alaska	0.222	0.072	0.734
Washington	Russia	0.015*	0.023*	0.639
Gulf of Alaska	Russia	0.222	0.008*	0.306

**Note:** Probability estimates considered significant following Bonferroni adjustments for three simultaneous tests per comparison are indicated with an asterisk (\*).



**Table 4.** Estimates of population subdivision ( $F_{ST}$  and  $R_{ST}$ ) for Pacific halibut.

Comparison		$F_{ST}$	$p(F_{ST})$	$R_{ST}$	$p(R_{ST})$
East Pacific	Gulf of Alaska	0.001	---	0.00831	0.101
northeast Pacific	northwest Pacific	0.003	---	0.00681	0.109
Gulf of Alaska	northwest Pacific	0.0009	---	-0.00256	---
GLOBAL		0.001	0.034*	-0.00053	---

**Note:** \* denotes significant test result.

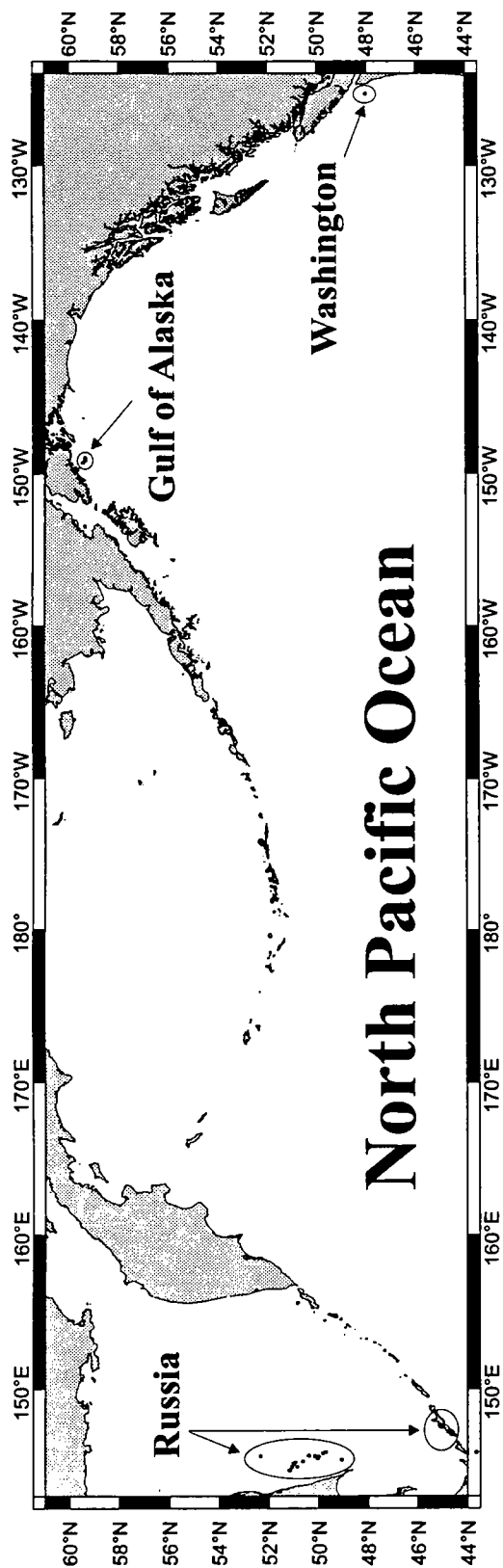


Figure 1. Collection areas for Pacific halibut samples used in the population survey.

