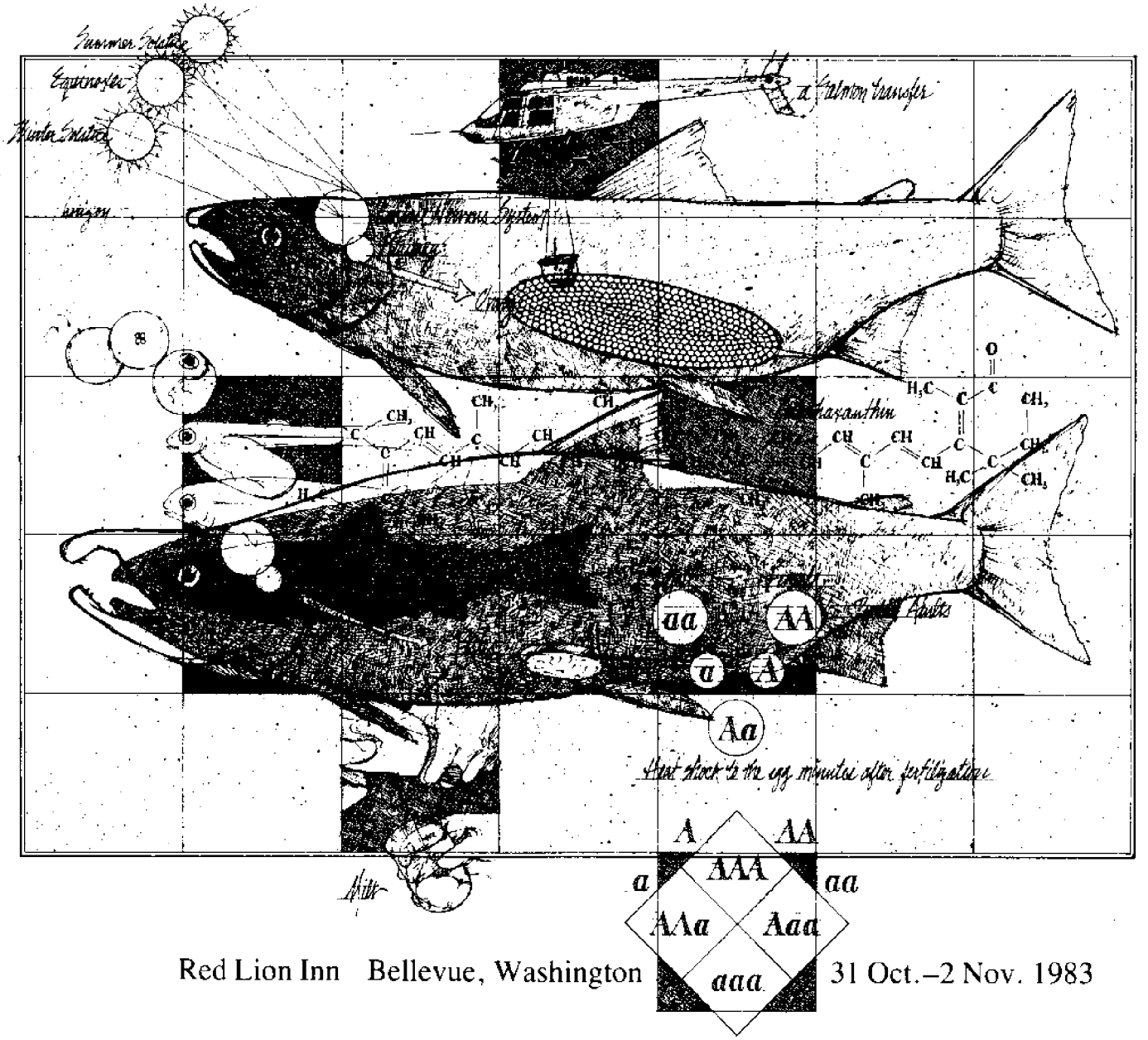
Salmonid Reproduction

An International Symposium



Red Lion Inn Bellevue, Washington 31 Oct.–2 Nov. 1983

Salmonid Reproduction
An International Symposium

This symposium will provide an international forum for examining the critical factors in five major areas that influence salmonid reproduction—genetics, endocrinology, nutrition, environmental factors, broodstock management and husbandry.

In addressing each area, an overriding concern will be the need for full evaluation of the current state of the art and feasibility of applying research results to modify salmonid reproductive capabilities.

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SYMPOSIUM SCHEDULE

Sunday evening, 30 October 1983

4:00–6:00 **Registration**
Lower Level Foyer
5:00–7:00 **No host reception**
King County East Room

Monday, 31 October 1983

7:30–8:00 **Registration**
Lower Level Foyer
8:15–11:15 **Endocrine session**
Idylwood/Marymoor
11:15–1:00 **Lunch break**
1:00–4:10 **Genetics session**
Idylwood/Marymoor
4:10–5:30 **Poster session I**
Factoria Room
6:30–8:00 **Symposium Reception**
Bellefield/Fairweather

Tuesday, 1 November 1983

8:15–11:30 **Environmental factors session**
Idylwood/Marymoor
11:30–1:00 **Symposium luncheon**
Bellefield/Fairweather
1:30–2:50 **Nutrition session**
Idylwood/Marymoor
2:50–5:00 **Poster session II**
Factoria Room
7:30–9:00 **Table talks**
Idylwood/Marymoor

Wednesday, 2 November 1983

8:15–9:50 **Endocrine session**
Idylwood/Marymoor
10:15–11:35 **Broodstock management and husbandry**
Idylwood/Marymoor
11:35–Noon **Symposium wrap-up**
Idylwood/Marymoor
1:30 **Buses leave from lower level lobby**
2:00–4:00 **Tour—University of Washington**
School of Fisheries

INFORMATION

REGISTRATION & INFORMATION

During the Symposium, the registration and information desk will be open during the following hours:

Sunday	October 30	4:00 pm to 7:00 pm
Monday	October 31	7:30 am to 5:30 pm
Tuesday	November 1	7:30 am to 5:30 pm
Wednesday	November 2	8:00 am to Noon

MESSAGE CENTER

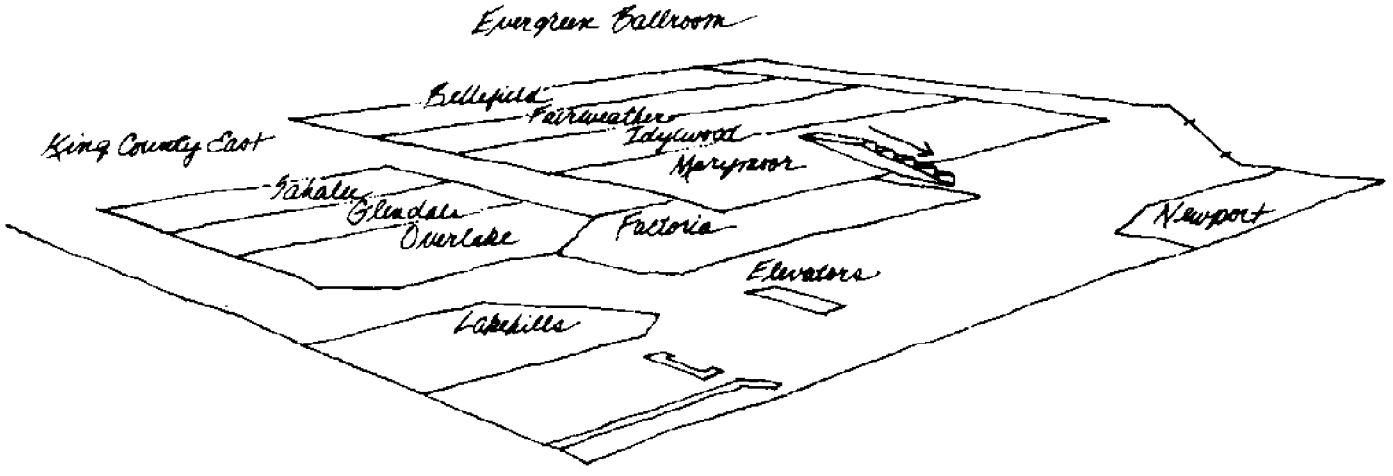
Symposium registrants may receive or leave messages at the Message Center in the registration area. Telephone calls for the message center will be received through the hotel switchboard, (206) 455-1300.

UNIVERSITY TOUR

On Wednesday afternoon November 2 following the Symposium, there will be a tour of the School of Fisheries at the University of Washington for Symposium participants. If you are interested in this tour, please sign up at the Symposium registration desk when you arrive.

SYMPOSIUM PROCEEDINGS

All papers—both oral and poster—are eligible for publication in either *Aquaculture* or in a proceedings to be published by the Washington Sea Grant Program. Each of these volumes will be a special issue devoted exclusively to the Symposium. One copy of the proceedings published by Washington Sea Grant will be provided to every Symposium participant who pays a full or limited registration fee. The proceedings to be published by *Aquaculture* will be available to Symposium registrants at a cost to be determined by the publisher.



MONDAY MORNING

31 October 1983
8:15 to 11:30

8:15 **Introduction and Welcome**
Louie S. Echols, Director, Washington Sea Grant Program

ENDOCRINOLOGY

Chairman
Yoshitaka Nagahama
National Institute for Basic Biology
Okazaki, Japan

8:30 **Endocrine Control of Final Gamete Maturation in Salmonids**
Yoshitaka Nagahama, National Institute for Basic Biology, Okazaki, Japan

Endocrine control of final gamete maturation has been investigated in our laboratory using four species of salmonids (amago salmon, *Oncorhynchus rhodurus*; chum salmon, *O. keta*; masu salmon, *O. masou*; rainbow trout, *Salmo gairdneri*). The maturation-inducing steroid (MIS) of amago salmon has been identified. Immature but full-grown folliculated oocytes of amago salmon were incubated with chum salmon gonadotropin (SGA). Ether extracts of the media from these incubates were fractionated (20 separate fractions) by reversed phase high performance liquid chromatography. The MIS activity based on *in vitro* germinal vesicle breakdown (GVBD) using immature, full-grown oocytes of amago salmon was found only in fraction 10 which had a retention time that coincided exactly with authentic 17-α,20-β-dihydroxy-4-pregnen-3-one (17-α,20-β-diOHprog). The purity and final characterization of the residue of fraction 10 were confirmed by the thin layer chromatography and mass spectrum. These results indicate that 17-α,20-β-diOHprog is the natural MIS in amago salmon. 17-α,20-β-diOHprog was the most potent inducer of *in vitro* GVBD in all salmonid species studied, and high concentrations were found in the plasma of all females undergoing final oocyte maturation and ovulation.

Incubations of testicular fragments from spermiating males with SGA or 17-α-hydroxyprogesterone resulted in a highly significant increase in 17-α,20-β-diOHprog levels in the incubation medium. In all males studied, plasma levels of 17-α,20-β-diOHprog were either not detectable or very low during testicular development and rapidly increased at the onset of spermiation. Furthermore, two successive intraperitoneal injections of 17-α,20-β-diOHprog into non-spermiating amago salmon induced precocious spermiation about one month prior to the normal spermiation period. In contrast, neither 11-ketotestosterone nor testosterone was effective in inducing precocious spermiation. These results will be discussed in relation to the possible involvement of 17-α,20-β-diOHprog in spermiation of male salmonids.

8:50 **Estrogen Synthesis in the Teleost Ovarian Follicles: The Two Cell-type Model in Salmonids**
Hirohiko Kagawa, National Institute for Basic Biology, Okazaki, Japan

The present study was conducted to assess the role of the thecal and granulosa layers of several species of salmonids, with particular emphasis on amago salmon, *Oncorhynchus rhodurus*, on estradiol-17-β (E_2) production *in vitro* in response to partially purified salmon gonadotropin (SG-G100). Four different follicular preparations from vitellogenic follicles of amago salmon (intact follicles, thecal layers, granulosa layers, and thecal layer-granulosa layer co-cultures) were incubated in the presence or absence of SG-G100. E_2 and testosterone (T) levels in the medium were measured by specific radioimmunoassay. SG-G100 stimulated E_2 production by intact follicles and co-culture preparations, but not by the isolated thecal or granulosa layers, indicating that both layers are necessary for gonadotropin-stimulated E_2 production. In contrast, SG-G100 greatly stimulated T production by thecal layers but only slightly by the other follicular preparations. E_2 precursors produced in the thecal layers in response to SG-G100 have been identified. The thecal layers were incubated with SG-G100 and ether extracts of the media from these incubates were fractionated (20 separate fractions) by reversed phase high performance liquid chromatography. Only two fractions added to the incubation medium were converted to E_2 by granulosa layers. These two fractions had retention times that coincided with authentic androstenedione (A) and T respectively; the purity and final characterization of these two fractions were confirmed by thin layer chromatography and mass spectrum.

These results indicate a two cell-type model for the production of follicular estrogen in amago salmon, the thecal layer contributing to E_2 production by synthesizing Δ^4 androgens (A and T) which are transferred to the granulosa layer and aromatized to E_2. A two cell-type model also seems applicable to the production of follicular estrogen in other species of salmonids (chum and masu salmon, and rainbow trout).

9:05 **The Application of Radioimmunoassays for Sex Steroids, Gonadotropin, and Vitellogenin to the Study of the Reproductive Cycle of Rainbow Trout *Salmo gairdneri***
A. P. Scott, J. P. Sumpter*, S. M. Baynes, and P. R. Witthames, Ministry of Agriculture, Fisheries, and Food, Lowestoft, United Kingdom
* Brunel University, Middlesex, United Kingdom

In rainbow trout females, significant changes occur in plasma levels of sex steroids, gonadotropin and vitellogenin during the reproductive cycle. While much attention has previously been directed to the levels of these hormones during advanced stages of maturity (i.e., exogenous vitellogenesis and ovulation), very little has been directed to the changes that occur during the very early stages of gonadal growth (i.e., the beginning of the secondary oocyte growth phase).

We present data on plasma hormone and vitellogenin levels in two-year old, virgin female rainbow trout over the months from January to June. This period encompasses the initiation of secondary oocyte growth phase as assessed histologically.

Data are also presented on hormone levels in triploid female rainbow trout. These fish are effectively sterile (the ovaries contain only oogonia), so the plasmas constitute a baseline for the present investigations which are concerned with small changes in hormone levels.

9:20 **Evolution of the Follicular Sensitivity *in vitro* to Maturation-inducing Hormones at the End of Vitellogenesis in Rainbow Trout *Salmo gairdneri*: Role of Estradiol-17β**
Bernard Jalabert and Alexis Fostier, Institut National de la Recherche Agronomique, Laboratoire de Physiologie des Poissons, Rennes Cedex, France

Trout ovaries exhibiting oocytes at a defined stage around the end of vitellogenesis (end of vitellogenesis—GV −, subperipheral germinal vesicle—GV ±, peripheral germinal vesicle—GV + were processed for *in vitro* incubations in standard conditions, in order to examine possible relationships between the following parameters: oocyte sensitivity to the maturing steroid 17α-hydroxy-20β-dihydroprogesterone (17α, 20β-OH-P), follicular sensitivity to the maturational gonadotropin s-GtH, inhibitory potency of exogenous estradiol-17β (E_2) over s-GtH-induced maturation, and level of E_2 in the plasma of donor fishes. The sensitivity to hormones was estimated by the median efficient dose (MED) for morphological oocyte maturation *in vitro*.

Globally, the follicular sensitivity and the oocyte sensitivity exhibit a significant covariation. However, when attention is paid to the oocyte morphological stage, the peripheral migration of the GV appear to coincide with a jump in oocyte sensitivity to 17α, 20β-OH-P.

A significant correlation can be observed between plasma E_2 level and the follicular sensitivity to s-GtH, but not between plasma E_2 and the oocyte sensitivity to 17α, 20β-OH-P. The inhibitory effect of E_2 on s-GtH-induced maturation *in vitro* appears all the more important as the follicular sensitivity is high, and can be demonstrated even with low doses, in the physiological range (0.05 μg/ml). Taken together these data lead to the conclusions that the peripheral migration of the GV is a morphological event which appears to coincide statistically with a jump in oocyte sensitivity and that E_2 is an important physiological regulator of follicular sensitivity to GtH.

9:35 **Plasma Steroid Profiles during Sexual Maturation in Salmonids**
Kouhei Yamauchi, Hokkaido University, Hakodate, Japan

Plasma levels of various steroids were examined at different stages of sexual maturity of three species of salmonids (amago salmon, *Oncorhynchus rhodurus*; masu salmon, *O. masou*; chum salmon, *O. keta*). Estradiol-17β (E_2), testosterone (T), 11-ketotestosterone (11-ketoT) and 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-diOHprog) were measured by radioimmunoassay. Plasma steroid hormone profiles in three species of salmonids were similar and will be treated together. In females, E_2 levels gradually increased during vitellogenesis and peaked at the end of the vitellogenic period and declined markedly in mature and ovulated fish. T levels lagged behind and followed that of E_2 during vitellogenesis, but remained high in mature and ovulated females. 17α, 20β-diOHprog levels were low during the major part of the preovulatory period and rapidly elevated in mature and ovulated females. Detailed changes in the plasma levels of E_2 and 17α, 20β-diOHprog levels were investigated in masu salmon during the course of final maturation and ovulation. E_2 levels were high at the beginning of the experiment (8 days prior to ovulation) and declined to basal levels 4–6 days prior to ovulation. In contrast, 17α, 20β-diOHprog levels were either not detectable or low when E_2 levels were high and rapidly increased after E_2 levels declined to basal levels, reaching a peak 2-4 days prior to ovulation. The levels gradually decreased thereafter.

In males, plasma levels of 11-ketoT increased during rapid testicular development, followed by a sharp drop during spawning period. A similar pattern was observed for T although T was consistently lower than 11-ketoT. 17α, 20β-diOHprog levels were very low during the ma-

jor part of the development of the testis, and rapidly increased at the onset of spermiation; the levels remained high during the period of active spermiation and sharply declined thereafter. The possible roles of steroid hormones in gamete development and maturation in salmonids will be discussed.

9:50 **Coffee break**

10:15 **Ovulatory and Steroidal Responses in Coho Salmon and Steelhead Trout Following Administration of Salmon Gonadotropin and D-Ala^6, Des-Gly^{10} Ethylamide(GnRHa)**
S. A. Sower, University of New Hampshire; R. N. Iwamoto, W. W. Dickhoff, and A. Gorbman, University of Washington

Hormone injection experiments were conducted for two years during the fall and early winter when adult coho salmon and steelhead trout, respectively, spawn. Successive injections of partly purified coho salmon gonadotropin and synthetic analogue of hypothalamic GnRHa advanced ovulation by two to three weeks in coho salmon and steelhead trout in all tests. In these same tests, all treatments of salmon gonadotropin followed by one of 4 doses of GnRHa (50, 5, 0.5, or 0.05 micrograms/kilogram) in coho salmon significantly depressed estradiol levels whereas androgen levels were significantly elevated compared to controls. Treatment with salmon gonadotropin followed by only one dose of GnRHa (60 microgram/kg) in steelhead trout caused a similar response of a significant decrease of estradiol. In summary, the doses of GnRHa (5, 0.5, or 0.05 microgram/kg) following injection of coho salmon gonadotropin are the lowest doses that have been reported to be effective for acceleration of ovulation in salmonids. Furthermore, part of the mechanism of normally accelerated ovulation is evident by a decrease in estradiol levels concomitant with an increase in androgen levels.

10:30 **The Use of Des-Gly^{10} (D-Ala^6) LH-RH-Ethylamide to Induce Precocious Ovulation in Adult Female Coho Salmon *(Oncorhynchus kisutch)***
M. S. Fitzpatrick, D. M. Oberbillig, B. K. Suzumoto*, and C. B. Schreck, Oregon Cooperative Fishery Research Unit, Oregon State University, Corvallis
* Oregon Aqua Foods, Inc., Springfield, Oregon

When adult hatchery coho salmon return to seawater or freshwater facilities, they must often remain in raceways for extended periods of time before ripening. This often results in significant mortality before ovulation. To circumvent this problem, as well as to simply obtain eggs when needed, we investigated means of inducing precocious final maturation.

Adult female coho salmon held in fresh water were given intraperitoneal injections of either the mammalian luteinizing hormone-releasing hormone analog (LH-RHa) des-Gly^{10} (D-Ala^6) LH-RH-ethylamide at various dosages or injections of saline. The mean number of days to ovulation in all groups of fish receiving only LH-RHa was significantly ($p<.01$) lower than that in saline injected controls. Within 10 days of the initial injection, more than half of the fish in groups receiving single injections of LH-RHa at various dosages had ovulated compared to 11% of the controls. Within two weeks of the initial injection, 85% of the fish in groups receiving two injections of LH-RHa at various dosages had ovulated while less than 50 percent of the controls had ovulated. This demonstrates that treatment of female coho salmon with LH-RHa can cause significant acceleration of final maturation leading to precocious ovulation.

In order to better understand the biology of salmon, our lab has also been involved in the development of endocrine profiles for adult fish. We are investigating the dynamics of various sex steroids in the plasma of salmon throughout the spawning run. Preliminary results from coho salmon and chum salmon *(O. keta)* indicate trends in the plasma titers of progesterone, estradiol, and 17α-hydroxy-20β-dihydroprogesterone which may elucidate the necessary criteria for the actions of maturation-inducing agents.