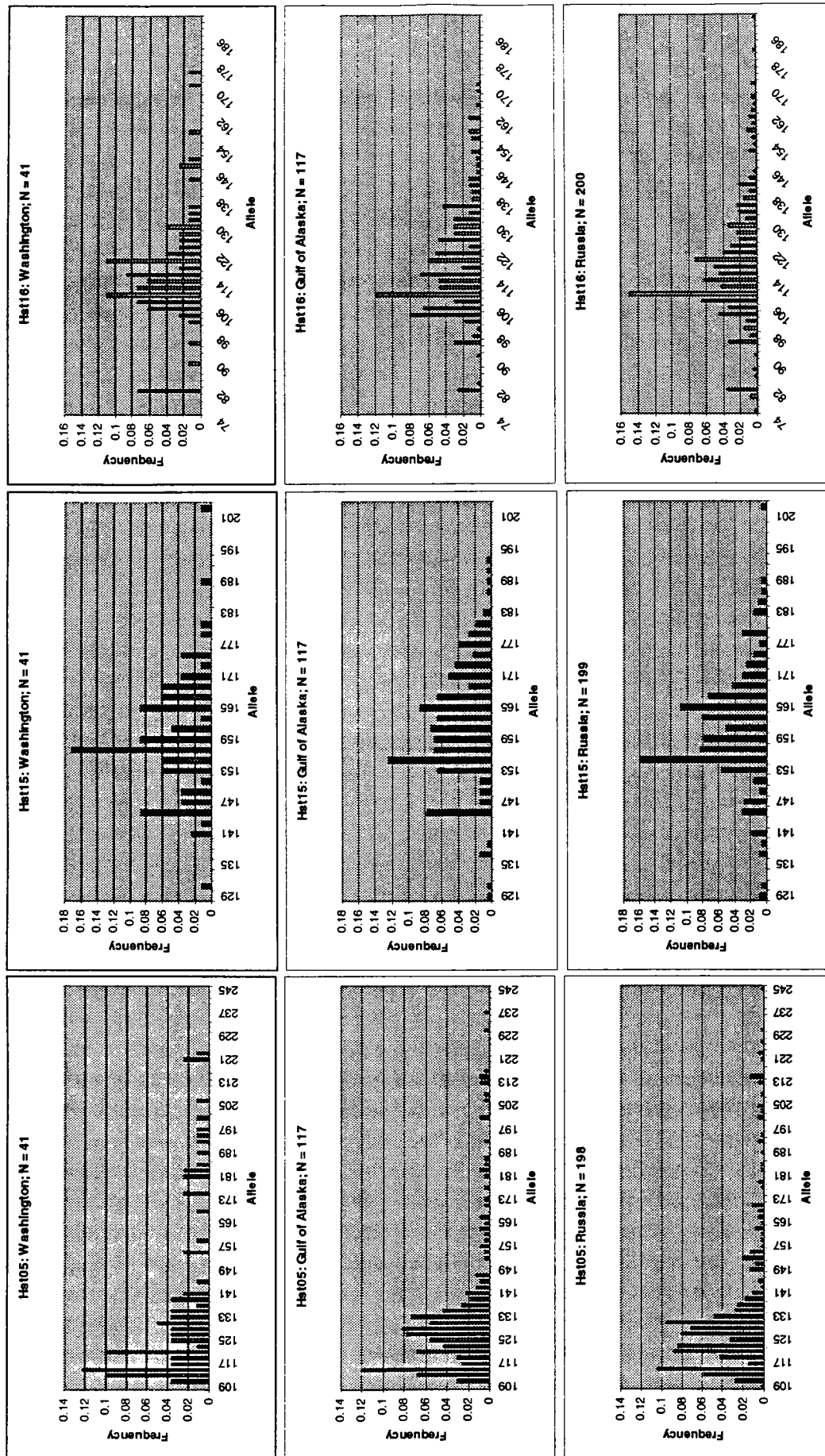


Figure 2. Allele frequency distributions of microsatellites in Pacific halibut.