10:45 **Changes in Plasma Estradiol-17-β and 17-α, 20-β Dihydroxy-4-Pregnen-3-One during Spontaneous and Induced Ovulation in Coho Salmon**
G. Van Der Kraak, University of British Columbia and E. M. Donaldson, Department of Fisheries and Oceans, West Vancouver, British Columbia, Canada

Current evidence suggests that in salmonids, vitellogenesis and oocyte maturation are mediated by estradiol-17β and 17α, 20β dihydroxy-4-pregnen-3-one (17α, 20βP) respectively. Few studies have examined the switch from estradiol-17β to 17α, 20βP production during the preovulatory period. In the present study, estradiol-17β and 17α,20βP were measured in plasma samples taken during spontaneous or gonadotropin induced reproductive activity to determine

the timing of steroid changes in relation to oocyte maturation and ovulation. Spontaneous reproductive activity was characterized by a rapid decline in plasma estradiol-17β commencing 12 days prior to ovulation and a large increase in plasma 17α, 20βP six days prior to ovulation. This preovulatory increase in 17α, 20βP coincides with the time of germinal vesicle breakdown. Fish injected with des-Gly^{10}DAla^{6}LH-RH-ethylamide alone or in combination with SG-G100 showed a biphasic increase in plasma 17α, 20βP levels. While plasma 17α, 20βP levels were elevated within three hours of injection, the surge associated with oocyte maturation was delayed four to eight days. In contrast, estradiol-17β levels while unchanged for 24 hours, declined to basal levels within 6 days. These results suggest that salmon have 20β hydroxysteroid dehydrogenase at least one month prior to the expected time of ovulation. It is not known if the concentration of this enzyme is low, necessitating the synthesis of additional enzyme or alternatively, the removal of an inhibitory factor to produce the high levels of 17α, 20βP associated with oocyte maturation. Analysis of plasma estradiol-17β and 17α, 20βP during induced ovulation indicates that the decline of estradiol-17β consistently precedes the surge in 17α, 20βP suggesting that estradiol-17β may function as an inhibitor of 17α, 20βP synthesis.

11:00 **Induction of Ovulation in Atlantic Salmon with Pelleted LHRH Analog**
L. W. Crim, Memorial University of Newfoundland, St. John's Canada; and B. D. Glebe, North American Salmon Research Center, St. Andrews, New Brunswick, Canada

Spawning in Atlantic salmon begins in the late fall and it may require several weeks for completion in all individuals of some stocks. Inducing spawning with LHRH or LHRH analogs (LHRH-A) may become the method of choice since these simple peptide hormones are commercially available at competitive prices relative to preparations of fish gonadotropic hormone. The objectives of our 1982 studies included an examination of the value and practicality of using pelleted, long-acting LHRH-A for advancing and synchronizing spawning of the female Atlantic salmon. Two separate experiments were conducted with sea cage-reared salmon beginning on September 14 and October 4, respectively. Females were treated with intraperitoneal implants of pelleted LHRH-A or control fish received the sham surgical procedures alone. The fish were frequently checked for signs of ovarian maturity and weekly blood samples were collected until the time of spawning. On the day of spawning, females were stripped and the eggs were fertilized with milt obtained from two males. The eggs were incubated in individual batches and fertility and viability to the eyed stage were recorded. In September ovary development was variable ranging from 5.9–19.7% GSI in a small group of initial controls. Plasma gonadotropic hormone (GtH) levels were elevated in females treated with pelleted LHRH-A but spawning was advanced (October 6) in only 3 (20% of group) fish. In experiment 2 beginning October 4, LHRH-A treatment increased plasma GtH and also accelerated and synchronized ripening of the treated females (94% of group) within 11 days. Compared with sham control females LHRH-A advanced maturation of females approximately 3 weeks. Whereas egg quality was extremely poor in the September trial, egg fertility and viability were acceptable for the LHRH-A stimulated fish in October. We conclude that pelleted LHRH-A can be successfully used to advance maturation and ovulation in Atlantic salmon, especially during the later phases of ovarian development. LHRH-A treatment also appears capable of synchronizing ovulation in female Atlantic salmon.

11:15–1:00 **Lunch break**

MONDAY AFTERNOON

31 October 1983
1:00 to 5:30

GENETICS

Chairmen
Graham A.E. Gall
University of California
Davis, California

Trygve Gjedrem
Agricultural University of Norway
As-NLH, Norway

1:00 **Quantitative Genetic Aspects of Reproduction in Salmonids: A Review**
Graham A.E. Gall, University of California, Davis

The reproductive phase of the salmonid life cycle has received little attention from quantitative geneticists even though artificial manipulation of the process could potentially have far reaching effects on performance during all phases of the life cycle. Most interest and research emphasis has been placed on spawning efficiency and the production of numbers of eggs for specific purposes. There has been considerable speculation concerning the effects of other aspects of broodstock management and fish husbandry; however, little definitive data are available.

It has long been accepted that egg size has an effect on hatchability and early fry survival and growth. Similarly, there is ample evidence of a phenotypic relationship between body size of

females and egg size and number, with larger fish generally producing greater numbers of larger eggs than smaller fish. These phenotypic relationships are difficult to interpret since the genetic effects involved are confounded with a number of environmental factors such as age of female, season of spawning, time of spawning within a season and annual fluctuation in climatic and other temporal variables.

The effect of growth rate and body size on rate and age of sexual maturation is currently receiving attention. How specific broodstock management practices, such as selection for time of spawning and age at spawning, affect overall performance of the stock in subsequent generations has not been studied. In addition, little is known about within-year or within-season effects that may stimulate environmental correlations which may be mistaken for genetic relationships or which have the effect of masking important genetic effects.

1:20 **The Influence of Genetic and Environmental Factors on Maturity in Farmed Atlantic Salmon**

R. Alderson, Marine Harvest Ltd., Lochailort, Inverness-shire, United Kingdom

The physiological changes associated with maturation in salmon can place a severe constraint on the farmer's options for selling his fish. If they are not to be used for breeding, the farmer must sell them before there is any deterioration in appearance and flesh quality. Problems can occur; if too many fish mature and cause a glut, or if the fish are too small for the market's requirements. These problems can be particularly acute with fish maturing as grilse only 12-14 months following transfer to sea.

In choosing and developing his stock the farmer therefore needs to understand to what extent genetic and environmental factors control the proportion of his fish which will mature each year. Only then can he predict, and eventually control, the pattern of harvest he will need to follow.

Marine Harvest Ltd., who have been farming Atlantic salmon in Scotland since the late 1960's, began a detailed programme in 1977 to compare the performance of various stocks of both farmed and wild origin. Each stock, taken as eggs, is reared in replicated tanks up to the smolt stage when fish are cold branded, mixed, and transferred to seawater cages. Up to 13 stocks are tested each year and aspects of performance, including the incidence of maturity in the first and second years at sea, are noted.

This programme has given a considerable body of information on the patterns of maturity shown by fish of different origins under farmed conditions. The results to be presented will show the relative importance of genetic and environmental factors in determining the pattern of maturity within the range of sites occupied by Marine Harvest on the west coast of Scotland.

1:35 **Genotypic and Environmental Effects on the Incidence of Sexual Precocity in Coho Salmon**

R. N. Iwamoto, B. A. Alexander, and W. K. Hershberger, University of Washington, Seattle

The relative effects of genotype and initial freshwater rearing on the incidence of sexual precocity in coho salmon males were examined. Eggs from normal males were fertilized with milt from both normal and precociously maturing males (jacks) in a partially nested factorial mating design. Progeny from each of the resulting full- and half-sib families were subsequently reared under two rearing temperature treatments (ambient and 15° C) during part of the hatchery phase of the life cycle.

Mean growth of progeny from the jack and normal-sired groups was equivalent during the majority of the initial freshwater rearing period, although a slight but significant increase in size of jack-sired progeny was detected at the end of the accelerated hatchery rearing period. Of the 4980 survivors at the termination of the experiment, 175 individuals or 3.75 percent of the population had precociously matured. These jacks were distributed disproportionately among jack-sired and normal-sired families with jack-sired families contributing a significantly higher 4.6 times as many jacks. Despite significant temperature effects in terms of initial freshwater growth, the subsequent incidence of jacks did not appear to be temperature related. These results and the genetic analysis of the relative importance of genotype (category of male parents and individual paternal and maternal effects) on the incidence of sexual precocity will be presented.

1:50 **Heritability and Early Expression of Ovarian Recruitment Processes in Different Forms of Atlantic Salmon *(Salmo salar)***

A. M. Sutterlin and D. A. MacLean, Memorial University of Newfoundland, St. John's, Canada

The two pure lines and the reciprocal hybrids between an early maturing, dwarf form of landlocked Atlantic salmon and a later maturing anadromous form were reared in captivity for a minimum period of 2 years. Ninety percent of female parr of the dwarf landlocked salmon matured at age 2-plus years (fork length 15 cm), while no females of the anadromous form are

expected to mature at this time. The size of the ovary differed in the two pure forms at age 0-plus, and the numbers, size and stages of previtellogenic oocytes also differed at age 1-plus. Ovarian patterns in the two hybrid forms more closely resembled that of the maternal parent. The implications of a genetically controlled predestinate influencing rates of sexual maturation, smoltification and subsequent fecundity are discussed.

2:05 **A Conceptual Fitness Model for Managing Pacific Salmon Fisheries: I. Description of the Model**

A.R.D. Kapuscinski and J. E. Lannan, Oregon State University, Newport

As information relating to the population genetics of Pacific salmon becomes more and more comprehensive, it is increasingly apparent that a conceptual framework must be developed which permits the translation of this information into management principles. This report describes a conceptual model for managing Pacific salmon fisheries to maintain a long term reproductive fitness in breeding populations. The model facilitates estimating the requirement for escapement to maintain reproductive fitness at a predetermined level, or determining if a given escapement results in a change of population fitness.

The model may be adjusted to accomodate the different life histories and reproductive strategies of the several species of Pacific salmon, and it considers both the mean and variance of fitness. Application of the model is demonstrated in the poster session.

2:20 **Coffee Break**

2:50 **Genetic Variation in Age at Maturity and Its Relationships to Growth Rate**

Trygve Gjedrem, Agricultural University of Norway, As-NLH, Norway

Age at sexual maturity is of large economic importance in sea ranching and in fish farming. In salmonids there is considerable variation between species as well as between strains within species. Therefore problems can partly be overcome by choosing stocks suitable for a particular production system.

It is remarkable that the number of years in fresh water, from one to seven depending on water temperature or latitude, seems to have very little influence on the time in sea before maturation takes place in Atlantic salmon. Maturation of parr is a heritable trait and it seems to be independently inherited from age at maturity. Genetic variation in age at maturity is quite large with a heritability of around 0.3. This means that it is possible to change this trait by selection.

There seems to be negative phenotypic as well as genetic correlation between age at maturity and body weight. This indicates that when selecting for increased growth rate the age at maturity may be reduced. In fish farming, when producing a large fish, this is not wanted. Combined selection for both traits simultaneously should therefore be practised.

3:10 **Production, Survival, and Growth of Triploid Trout**

G. H. Thorgaard, Washington State University, Pullman

There has been considerable interest recently in induced triploidy in trout because sterile triploids might show better growth and survival at maturity than normal fish, and because of the potential for control of reproduction. Triploidy can be induced by heat shock in several trout species. Heat shock techniques can be readily adapted to large scale production. Triploids within a species show slightly lower survival than diploids, while triploid hybrids show better survival than diploid hybrids. Triploid rainbow trout show evidence of better growth than normal fish after sexual maturity.

3:25 **Chromosome Set Manipulation in Three Species of Salmonids Using Hydrostatic Pressure**

S. K. Allen, Jr., and J. M. Myers, University of Washington, Seattle

The use of hydrostatic pressure to affect early developmental events has been examined in various taxa, but few investigations have dealt with the potential practical utility of this method. Intensity and duration of hydrostatic pressure treatments were varied to estimate the optimal parameters for production of triploid and gynogenetic salmonids. These parameters were then applied to 5 families in 3 different species: chinook *(Oncorhynchus tshawytscha)* and coho salmon *(O. kisutch)* and rainbow trout *(Salmo gairdneri)*. Pressures of 10,000 psi for 8-10 minutes yielded the best compromise between conversion and mortality. Conversion rate across fam-

ilies was more consistent than parallel heat treatments and generally exceeded 90% in all groups. Mortality was more variable in pressure treatments than heat treatments. At present, pressure treatments offer an alternative to heat for experimental chromosome set manipulation, especially where consistency among replicates is desired. Prospects for the application of pressure to production schemes are discussed.

3:40 **Production of Triploid Landlocked Atlantic Salmon (*Salmo salar* L.) and the Implications of Their Haemotology to Oxygen Utilization**

T. J. Benfey and A. M. Sutterlin, Memorial University of Newfoundland, St. John's, Canada

Triploidy was induced in landlocked Atlantic salmon with 100% success and 70–90% survival (relative to controls) using either heat shocks or hydrostatic pressure. Triploidy was rapidly and accurately assayed at either the alevin or parr stage using a channelized Coulter Counter to measure the volume of a minimum of 150,000 erythrocytes per fish from a 1 µl sample of whole blood. Triploids had a larger mean erythrocyte volume (MCV) but lower erythrocyte count than diploids, and haematocrit was the same for both diploids and triploids. The haemoglobin content of the triploid blood was lower than that of the diploids, however, mean corpuscular haemoglobin (MCH) was higher due to the larger MCV. Triploids had a lower mean corpuscular haemoglobin concentration (MCHC), however, this was complicated by the fact that the nucleus of triploid erythrocytes occupied a greater percentage of corpuscular volume than did the diploid nucleus. MCHC was found to be the same for diploids and triploids when recalculated on a cytoplasmic rather then corpuscular level. The increase in MCV of triploids was due mainly to an increase in cell length; there was only a minor increase in cell width and no increase in cell height. Triploids had a lower rate of oxygen consumption than diploids; however, there was no difference in the oxygen tension at asphyxiation.

3:55 **Artificial Polyploidization of Salmonids by Hydrostatic Pressure**

Hiroshi Onozato, Hokkaido University, Hakodate, Japan

Artificial polyploidization technique is important to produce parthenogenetic diploids and polyploids. Thermal shock is most widely used to duplicate the chromosome set and it is very successful in some species. In salmonids, however, many eggs subjected to the shock remain unaffected.

In the present study, it is found that hydrostatic pressure has a marked effect on polyploidization of salmonid eggs. Gynogenetic diploids, androgenetic diploids, triploids and tetraploids were successfully produced in rainbow trout, masu salmon and chum salmon using hydrostatic pressure.

Optimum amount of pressure and the duration of treatment were tested on rainbow trout eggs inseminated with genetically inactivated sperms by UV irradiation. Eggs were treated 5 min. after insemination. 100% of eggs were diploidized when eggs were subjected to hydrostatic pressure (more than 650 kg/cm^2) for more than 6 min. Although all embryos treated were diploid, 36.9 to 58.9% of embryos were abnormal in that their body shape was similar to haploid embryos. Optimum time after insemination at which eggs were subjected to pressure shock (650 kg/cm^2, for 6 min.) was determined in chum salmon and masu salmon. Eggs were inseminated with genetically inactivated sperms and maintained at 10° C until treatment. Normal diploid embryos were encountered when eggs were treated during 5 to 40 or 270 to 330 min. after insemination. The ratios of normal embryos to developing ones were 58.3 to 78.6% and 16.7 to 40.0%, respectively.

When rainbow trout eggs inseminated with normal sperms were subjected to hydrostatic pressure (700 kg/cm^2, for 7 min.) at 15 min. after insemination, 100% of embryos were triploid. The ratio of normal embryos at eyed stage varied from 58.6 to 83.0%. Tetraploids were induced when eggs were treated (700 kg/cm^2, for 7 min.) at 300 or 330 min. after insemination. The ratio of normal embryos to total eggs used was only 7.2%.

3326 eggs of masu salmon were irradiated with 5 to 10 x 10^4 rads of gamma rays and were inseminated with normal sperms. Eggs were maintained at 10° C and subjected to hydrostatic pressure (650 kg/cm^2, for 6 min.) at various times (during 210 to 370 min. after insemination). The best results, 7.2% of treated eggs were normal at eyed stage, were obtained when eggs were treated at 310 min. after insemination. A total of 53 normal androgenetic diploids were produced and 46 of them hatched out. They were viable and grew up normally.

4:10 POSTER SESSION I
Endocrinology and Environmental Factors

Factoria Room

Salmonid Redd Dewatering: What Do We Know?

C. D. Becker and D. A. Neitzel, Battelle, Pacific Northwest Laboratory, Richland, Washington

Spawning areas used by adult salmonids may be dewatered by environmental manipulation during the critical period when embryonic developmental phases are intergravel. As a result, eggs and yolk-sac alevins may be exposed to altered physical conditions imposed by withdrawal

of water from the gravel. Dewatering may be periodic and short-term, as imposed by daily power peaking operations of a hydroelectric generating facility, or sporadic and long-term, as imposed by drawdowns for irrigation or construction activities.

We have conducted dewatering experiments with chinook salmon and rainbow trout eggs in artificial redds at our laboratory since 1979. The data indicate that, relatively, egg phases are tolerant of dewatering while alevin phases are intolerant. Comparative quantitative data are available. A number of physical factors that influence survival of intergravel development phases during dewatering were examined experimentally. Other factors that might influence survival of development phases were identified from the literature. Many of these factors, such as subsistent flow from bank storage after drawdown, will vary with seasonal and site-specific conditions. Knowledge of relative tolerance of intergravel phases to dewatering, and of the effect of various abiotic conditions associated with dewatering, can be used in developing and implementing mitigation measures.

Artificial Insemination in Salmonids

Rolland Billard, Laboratoire de Physiologie des Poissons, Institut National de la Recherche Agronomique, Jouy-en-Josas, France

It is strange that after almost a century of salmonid culture, we continue to use one male for only several females in artificial insemination since we know that milt production is high (about 5.10^{12} spermatozoa/kg body weight) and that 20 to 40% of the spermatozoa, or $1\text{-}2.10^{12}$, are utilizable during reproduction. Moreover, using an appropriate diluent conforming to the requirements of ova and spermatozoa permits higher dilution of the spermatozoa and a slight increase in the duration of motility. In these conditions, 30,000 to 300,000 spermatozoa per ? ova can be used, depending on sperm quality which decreases at the end of the reproductive season. The milt production then of one male can fertilize the ova of a very large number of females, reducing the number of males needed in aquacultures, providing that the brood stock is well managed genetically.

Advancement and Synchronization of Spawning in *Salmo gairdneri* and *S. trutta* Following Administration of Pimozide and LHRH-A

Rolland Billard, P. Reinaud, M. G. Plouidy, B. Breton, Laboratoire de Physiologie des Poissons, Institute National de la Recherche Agronomique, Jouy-en-Josas, France

The spawning of rainbow trout was advanced by more than 1 month in 60% of females after pimozide injection (10 mg/kg body weight) followed by LHRH-A injection (1 µg/kg) 6 h. later. Advancement was clearly less marked when we injected either LHRH-A or pimozide alone and when the controls were given only saline solution. Ovum fertility was comparable in all groups.

With the aim of avoiding two successive injections, we tried administering pimozide (10 mg/kg in an injection) and LHRH-A (implant containing 50 and 5 µg/kg body weight) simultaneously in brown trout during full spawning. The layings of females given pimozide plus LHRH-A were grouped over a 4-day period and those of females given only pimozide or a blank implant over 8 days.

Measurement of a Sex-specific Protein in the Skin Mucus of Premature Coho Salmon *(Oncorhynchus kisutch)*

M.R. Gordon, T. Owen*, and L. Hildebrand, B.C. Research, Vancouver, B.C.
* Helix Biotech Ltd., Richmond, B.C., Canada

The work reported here is the initial experiment we undertook to confirm our working hypothesis that a simple, rapid, non-interventive technique for sexing premature salmonids was possible without blood analysis. The final objective is to develop a "litmus paper" type of method which is harmless and unstressful and, most importantly, will determine the sex of fish several months before spawning.

Analysis of skin mucus specimens from fish sampled up to eight months prior to spawning revealed the presence of vitellogenin, a female-specific protein. The occurrence of this protein in the skin mucus of female fish was coincident with the initial development of the female gonads. These preliminary findings indicated that the concentration of vitellogenin in the mucus was greatly increased and readily discernible when female gonad weight was >1% and >6% of body weight, some 4–5 months prior to spawning. At this time no visible external signs of maturation was evident in male or female coho from this stock.

A practical method for sex determination that can be used by fish farmers, ranchers and hatchery biologists is currently under development.

Artificial Hatching Substrate: Effect on Yolk Sac Absorption and Growth Rate During Startfeeding of Atlantic Salmon *(Salmo salar)*

Tom Hansen and Dag Möller, Institute of Marine Research, Direktorate of Fisheries, Matredal, Norway

The Norwegian fish farming industry experiences serious mortality problems both in the hatcheries and during the start feeding period. Both natural and artificial substrates have been reported to increase fry size and to lower mortality rate. The effect of these substrates has however not been studied in detail. The purpose of this experiment was to investigate to what extent an artificial substrate affects the growth rate, survival rate and yolk conversion efficiency of the fry.

In this experiment groups of pooled Atlantic salmon *(Salmo salar)* eggs were hatched in a California hatching system with and without an Astro-turf artificial substrate. At 41 days after hatching each group was transferred to separate feeding units and was fed dry feed pellets for a period of 46 days. Alevins reared in Astro-turf absorbed their yolk sac faster and more efficiently than alevins reared on a flat screen. The alevins reared in Astro-turf showed no constrictions in their yolk sacs, a feature which was notable in the yolk sacs of the alevins reared on a flat screen. The fry hatched without Astro-turf grew faster than the fry hatched with Astro-turf during the first startfeeding period. However, the growth rate during the first startfeeding period is highly influenced by the amount of yolk left in the yolk sac. In this experiment the early growth of the fry hatched with Astro-turf probably could have been improved by introducing food earlier. From startfeeding day 18 and to the end of the startfeeding period the fry hatched with Astro-turf grew better than fry hatched without Astro-turf. The mortality of alevins and fry hatched with Astro-turf was lower both in the hatchery and during startfeeding.

Manipulating Photoperiods in Hatchery Reared Rainbow Broodfish to Control Spawning Activities

Larry Harris and Tom Mandis, Colorado Division of Wildlife, Fort Collins, Colorado

Abstract not available at press time.