# **Evaluation of Maintaining the IPHC Closed Area in the Bering Sea**

by

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## **ABSTRACT**

The existing IPHC closed area in the Bering Sea provides little biological benefit to the halibut resource or fishery. In spite of the weak Bering Sea data set, the very low directed fishery exploitation on legal-sized fish has little effect on halibut abundance. Except for bycatch mortality from groundfish fisheries, which is substantial, the nearly unfished Bering Sea shelf may function as a reserve. Marine reserves may be appropriate for areas of high exploitation or high data uncertainty. At this time, only data uncertainty provides justification for a reserve in the Bering Sea. Should circumstances make a reserve potentially desirable, a special project to develop a purpose and criteria for a reserve should occur.

## **INTRODUCTION**

Over the years, the International Pacific Halibut Commission (IPHC) has closed and reopened areas to halibut fishing, and worked with the U.S. and foreign governments to close areas to groundfish fishing (Skud 1977). Halibut nursery areas in Canadian waters closed and reopened to halibut fishing, and a nursery area established in 1967 in the eastern Bering Sea (Fig. 1) remains closed to the present. Other areas of the Bering Sea-Aleutian Islands and Gulf of Alaska with high halibut bycatch had closed to groundfish fisheries, at least seasonally, to foreign groundfish fisheries. All of the Bering Sea and Gulf of Alaska halibut bycatch closed areas subsequently reopened as the groundfish fisheries converted to American fleets.

During development of the groundfish fisheries of the Bering Sea by foreign and U.S. vessels, bycatch of halibut occurred throughout the Bering Sea, including the Bering Sea closed area. During the five years that preceded the closure, the commercial halibut fishery caught a total of 103,000 pounds from what became the closed area, and 97,000 pounds occurred in 1962 (IPHC 1967). No commercial harvest occurred in the area during 1966, the last year before the closure. Commercial halibut fishing on the continental shelf in the Bering Sea adjacent to the closed area is now about 300,000 pounds. An additional 1.6 million pounds of harvest occurs around the Pribilof Islands, an area of relief northwest of the closed area. Given the large halibut mortality caused by bycatch and the apparent lack of interest in commercial fishing in the closed area, the IPHC staff proposed in 1998 to review the purpose and need for the closed area. The IPHC asked the staff to prepare a report on the consequences of eliminating the closed area.

## **BACKGROUND**

Among its earliest actions to reverse a perceived decline in halibut stocks, the IPHC in 1932 established permanent closures of two areas in Canadian waters defined as halibut nursery grounds.

On these grounds, small halibut dominated, and larger halibut occurred only as strays (Babcock et al. 1931). The IPHC considered the closures superior to minimum size limits and prohibitions on small hooks. The closure was intended as a reserve with total protection of small halibut, by eliminating culling of undesirable, small fish during the fishery. Small fish in the closed areas could grow to more desirable sizes, but no restrictions would be placed on small halibut captured outside of the closed areas. Economic inefficiencies of prohibiting small hooks would not occur. The IPHC considered the nursery closures as economic, but not biological, measures. Overfishing of larger halibut in open areas was viewed as the chief threat to the productivity of the resource.

The nursery area closures remained until reopened in 1960. Surveys during the late 1950s demonstrated an "accumulation of old and large fish" such that the closed areas "do not currently qualify for closure as nursery grounds" (IPHC 1960).

During the early 1960s, directed halibut fishing by foreign fleets and heavy fishing by fishermen of the U.S. and Canada caused a significant decline in abundance of halibut in the Bering Sea-Aleutian Islands. In 1966, the IPHC staff recommended management measures for the halibut fishery in the Bering Sea that included a proposal to close an area of the eastern Bering Sea to all halibut fishing (IPHC 1967). A "closed nursery ground would aid in the protection of the large population of small, immature halibut in that area" (IPHC 1997). The IPHC implemented the Bering Sea closed area in 1967, and it has remained in place since with small modifications. The IPHC also recommended closures to foreign groundfish fishing in areas of high abundance of halibut. As a result of negotiations through the International North Pacific Fisheries Commission and bilateral meetings with foreign governments, Japan and Russia agreed to closures for groundfish fisheries that included the IPHC closed area (Skud 1977).