Biochemical Changes Occurring in Female Salmon *(Salmo salar)* During Vitellogenesis

Carl Haux, University of Göteborg, Göteborg, Sweden; and Ulf-Peter Wichardt, Salmon Research Institute, Älvkarleby, Sweden

During a period of the reproductive cycle in female fish, a specific lipophosphoprotein, vitellogenin (VG), is synthesized in the liver, transported in the blood to the ovaries and incorporated in the oocytes. Estradiol-17β appears to be the main steroid involved in the induction of VG synthesis. This is supported by findings in adult female Atlantic salmon, where a correlation between plasma VG and estradiol-17β has been reported (Idler et al., Can. J. Fish Aquat. Sci. 1981, 38, 405-413). Since VG is a large multicomponent protein, its appearance in the blood alters the biochemical plasma composition dramatically.

The object of the present study was to follow changes in some plasma parameters that are related to vitellogenesis by repeated blood sampling on female salmon undergoing sexual maturation and spawning. Increased knowledge of these processes could have applications in brood fish handling, where environmental factors, such as temperature, handling stress, infections, prophylactic treatments etc., may influence vitellogenesis and subsequently the production of eggs and fry.

In the present study, females were trapped near Älvkarleby on their spawning migration in the river Dalälven during July. Totally eight females were tagged and repeatedly sampled roughly every second week, starting in August and ending in December. All females were stripped of eggs within one week in early November.

The amount of plasma VG, measured as phosphate bound to protein (PP), was about 2.4 mM in August, increased to 5.7 mM in September, and decreased successively to 2.4 mM at the time of spawning. The level of PP decreased then further to 1.3 mM in December, which is close to the amount of PP present in adult male salmon (approximately 1 mM). Elevated levels of the total plasma protein content (PR), which reached a maximum of 6.8 g/100 ml in late August and decreased to 4.5 g/100 ml at spawning, presumably reflected the presence of VG in the plasma. This assumption is further supported by a correlation between plasma PP and PR ($r = 0.54$). Similarly, total plasma calcium (CA), and to a lesser extent, total plasma magnesium (MG) followed the increase and decrease in plasma VG closely: CA peaked at 4.7 mM in September and MG at 1.3 mM in late August, were reduced to 3.2 and 0.9 mM respectively during spawning, and reached normal values of 2.4 and 0.8 mM in December. The increase in total plasma CA as well as the close correlation between plasma PP and CA ($r = 0.82$), indicate that salmon VG binds CA in a similar way to that observed in other species. Possibly, also MG could bind to VG ($r = 0.44$). During the most intense period of vitellogenesis, the amount of plasma phospholipids (PL) were markedly elevated (by 30 to 50%). This increase could probably be attributed to VG, which usually contains both triglycerides and PL.

Effects of Sediment on the Reproduction of Salmonids

K V. Koski, Auke Bay Laboratory, National Marine Fisheries Service, Auke Bay, Alaska

Salmon and trout have evolved similar strategies for reproduction. The construction of redds in gravel substrates is a means of protecting their eggs from predation and ravages of the environment. Fry recruitment is dependent on the intra-gravel environment of the redd, and natural or man-induced events can cause significant alteration resulting in high mortality. A natural component of the redd is sediment (i.e., coarse sand), however, in high quantities it can significantly reduce the number of emerging fry. Sedimentation of streams can occur as a result of industrial development, logging, road-building, etc. and is one of the major factors affecting spawning habitat. Sediment causes mortality by physically blocking the emergence of fry from the gravel or by reducing the amount of dissolved oxygen in the intergravel water by preventing interchange with surface water. Sediment content of the spawning gravel sets the upper limit on survival, and other factors such as dissolved oxygen, freezing, scouring, etc. impose additional mortality. The stage of development, condition, and timing of emerging fry are altered by increases in sediment in the redd. Egg size may form the basis of preferred spawning habitat by the different species of salmonids.

Alteration of spawning habitat is likely to continue and improved methods of evaluating impacts are essential. Enhancement of spawning habitat must address substrate composition. Research during the last 20 years in Alaska, Oregon and Washington has resulted in equations expressing the relationship between sediment and survival to emergence of salmonids. New methods of gravel analysis have been developed. New expressions of gravel composition and survival are developed, enabling one to predict survival to emergence and to determine quality of spawning habitat.

On Modifications of Gonadotropic Cell Ultrastructure in the Intact Mature Dwarf Male *Oncorhynchus formosanus* and Juvenile *O. masou* after Injection of Salmon Gonadotropins

A. A. Maximovich, V. V. Pljusnin, and V. N. Poljakov, Laboratory of Functional Morphology of Cells, Institute of Marine Biology, Vladivostok, U.S.S.R.

The ultrastructure of gonadotropic cells (GTG-cells) of adenohypophysis in the dwarf male salmon *Oncorhynchus formosanus* during gonadal maturation and in the juvenile masu salmon *(Oncorhynchus masou)* in 6, 12, and 24 hours after a single injection of salmon gonadotropin (GTG) was studied.

Two fractions of GTG isolated from the hypophyses of mature males (fraction M_2) and females (fraction F_1) of the pink salmon *Oncorhynchus gorbuscha* and purified by column gel-filtration with Sefadex G-100 have been used in this study.

In all the fish individuals granular, vesicular and globular GTG-cells were detected. It was established that the percentage of these GTG-cells in the adenohypophysis distinctly changed during sexual maturation and after injecting the fractions F_1 and M_2 to immature salmon. Accordingly, the question is discussed whether the detected types of GTG-cells are different functional stages of gonadotropocytes or they are responsible for production of different gonadotropic hormones.

The Relationship between Fertility of Rainbow Trout Eggs and Motility of Spermatozoa as Evaluated by Quasielastic Light Scattering Techniques

R. D. Moccia and K. R. Munkittrick, International Aquaculture Developments, Erin, Ontario; T. Craig and F. R. Hallet, University of Guelph, Canada

In many vertebrates, it is commonly assumed that spermatozoan motility is a reasonable indicator of fertility. Although there have been several investigations into the relationship between fertility and motility, few of these have utilized objective motility estimation techniques, and in general, results have been of low predictive value. It is possible that such a relationship may be obscured by the limitations of subjective motility estimation. Historically, subjective motility estimation is variable, not highly repeatable, and inaccurate when the sample contains either large numbers of dead sperm or sperm which are swimming abnormally.

Quasi-elastic light scattering (QELS) techniques offer repeatable, objective, quantitative evaluations of large numbers of sperm simultaneously. This study proposed to evaluate the relationship between fertility and motility in rainbow trout *(Salmo gairdneri)* using QELS techniques.

Gametes were collected from brood stock at a commercial trout hatchery. Semen was evaluated for spermatocrit, density, subjective motility, objective (QELS) motility and ability to fertilize pooled, replicated egg samples. A second trial late in the spawning season also involved

cryopreservation of semen and fertilization of pooled egg samples.

There was a statistically significant relationship between sperm density and spermatocrit, although neither parameter could be related to subjective motility, objective motility or fertility. Objective estimates of motility were repeatable but needed standardization with respect to density and dilution. There was day-to-day variability in fertility and in the relationship between both subjective and objective motility estimates. Estimates of fertility were also variable. Cryopreserved samples showing the best subjective motility pre-freezing gave fertility, while those with poor pre-freezing motility gave none.

Integration of Salmon Enhancement in Water Quality Planning: A Case Study
Thomas B. Murdoch, Snohomish County Public Works Department, Everett, WA

Snohomish County, Washington, is a rapidly urbanizing area in western Washington that contains over 3,000 miles of streams and 600 lakes. Most of these bodies of water support salmonids; however, increased development without proper drainage management is destroying spawning and rearing habitats at an alarming rate.

To further public appreciation for watershed management programs that avert stormwater damage to property and also protect natural resources, the Snohomish County Public Works Department has sponsored stream enhancement projects for adoption by community groups. Such organizations as schools, sportsmen's clubs, ad-hoc committees and civic organizations have reared salmon in egg boxes, replanted streambanks, removed debris blocking stream channels, and identified unmapped small streams.

Results are provided to officials making land use decisions. Of particular importance is the identification of formerly unknown streams and the incorporation of these data into local zoning maps. Streams "adopted" by a neighborhood group have an improved chance for survival. When organizations become actively involved in local water quality projects, dramatic positive results can be achieved in short periods, with salmon being the primary beneficiary.

Effects of Estradiol-17-β on Gonadal Differentiation in Two Species of Salmonids, the Masu Salmon, *Oncorhynchus masou*, and the Chum Salmon, *O. keta*
Masaru Nakamura, Faculty of Medicine, Teikyo University, Tokyo, Japan

The effect of estrogen treatment on the induction of gonadal feminization was investigated in two species of salmonids, masu and chum salmon. Histological examination reveals that the two sexes can clearly be distinguished in masu salmon 13 days after hatching and in chum salmon 25 days. Masu salmon fry were immersed in water containing estradiol-17β (E_2) at concentrations of 0.25, 0.5, 1, 2, 5, 10, 20, 50, 100 and 200 μg/l for 18 days starting from 5 days after hatching. Sex differentiation was assessed histologically at 30, 50, 90 and 360 days after hatching. Histological examination of fish at 30 days revealed that in the 0.5 to 5 μg/l treated groups, nearly 100% fish were female with gonads which closely resembled those of female controls; retarded ovaries were observed only in one or two fish in each group. In addition, inspection of gonads of 0.5 to 5 μg/l groups at 360 days confirmed complete feminization. Most fish of these groups were grown to maturity. On the other hand, masu salmon receiving higher doses of E_2 (10 to 200 μg/l) resulted in high mortality while fish with the lowest concentration (0.25 μg/l) had intersexual gonads.

Chum salmon fry at various stages of gonadal sex differentiation were immersed in water containing E_2 at concentrations of 0.5, 1 and 2 μg/l for a period of 15 to 67 days. Unlike the situation in masu salmon, complete feminization could not be induced in chum salmon by these treatments. However, partial feminization was observed in fish treated at the lowest concentration (0.5 μg/l) during the period from 6 to 34 days after hatching. The testes of this group consisted of small oocytes interspersed with undeveloped germ cells with stroma. These results will be discussed in relation to the effective dose and period of estrogen treatment for the successful feminization of gonads in two species of salmonids.

Temperature Effect on Egg Viability and Spawning Time of Cutthroat Trout
Robert G. Piper, U.S. Fish & Wildlife Service, Bozeman, Montana

Westslope cutthroat trout egg viability and spawning time were compared in colder, fluctuating temperature creek water and constant 50°F temperature spring water.

Eggs from broodfish held in creek water were easily expelled and normal appearing. Eggs from broodfish held in spring water, however, were usually difficult to expel, and broken and opaque eggs were common. Egg viability was greatly affected by water temperature: there was a 77% eye-up in eggs from broodfish held in creek water, while only a 25% eye-up occurred in eggs from broodfish held in constant temperature spring water.

Duration of spawning varied; groups of broodfish held in creek water spawned over a 20 to 26 day period, while 44 to 57 days elapsed before all groups of broodfish held in spring water had spawned.

Development of Atherosclerotic Lesions in Coronary Arteries of Atlantic Salmon During Sexual Maturation

R. L. Saunders, Department of Fisheries and Oceans, St. Andrews, New Brunswick; A. J. Farrell, Mount Allison University, Sackville, New Brunswick; H. C. Freeman, Department of Fisheries and Oceans, Halifax, Nova Scotia, Canada

Atherosclerotic lesions have been observed in coronary arteries of Atlantic salmon and other salmonids at the time of sexual maturation. It has been suggested that these lesions are in association with elevated levels of sex hormones. The purpose of our study is to document development of atherosclerotic lesions in Atlantic salmon in relation to the natural maturation cycle between June and October. Samples of maturing and non-maturing individuals were killed monthly for determination of blood levels of cholesterol and high density lipids as well as the hormones testosterone, 11-keto-testosterone and estradiol; heart-ventral aorta samples were taken for histological examination. The determination of sex hormone levels and histological examination of coronary arteries is in progress. Additional samples will be collected during 1983 to learn whether or not atherosclerosis is confined to maturing individuals and, if so, to study the time course of its development and related patterns of sex hormone levels.

Photoperiod Control of Spawning Time in Rainbow Trout *(Salmo gairdneri)* Using Constant Long Daylengths

A. P. Scott, O. Skarphedinsson, S. M. Baynes, and V. J. Bye, Ministry of Agriculture, Fisheries and Food, Lowestoft, United Kingdom

Long daylengths of 18-20 hours have been shown to stimulate precocious maturation in male underyearling trout and to induce a six month reproductive cycle in adults. Two trials, based upon the latter observation, have been carried out on a commercial fish farm in order to obtain out-of-season production of gametes. Both have been successful, in one case yielding gametes in July/August and in the other April/May.

The system we have used is cheap and simple to run, requiring minimal equipment and supervision. Lightproof facilities are not required. Light bulbs suspended above the broodstock tanks and controlled by time clocks are switched on from 0400 to 2200 hours every day, starting at or about the time of spawning.

One consequence of the constant long daylengths is that the growth of the broodstock is reduced. The reasons for this will be discussed (with supporting experimental data). The mechanism of action of long daylengths on the reproductive cycle of the rainbow trout will also be discussed.

Sex Reversal in Atlantic Salmon: Problems with High Doses of Estradiol and DES

S. A. Sower, University of New Hampshire; W. W. Dickhoff, T. Flagg, J. Mighell, University of Washington; and C. Mahnken, National Marine Fisheries Service, Seattle

Atlantic salmon were fed Oregon Moist pellets incorporated with different doses of estradiol (20 or 2 mg/kg diet) or diethylstilbesterol (DES) (10, 1 or 0.1 mg/kg diet) in an attempt to reverse the sex of the salmon from male to female during the time of sexual differentiation. Atlantic salmon treated with a high dose of estradiol at 20 mg/kg had the highest mortality compared to controls and other treatment groups. Also, the salmon treated with the highest dose of the estrogens contained the highest percentages of female fish.

It is not clear from this study, whether differential mortality occurred, causing male mortality resulting in survival of the females, or whether sex reversal had occurred. These possibilities will be discussed. We will also present data on the occurrence of precocial sexual development in 42% of the control males which were only six months old. Precocial sexual development did not occur in the estrogen-treated male fish. The implications will be presented.

Purification Studies of Coho Salmon Pituitary Glycoprotein Hormones

P. Swanson, W. W. Dickhoff, A. Gorbman, University of Washington, Seattle

Reproductive processes in salmon may be regulated by both the thyroid and reproductive endocrine systems. Important components of these two systems are the pituitary glycoprotein hormones, gonadotropic hormone (GtH) and thyroid stimulating hormone (TSH). Research designed to examine the specific physiological roles of GtH and TSH in salmon reproduction has been limited by (1) the lack of salmon GtH which has been demonstrated to be uncontaminated with TSH and (2) the lack of pure salmon TSH. The goal of this investigation was to isolate coho salmon TSH and GtH.

Two sources of pituitary glands were used. Pituitaries were collected from sexually mature coho salmon at the time of spawning and from immature coho salmon held in seawater net pens. Chemical fractionation procedures included extraction in acid alcohol and chromatography with

Sephadex G-100, Sulphopropyl Sephadex C-50, and DEAE- Sephacel. During the purification steps assays for gonadotropic and thyrotropic activities were based on the ability of test materials to elevate plasma levels of thyroxine (T_4) and estradiol 17-β after injection into underyearling coho salmon (body wt. 15–30 g). Fractions from both sources of glands contained thyrotropic and gonadotropic activities. A fraction from the pituitaries of seawater-maintained salmon had greater TSH activity than an approximately equivalent fraction from the pituitaries of spawned adult fish. Extracts of salmon pituitaries were more than twice as potent as bovine TSH (Sigma Chemical Co.) in elevating plasma T_4. A seasonal variation in responsiveness to test fish TSH and GtH fractions was observed.

Factors Related to the Relatively Low Hatching Output in the Production of Salmon and Trout in Norwegian Commercial Hatcheries

Yngve Ulgenes and Gunnar Nævdal, Institute of Marine Research, Direktorate of Fisheries, Matredal, Norway

Abstract not available at press time.

Effects of Low pH on the Reproduction of Rainbow Trout *(Salmo gairdneri)*

G. S. Weiner, C. B. Schreck, and H. W. Li, Oregon Cooperative Fishery Research Unit, Oregon State University, Corvallis

Surface water acidification, presumably due to acidic precipitation, has been correlated with the decline of fish populations in regions of Europe and North America. Field studies indicate that failure in recruitment of early year-classes is an important contributor to the gradual demise of fish populations in impacted waters. Such recruitment failure may be due, in part, to disruption of adult reproductive physiology and mortality of early life history stages. Salmonid fishes are generally very sensitive to environmental acidification. We address the potential effects of acidification on the reproduction of salmonids, using the rainbow trout *(Salmo gairdneri)* as a model.

Male and female adult rainbow trout were exposed to pH levels 4.5, 5.0, 5.5, and control over the final six weeks of reproductive maturation. Fertilization was then performed within each treatment group (with rearing of offspring at the respective pH level) and between treatment fish and controls (with rearing in control water). Crosses between control fish were reared in groups at each pH level. Offspring were monitored for rates of survival to eye-up, hatching, and absorption of the yolk-sac.

Analysis of the results of the cross-breeding matrix yields a comparison of the effects of low pH on gametogenesis with effects on developing embryos and larvae. Plasma samples collected from adults prior to acid exposure, during the exposure, and at spawning are being analyzed for concentrations of reproductive hormones and electrolytes as indicators of effects on reproductive physiology. Indicators of effects on egg and sperm quality are also being evaluated.

MONDAY EVENING

6:30-8:00 **Symposium reception**
Bellefield/Fairweather

TUESDAY MORNING

1 November 1983
8:15 to 11:30

ENVIRONMENTAL FACTORS
Idylwood/Marymoor

Chairman
Rolland Billard
Institut National de la
Recherche Agronomique
Jouy-en-Josas, France

8:30 **Salmonid Reproduction and Environmental Factors**
Rolland Billard, Institut National de la Recherche Agronomique, Jouy-en-Josas, France

A number of environmental factors affect the reproductive function in fish. The effects of these factors vary with the species and its developmental stages. The most visible factors implicated are photoperiod and temperature. Photoperiod acts on the establishment of puberty, gametogenesis and ovulation. Temperature, being an environmental signal, has similar effects but they are usually short-termed; this signal coordinates various stages (gamete release, fertilization, embryogenesis) with the most favorable conditions for the progression of each stage. Temperature also acts on sex differentiation, puberty and gametogenesis. Other factors that are also important are salinity which affects puberty, gametogenesis, the quality and survival of gametes, fertilization and embryogenesis and the diet which affects puberty and gametogenesis. The social environment also acts on puberty, gametogenesis and the efficiency of gamete release. The biological environment is sometimes a determining factor in laying (e.g. plant substrates).

Anthropic factors (change in water flow rate, pollution, river navigation) also affect reproduction, usually in a negative way. Environmental factors often have an indirect effect via sensorial functions and the central nervous system but may also directly affect gonads, gametes and eggs. Some rhythmic-type factors in the environment also entrain the internal cycle.

A basic question in aquaculture is: should the environment be adapted to the animal, as in classical animal husbandry, or the animal to the environment? The answer depends on the type of aquaculture practised.

8:50 **The Use of Altered Seasonal and Constant Photoperiods in the Production of All-Year Round Supplies of Eggs in the Rainbow Trout**
N. Bromage, J. A. K. Elliott, and J. Springate, University of Aston, Birmingham, United Kingdom

A major constraint to the more efficient commercial production of trout is the difficulty in supply of eggs. In the U.K. eggs are commonly only available from home hatcheries from November to February/March and the season can only be extended by the importation of eggs from overseas particularly the U.S.A. and Denmark. Similar difficulties imposed by seasonal supplies of eggs are also experienced by other producing countries. Ideally, eggs should be available throughout the year or at least at times prescribed by the industry. Improvements in the availability of eggs would ensure a more effective use of farm facilities and staff and guarantee an all-year round supply of table fish.

One method of altering the time of spawning of salmonids, and hence the supplies of eggs, is to manipulate the daylength to which the broodstock fish are exposed. The present paper discusses the use of both seasonally-changing and constant light regimes in the advancement and delay of the natural spawning times of different strains of rainbow trout. Up to 4 months advancement and 3 months delay in spawning can be achieved during one reproductive season in parallel groups of broodstock using relatively simple photoperiod regimes. Subsequent manipulations over successive cycles can provide spawnings during any month of the year. it is also possible to have up to three batches of eggs from the same fish over a 16-month period.

A variety of experiments are described including pilot-scale studies, and also more importantly data from facilities on a number of commercial farms where many hundreds of broodstock are under photoperiod control. The effects of altered photoperiod on the time of spawning and fecundity and the quality of the egg are discussed.

9:05 **Control of Maturation in Masu Salmon by Manipulation of Photoperiod**
Fumio Takashima and Yoshiaki Yamada, Tokyo University of Fisheries, Tokyo, Japan

In order to change the spawning time in landlocked variety of masu salmon, *Oncorhynchus masou*, by manipulation of photoperiod, the effective light regime was investigated.

O. masou usually spawn in October at the age of two under natural conditions. However, maturation and spawning were accelerated two to three months earlier when reared under long photoperiod (18L-6D or 24 L-0D) for one or two months during initial course of reproductive cycle, and under short photoperiod (6L-18D) during successive periods of the cycle. The maturation, on the other hand, was delayed when the brood fish was subjected to continuous long pho-

toperiod. Long photoperiod during initial course of the cycle seemed to stimulate early process in the accumulation of fatty yolk. At least, one month of continuous long photoperiod is necessary to initiate maturation. Rapid vitellogenesis, as in the other salmonid species, was apparently stimulated by successive short photoperiods.

The fecundity of matured females under artificial photoperiod was significantly higher than that of naturally matured ones. Although the size of the eggs was smaller, percentage of eyed eggs did not differ with that of naturally matured eggs. Serum gonadotropin did not change during long photoperiod and increased at the time of ovulation that was triggered after transferring to short photoperiod.