The intent of the IPHC for the Bering Sea closed area, to protect small, immature halibut, was violated when the area opened to U.S. groundfish fisheries, which catch large numbers of these small halibut as bycatch. A large component of the halibut bycatch mortality in the Bering Sea-Aleutian Islands region comes from the IPHC closed area. Since the early 1990s when the Americanization of the groundfish fisheries occurred, bycatch mortality documented by samples from observers in the IPHC closed area has increased from about 20 percent to about 40 percent of the Bering Sea-Aleutian Islands total (NMFS unpublished data). Of the groundfish catch monitored by observers, catch in the IPHC closed area during this period increased from about 10 percent to about 40-50 percent of the total.

## MARINE PROTECTED AREAS

Marine protected areas (MPA), which encompass such terms as reserves, sanctuaries, and closed areas, are gaining international favor as a mechanism for ecosystem and fishery management (Attwood et al 1997a). In many cases, insufficient information precludes proper management under the pressure of intense fishing or attempts to modify the environment of an area. Under the Precautionary Principle, MPAs offer an opportunity to maintain marine environments intact while further study occurs. Attwood et al. (1997a) further suggest that MPAs may enhance fish yield, if substantial spill-over of fish occurs from the MPA. They note that evidence for such enhancement comes from conceptual arguments and theoretical models, rather than from direct observations.

Attwood et al. (1997b) summarized the role of MPAs in fisheries management with "recognition of:

- (i) the failure of conventional single-species management to control bycatch and habitat destruction;
- (ii) the failure of conventional fishery control methods for fish with certain types of life-history characteristics;
- (iii) the importance of conserving ecosystem structure as the context for stable fishery production;
- (iv) the value of undisturbed ecosystems for comparative study."

Lauck et al. (1998) extended the concept of MPAs (or marine reserves in their terminology) to fisheries management. They noted the widespread failure of stock assessment models to provide accurate and timely advice and the failure of management to prevent stock collapse, as a result of irreducible scientific uncertainty and inability to control catches. These authors liken a marine reserve for fisheries to an insurance policy, in which a premium paid (lower overall harvest because of the closed area) minimizes the risk of a fishery collapse. In rough terms, they recommended that the size of the reserve should include on the order of 50 percent of the fish stock abundance. As exploitation rates decline, the necessary size of the reserve becomes smaller. The proportion of harvest lost because of a marine reserve is less than the proportion closed, because exploitation in the remaining open area can increase.

## **RELEVANCE OF MPA/RESERVES TO THE BERING SEA CLOSED AREA**

MPAs are an attractive concept for many situations in fishery management, especially those with limited or insufficient information. However, evaluation of the concept is generally lacking, and criteria for selecting MPAs are generally vague. Even so, the IPHC closed area meets few of the justifications for an MPA.

The closed area does not reduce halibut bycatch mortality. Bycatch is managed with bycatch mortality limits through the North Pacific Fishery Management Council, and with quota reductions and harvest rate reductions by the IPHC.

Ecosystem effects from the IPHC closed area have little benefit. The fishing by other gear types throughout the Bering Sea-Aleutian Island area, especially on the Bering Sea shelf, preclude an undisturbed ecosystem. A small no-trawl zone occurs on the eastern edge of the IPHC closed area. Evaluation of ecosystem stability in the Bering Sea must include the other fisheries, both in and out of the IPHC closed area and the no-trawl zone.

Of the issues favoring development of MPAs, only uncertainty of the stock assessment and concomitant management program apply to Pacific halibut. Stock assessment results in the Bering Sea are currently inadequate because of insufficient time series of catch and survey data (Sullivan and Parma 1998), and because exploitation rates are low. Questions still remain on stock assessment issues in the Gulf of Alaska.

### **Uncertainty**

In the Gulf of Alaska, two estimates of exploitable biomass occur for Area 3B. The stock assessment model (Sullivan and Parma 1998) gives a value of exploitable biomass about half that estimated from CPUE ratios scaled with biomass of areas with good data (Trumble and Hoag 1998). Retrospective analysis of halibut abundance demonstrated that the age-based model formerly used

for halibut stock assessment underestimated exploitable biomass (Parma 1993), and helped document the need for length-age-based model. Clearly, a degree of uncertainty exists for stock assessment in all or part of the IPHC management areas.

### **Exploitation rates**

Halibut fishing mortality contributes very little to total mortality in the Bering Sea (W. G. Clark, IPHC, pers. comm.). Estimates of total mortality (fishing plus natural) exceed the estimate of natural mortality currently used.

Data available from the Bering Sea are the weakest of any IPHC regulatory area, but exploitation is so low that the effect hardly registers. Exploitation is higher in the Gulf of Alaska, but the strongest data set occurs there. The present IPHC closed area is insufficient to offer the degree of insurance suggested by Lauck et al. (1998). The closed area is far too small and accounts for too few halibut to offer significant benefits. However, it costs the fishery virtually nothing because of little or no interest in fishing there. The Bering Sea shelf functions as a closed area to halibut fishing, because the density is so low that halibut fishermen have little interest in fishing in any but a few spots. Yet because of the large surface area, the halibut abundance on the shelf amounts to about a third of the total abundance in the Bering Sea (Clark 1998). Lauck et al. demonstrated that the need for a reserve diminishes as exploitation decreases. The existing closed area in the Bering Sea provides little biological benefit to the halibut resource or fishery.

### **ALTERNATIVE ACTIONS**

The IPHC staff has several options concerning the closed area and the MPA concept.

1. Status quo. Leave the closed area as it is. This action requires no further evaluations.
2. Push for expansion of the closed area/no-trawl zone to make a reserve of a meaningful size. This action would require substantial evaluation.
3. Develop an alternate closed area. This action would require substantial evaluation.
4. Eliminate the IPHC closed area. This action would require substantial evaluation.

We cannot develop a justification for any specific MPA/Reserve in the Bering Sea or Gulf of Alaska at this time. Should circumstances develop that make an MPA/Reserve potentially desirable, then a special project to establish objectives and criteria for a halibut-specific MPA should occur.



## REFERENCES

- Attwood, C. G., J. M. Harris, and A. J. Williams. 1997. International experience of marine protected areas and their relevance to South Africa. *S. Afr. J. mar. Sci.* 18: 311-332.
- Attwood, C. G., B. Q. Mann, J. Beaumont, and J. M. Harris. 1997. Review of the state of marine protected areas in South Africa. *S. Afr. J. mar. Sci.* 18: 341-367.
- Babcock, J. P., W. A. Found, M. Freeman, and H. O'Malley. 1931. Report of the International Fisheries Commission. *Int. Fish. Comm. Rep. No. 1.* 31 pp.
- Clark, W. G. 1998. Coastwide distribution of exploitable biomass according to 1997 setline surveys. *Int. Pac. Halibut Comm. Report of Assessment and Research Activities.* pp. 161-166.
- IPHC. 1967. Regulation and investigation of the Pacific halibut fishery in 1966. *Int. Pac. Halibut Comm. Rep. No. 46.* 23 pp.
- Lauck, T., C. W. Clark, M. Mangel, and G. R. Munro. 1998. Implementing the precautionary principle in fisheries management through marine reserves. *Ecol. Applic.* 8(1) supplement. pp. S72-S78.
- Parma, A. M. 1993. Retrospective catch-at-age analysis of Pacific halibut: implications on assessment of harvesting policies. In: *Proceeding of the International Symposium on Management Strategies for Exploited Fish Populations.* G. Kruse, D. M. Eggers, R. J. Marasco, C. Pautzke, and T. J. Quinn II (editors). Alaska Sea Grant College Program Report No. 93-02. pp. 247-265.
- Skud, B. E. 1977. Regulations of the Pacific halibut fishery, 1924-1976. *Int. Pac. Halibut Comm. Tech. Rep. No. 15.* 47 pp.
- Sullivan, P. J. and A. Parma. 1998. Population Assessment, 1997. *Int. Pac. Halibut Comm. Report of Assessment and Research Activities.* pp. 83-99.
- Trumble, R. J. and S. H. Hoag. 1998. A proposed method for setting Area 4 and Area 3B catch limits. *Int. Pac. Halibut Comm. Report of Assessment and Research Activities.* pp. 203-210.