9:20 **Effects of Temperature and Food Availability on Maturation of Sockeye Salmon in the Ocean**

T. Nishiyama, University of Alaska, Fairbanks; and T. Minoda and T. Fujii, Hokkaido University, Hakodate, Japan

The return timing of salmon to fresh water is believed to result from the effects of oceanographic conditions during the last marine life stage. Specifically it has been found that the return timing of Kvichak river sockeye is inversely related to the mean sea surface temperature in the central Bering Sea during June, a month prior to the return migration. Nishiyama (1982) offered an explanation of the mechanisms determining the return timing. First, the return timing is controlled by the maturity condition of salmon which is directly regulated by sea temperature. Second, the temperature influences upon food availability and growth must affect the maturity condition and thereby the return timing.

To examine these mechanisms, the relationships between return timing, temperature and zooplankton biomass in the basin and shelf areas of the Bering Sea were investigated based on data collected during June and July from 1957 to 1981.

The zooplankton biomass was low from 1957 to 1963, but high between 1965 and 1969. Since 1974 the zooplankton biomass has tended to decrease slightly. Mean zooplankton biomass has generally been 25% higher in the shelf area than in the basin area. In the basin area, the zooplankton biomass has tended to decrease linearly with an increase in temperature, indicating that the zooplankton production was higher in the colder years. In the shelf area, the zooplankton biomass was low both in the colder and warmer years, but high in the moderate years. The peak return date of Kvichak river sockeye appeared to be related to the zooplankton biomass in the Bering Sea. A high zooplankton biomass in the basin area was associated with delayed return timing; whereas, in the shelf area, the inverse was true. Finally, the effects of temperature and food availability will be discussed in relation to the vertical distribution patterns of sockeye and zooplankton.

9:35 **Developmental Rate, Fecundity, and Egg Size in Atlantic Salmon, *Salmo salar* L.**

J. E. Thorpe, Department of Agriculture and Fisheries for Scotland, Pitlochry, Perthshire, United Kingdom

Egg number and egg size vary widely between individual spawners within stocks, and between stocks in Atlantic salmon. Early papers attempted to establish mean fecundity measures to characterize particular populations, or to supply rule-of-thumb estimating methods for fishery managers. More recently interest has been focussed, especially in the Soviet Union and Scandinavia, on temporal variation in size-fecundity relationships, and in age-fecundity relationships, within individual stocks. These studies are reviewed, and new data presented, evaluating the inter-relationships of developmental rate (river and sea age) on egg number and egg size in the River Almond salmon stock, Tayside, Scotland. In particular, it is shown that the rate of development of females during the juvenile riverine phase influences both the number and size of eggs produced subsequently. The general life-history strategy significance of this is discussed.

9:50 **Coffee Break**

10:15 **Fertilization Success and Sperm Motility of Atlantic Salmon *(Salmo salar)* L. in Acidified Waters**

P. G. Daye, Daye Atlantic Salmon Corporation, Armdale, Nova Scotia; and B. D. Glebe, North American Research Center, New Brunswick, Canada

Fertilization success of Atlantic salmon *(Salmo salar)* eggs by large sea-run males and precocious male parr in acidic waters was determined. Values of pH for milt, ovarian fluids, acidic media during fertilization, and acidic media during water hardening are presented. Duration of spermatozoa motility in acidic media is also given and discussed in relation to fertilization success. There was no effect on fertilization success at pH 5.0 or above. There was a steady decline

in fertilization success from pH 5.0 to 4.0. No eggs were fertilized below pH 4.0. The pH LL50 was 4.5 for fertilization success. Duration of spermatozoa motility constantly declined at all tested levels until about pH 4.5 where the decline became extremely rapid with zero seconds of motility occurring near pH 4.0. The duration of spermatozoa motility of precocious male parr was longer than that for the large sea-run males at levels above pH 4.4. The significance of all results is discussed in respect to the recruitment of young into fish populations in acid stressed waters and acid precipitation.

10:30 **The Effect of Size, Age, Growth Rate and Photoperiod on Maturation in the Brook Trout *(Salvelinus fontinalis)***

Stephen D. McCormick and R. J. Naiman, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

Brook trout *(Salvelinus fontinalis)* were raised at constant temperature (10°C) under two photoperiod conditions: one cycled normally with calendar date and the other was three months delayed from the norm. Within each photoperiod two growth groups, one fast (0.246 g/d over one year) and one slow (0.046 g/d), were created by controlling the amount of food offered. Photoperiod controlled the temporal phasing of the maturation cycle. Within each photoperiod, size and/or growth rate, and not age, determined the proportion of maturing individuals during their first autumn (0+). The largest proportion of mature individuals occurred in the delayed photoperiod, fast-growing fish. Plasma thyroxine levels were generally lower in slower growing fish and showed an annual, photoperiod controlled pattern in all groups. In each experimental group, the proportion of 0+ mature females was always significantly ($p<0.05$) lower than the proportion of mature males. All fish in each experimental group became mature in their second autumn (1+). A size related threshold which is sexually dimorphic and which interacts with photoperiod can best explain the 0+ and 1+ maturation of brook trout under artificial culture.

10:45 **Photoperiod Induced Delayed Spawning of Freshwater Reared Chinook Salmon**

W. S. Johnson, Ministry for Conservation, Fisheries & Wildlife Division, Alexandra, Victoria, Australia

Chinook salmon have been cultured at the Snobs Creek Freshwater Fisheries Research Station and Hatchery, Victoria, Australia, for more than 14 years. The salmon live their entire life cycle at the station in freshwater. Breeding has been relatively successful and two lakes are regularly stocked with salmon which grow quickly to a large size and provide excellent angling. In 1980 a five year program was initiated to identify factors adversely affecting breeding success and to make improvements where possible to fish husbandry.

The effect of relatively high water temperature during the maturation of adults and the incubation of ova has been studied, and related to embryonic deformation and death. An artificial photoperiod regime has been used in an effort to delay adult maturation to later in the autumn when water temperatures are considered to be more favourable.

11:30 **Symposium Luncheon**

Bellefield/Fairweather

Salmon Recipe: One Part Science, Two Parts Technology, Three Parts Economics, and Ten Parts Politics

William McNeil, General Manager, Oregon Aqua Foods, Springfield, Oregon

TUESDAY AFTERNOON
1 November 1983

NUTRITION

Idylwood/Marymoor

Chairman
Ronald W. Hardy
University of Washington
and Northwest & Alaska Fisheries Center
National Marine Fisheries Service
Seattle, Washington

1:30 **Proximate and Elemental Composition of Developing Eggs of Pen-reared Coho Salmon *(Oncorynchus kisutch)* Fed Production and Trace Element Fortified Diets**
R. W. Hardy, University of Washington and National Marine Fisheries Service; K. D. Shearer, National Marine Fisheries Service; and I. B. King, University of Washington, Seattle

Two groups of coho salmon, raised in marine net-pens, were fed standard Abernathy diet 19-2 or Abernathy diet 19-2 fortified with additional cobalt, copper, iron, manganese, and zinc during the 6-month period before spawning. Monthly ovary samples and whole fish minus the ovary samples from each group were analyzed for proximate and elemental composition. Whole body wet weights increased about 30% during the experimental period but most of the gain was in ovary weight. Analysis of the proximate changes showed a reduction in lipid levels of the fish during ovarian development, elemental levels of the fish did not decrease. Proximate constituents were deposited in the eggs throughout ovarian development but the rates of deposition varied between constituents from month to month. Elemental deposition in the ovaries was continous but the rates of deposition varied between elements. No evidence of maternal somatic elemental depletion was observed, and no differences were detected between the two groups in elemental composition of the maternal tissues or ovary at spawning. Analysis of the results of this study and comparisons with observations from pen-reared and wild coho salmon eggs in previous years indicated that additional elemental fortifications of Abernathy diet 19-2 is unnecessary for pen-reared coho salmon.

1:50 **Mineral Supplementation of Atlantic Salmon Broodstock Diets**
H. G. Ketola, U.S. Fish and Wildlife Service, Cortland, NY

Spawning of wild-kelts reconditioned on hatchery diets and of hatchery reared Atlantic salmon *(Salmo salar)* has been much less successful than that for sea-run salmon in various recent attempts in hatcheries in northeastern states. The "eye-up" of eggs from sea run salmon ranges from 83 to 92%, in contrast to that for reconditioned salmon which ranges from 45 to 75% and that for hatchery-reared salmon, 17 to 80%. Chemical analyses of eggs and diets suggested that mineral dificiency could be the cause of poor eye-up.

A 3-month pilot study was conducted to test the effect of feeding a fish meal-containing diet with and without a mineral mixture formulated to provide sodium chloride at 0.75% of diet and magnesium, zinc, manganese, iron, copper, and iodine at 400, 150, 100, 100, 5, and 8 mg/kg of feed, respectively. Each diet was fed at about 0.5% of body weight daily to duplicate lots (40 fish each) of mixed-sex, domestically-reared 4 3/4 year-old Atlantic salmon.

The results showed that the mineral supplement had no significant ($P > 0.05$) effect on egg production, fertilization rate, eye-up, hatchability or swim-up. However, there was considerable variability between replicate for all of these measurements. Semen of salmon was microscopically active and contained about 13.4 billion sperm/ml. Spermatocrit values (packed sperm cell volume) were not significantly affected by diet, nor were hematocrit values for spent females. Chemical analyses of various minerals in eyed eggs and fresh semen showed no appreciable effect of diet on any of the minerals measured.

A recent 30-month study on mineral supplementation (Zn, Mn, Fe, Cu) of fish meal diets fed to broodstock rainbow trout showed that supplementation with minerals dramatically increased eye-up and hatachability (Takeuchi et al., 1981, Bull. Jap. Soc. Sci. Fish. 47:645). This suggests that a long-term study may be essential to show any effect of supplemental minerals on reproduction in Atlantic salmon. Therefore, the lack of significant effects in this short-term study should not be considered as evidence for the non-essentiality of supplemental minerals in fish meal diets. A long-term experiment appears to be needed.

2:05 **Pigmentation of Salmonids: Effect of Carotenoids in Eggs and Startfeeding Diet on Survival and Growth Rate**

Ole Torrissen, Institute of Marine Research, Direktorate of Fisheries, Matredal, Norway

Salmonids mobilise their carotenoid pigments, astaxanthin and cantaxanthin, in the flesh and deposit them in eggs and skin during sexual maturation. These active metabolisms of carotenoids indicate that they have a specific function either during reproduction, early life, or both.

Carotenoids are reported to enhance growth rate, maturation rate and fecundity and to reduct mortality rate. However no biological function of astaxanthin and cantaxanthin have so far been documented by adequate scientific data.

To study the effect of carotenoids on survival, differently pigmented Atlantic salmon *(Salmo salar)* eggs and alevins were exposed to light of different wavelengths. Differently pigmented eggs and alevins incubated in darkness have also been studied. Highly pigmented eggs were found to be sensitive to light. In darkness there was no significant effect of the carotenoid level on survival of eggs and alevins. Diets supplemented with synthetical astaxanthin and cantaxanthin promoted growth rate during the early startfeeding period.