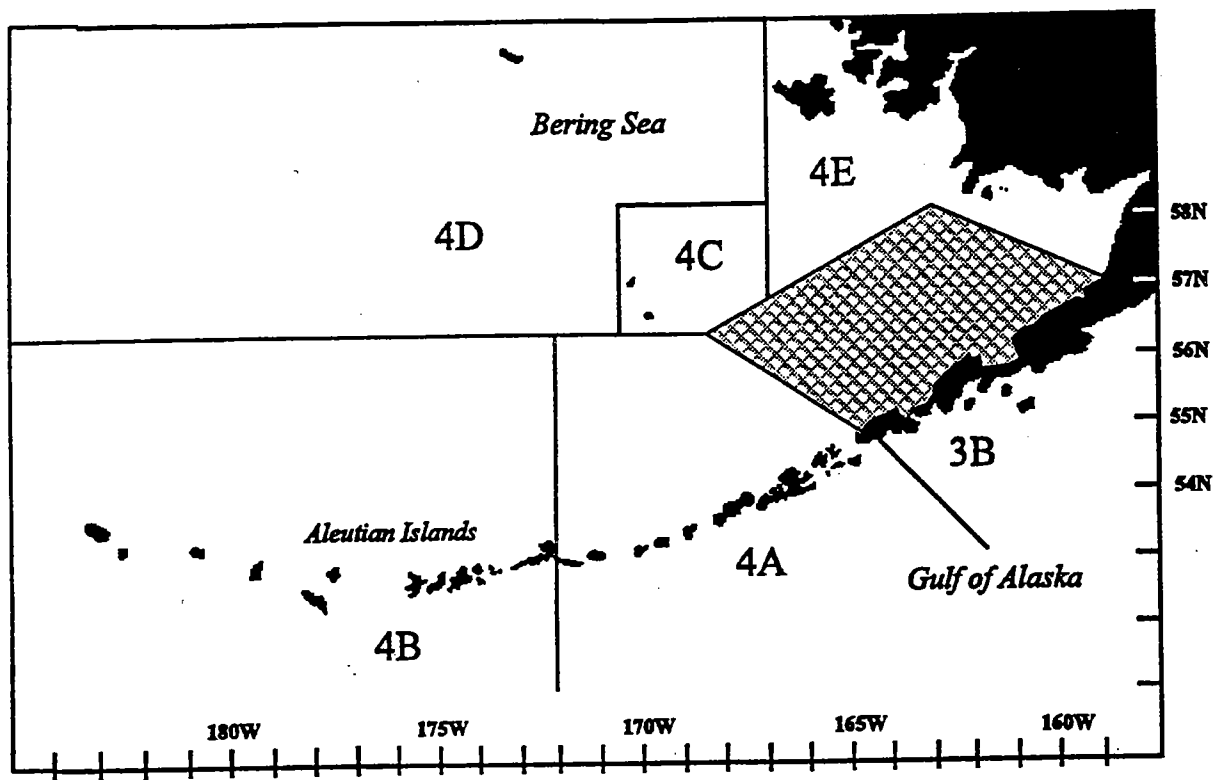


Figure 1. Area closed by IPHC to longline fishing for Pacific halibut.