2:20 **Effect of Nutritional Quality of Broodstock Diets on Reproduction of Rainbow Trout and Their Egg Quality**

Takeshi Watanabe, Tokyo University of Fisheries, Tokyo, Japan

Economically productive aquaculture is heavily dependent upon an adequate supply of seed, of fertile eggs and juvenile fish, with which to stock the ponds, enclosures, and other cultivation systems. One of the most important and fundamental approaches to the artificial seed production to satisfy the ever-growing demand of fish breeders is to ensure a year-round, rather than a seasonal, supply of enough fertile eggs with high qualities which produce higher survival and growth rates than those naturally occurring.

Nutrition is known to have a profound effect upon gonadal growth and fecundity. Although precise information on the nutritional requirements for gonadal maturation in broodstock is lacking, it has been generally agreed that quality and quantity of the feed, as well as the feeding regimen, are important for reproduction and egg quality. Thus this study was conducted in order to develop an adult fish diet suitable for reproduction of rainbow trout by ensuring a relationship between feed quality and egg quality.

Adult or fingerlings of rainbow trout were fed on various test diets for 3 months or 3 years to examine the effects of low protein-high calorie or EFA-deficient diets and the total deletion of trace elements from the mineral mixture in white fish meal diets on reproduction and chemical components of eggs produced.

Eggs produced from the fish fed on the low protein diet with a high energy value gave good yield of eyed eggs with high hatchability compared to those fed on the control commercial diet. But, eggs from the fish fed on the diet without supplement of trace elements or EFA were significantly low in both percentages of eyed eggs and hatchability. These results have demonstrated that the low protein–high caloric diets supplemented with beef tallow have no adverse effects on reproduction of rainbow trout, and that a supplement of trace elements to fish meal diets is indispensable for reproduction, although white fish meal contains various kinds of minerals. The same kinds of results were also obtained in red sea bream.

2:35 **Effect of Increased Ascorbic Acid Supplementation in Broodstock Feed for Reproduction of Rainbow Trout**

Kjartan Sandnes, Yngve Ulgenes, Olaf R. Braekkan, and Finn Utne, Institute of Nutrition, Directorate of Fisheries, Nygardstangen-Bergen, Norway

Abstract not available at press time.

2:50 POSTER SESSION II
Genetics

Factoria Room

Cryopreservation of Milt and Its Application to Atlantic Salmon Farming

R. Alderson and A. J. MacNeil, Marine Harvest Ltd., Lochailort, Inverness-shire, United Kingdom

Recent studies by Stoss and others have shown the feasibility of long term storage of Atlantic salmon milt by cryopreservation. This technique could have value in the commercial farming of salmon by enabling milt of one year class to be used to fertilise eggs of the following year classes. This would improve the scope for rotational line crossing to avoid inbreeding depression in highly selected broodstock lines. The opportunity for using the technique will however be limited by the ease with which high levels of fertility can be achieved in full sized egg batches.

Initial trials using milt frozen in straws and batches of 300-400 eggs gave up to 90% of control fertilisation with certain combinations of freezing conditions and cryoprotectants. En-

couraged by these results improvements were made in the method of milt collection which enabled large quantities of uncontaminated milt to be obtained. A further test with batches of 4,000 eggs, which were stripped and fertilised with cryopreserved milt on the sea cages holding the broodstock, gave fertilisation rates of 30% and 70% of control levels. These results will be discussed further in the context of the difficulties which still remain for the wider use of cryopreserved milt in the salmon farming business.

Flow Cytometry vs. Nuclear Area: Analysis of Induced Polyploidy in *Ctenopharyngodon idella* x *Aristhichtys nobilis* Hybrids

S. K. Allen, Jr. and J. M. Myers, University of Washington, Seattle

Assays for ploidy have suffered from a lack of an unequivocal and economic assay for polyploidy. Putative triploid hybrids between the grass carp *(C. idella)* and bighead carp *(A. nobilis)* were obtained from J.M. Malone and Sons, Lonoke, Arkansas in order to make direct comparisons between the current methods for evaluating ploidy in these hybrids: measuring (1) erythrocyte nuclear volume and (2) DNA content via flow cytometry.

Blood samples were drawn from each fish and processed for nuclear volume and flow cytometric analysis. To measure nuclear volume, blood was smeared, stained, and photographed at 1000x; photomicrograph negatives were mounted in 35 mm slide frames and projected onto a screen. Nuclear areas were traced and then later retraced on a digitizer. Blood cells for flow cytometry were stained in the DNA flourochrome DAPI and flouresecence from 1000-5000 individual cells was quantified for each fish.

Results indicate that flow cytometric analysis achieved a three-fold reduction in the coefficient of variation for estimates of DNA content. The increased precision obtained via flow cytometry allowed distinct ploidy groups to be isolated whereas nuclear volume measurements failed to provide clear demarcation between ploidy levels. Flow cytometry costs compared favorably with nuclear volume, being 17% less expensive per sample and providing more immediate results. We recommend cytoflourometric analysis as the most efficient and definitive analysis method for ploidy analysis.

Evaluating the Consequences of Reproduction in Complex Salmonid Life Cycles

Hal Caswell, R. J. Naiman, Roderick Morin, Woods Hole
Oceanographic Institution, Woods Hole, Massachusetts

The demographic methods used in modern life history theory were originally developed for human populations. The life cycles of many organisms, however, differ significantly from that of man, and thus are difficult to analyze by traditional means. In salmonids, these differences include the partition of the life cycle between two distinctly different environments, plasticity in growth which makes maturation an ill-defined function of age, the existence of multiple reproductive pathways, and the divergence of the life cycles of males and females. Recent developments in evolutionary demography now make it possible to analyze such complex life cycles in great detail. This paper outlines these developments in general terms, and presents preliminary results of their application to the Atlantic salmon *(Salmo salar)* L. of the Matamek River, Quebec.

In particular, we focus on the problem of evaluating the relative contribution to population dynamics of reproduction by different stages in the life cycle. We show that quantitative estimates of these contributions can be made, and that they depend on both the stable stage distribution and the reproductive value distribution of the population. In an evolutionary perspective, this provides a measure of the selective importance of the different reproductive pathways. Recent changes in the reproductive life history of the Atlantic salmon (increased incidence of precocious maturation by males and of spawning by one sea year females) may be explicable on this basis.

Enhancing the Reproductive Potential of Captive Coho Salmon Broodstock

R. N. Iwamoto and W. K. Hershberger, University of Washington; and Carlin McAuley, Domsea Farms, Inc., Bremerton, WA

An integrated program has been designed and implemented to provide coho salmon broodstock for the controlled environment and production requirements of marine net-pen culture. While substantial progress relative to non-selected controls has been achieved in sub-adult performance traits (fresh- and seawater growth, smoltification, and seawater survival), the management of adults under captive conditions and their requirements for subsequent survival and maturation require definition. The parameters currently used to evaluate the status of broodstock include physiological measurements, effectiveness of prophylactic disease treatments, and determination of appropriate holding conditions. Results of the use of these techniques and genetic manipulation will be presented.

Comparison of the Reliability of a Coulter Counter with a Flow Cytometer in Determining Ploidy Levels in Pacific Salmon

O. W. Johnson and P. Rabinovich, University of Washington; and F. M. Utter, National Marine Fisheries Service, Seattle

The reliability for differentiating ploidy levels of an ICP-22 Flow Cytometer using a DNA flourescent dye and a Coulter Counter and Coulter Channelyzer measuring cellular volume was compared.

Fish from each of four groups of Pacific salmon—*Oncorhynchus tshawytscha*; *O. gorbuscha*; and two groups of hybrids *O. tshawytscha* (female)/*O. gorbuscha* (male) and *O. gorbuscha*(female)/*O. tshawytscha* (male)—which had been heat treated at fertilization to produce triploid fish were analyzed at 15 months of age to determine ploidy using both techniques. Results are discussed with the objective of using the Coulter Counter as an inexpensive and more commonly available alternative to the flow cytometer.

Studies on Salt Water Adaptability and Sexual Maturity of Triploid Pacific Salmon

O. W. Johnson, F. M. Utter*, W. W. Dickhoff, and R. N. Iwamoto, University of Washington
* National Marine Fisheries Service

Fish from four groups of Pacific salmon which had been heat treated at fertilization to produce triploid fish, and whose ploidy levels had been monitored throughout development, were introduced into salt water. Blood samples were taken at two week intervals prior to and following the introduction of the fish into salt water, to analyze estradiol and thyroid hormone levels and to monitor ploidy. Relative sexual development of diploid and triploid individuals was also examined at regular intervals.

Significant differences in hormone levels, sexual development, and mortality were observed between diploid and triploid fish, suggesting that alternation in ploidy levels has a profound effect upon development and salt water adaptability.

Development of Biannual Spawning Behavior in a Rainbow Trout Population

Harold L. Kincaid, U.S. Fish and Wildlife Service, Kearneysville, W.V.

During December 1974, hybrid matings between males from normally fall-spawning (October) strains and females from normally winter-spawning (January) strains were produced. The F_1 progeny matured as two year fish in December, 1976 as expected. In June 1976, five females were found mature after a 6-month spawning interval. A selection and hybridization program was established that has resulted in an increased frequency of fish exhibiting the biannual spawning trait since 1976. Data from the 1980 year class through three years of age show that 96.9, 86.5, and 93.0% of the fish spawned at 2.0, 2.5, and 3.0 years of age. Fifty-eight individually identified fish were evaluated after the 2.5 year spawning period yielding 36 females (75% mature at both periods), 11 males (64% mature at both periods), and 5 fish not mature at either spawning period.

Egg quality measured in terms of egg hatchability during the May-June period has been low, ranging from 0 to 56% in 56 lots spawned in 1982. Fecundity of the fish at 2.0, 2.5, and 3.0 years was 2197, 2153, and 3623, respectively. Management implications of biannual spawning is discussed.

A Conceptual Fitness Model for Managing Pacific Salmon Fisheries: II. Application of the Model

J. E. Lannan and A. R. D. Kapuscinski, Oregon State University, Newport

Application of the model presented in the previous paper to the management of hypothetical salmon populations is demonstrated by simulation. The demonstration includes the estimation of required escapement to maintain reproductive fitness and the pre-selected levels of escapement which will result in changes in reproductive fitness of a hypothetical population. Algorithms employed in the model are briefly reviewed.

The Consequences of Maintaining Families in a Commercial Breeding Program for Atlantic Salmon

G. F. Newkirk, Dalhousie University, Halifax, Nova Scotia, and and S. Merrill-Stavostrand, IMA Aquatic Farming Ltd, Argyle Head, Nova Scotia, Canada

In a study designed to evaluate a breeding program for Atlantic salmon, *Salmo salar*, a stock establishment and evaluation program was started in a commercial hatchery. Stock was maintained as separate full sib families. The facilities were designed to hold the eggs and fry in separate tanks until the fingerlings were large enough to be given a family brand and mark. The resulting loss in growth due to this procedure has meant a serious loss in yield of 1+ smolts

compared to contemporaneous fish handled according to conventional hatchery procedures. The advantages of family structure in a breeding program is discussed in terms of control of pedigree and obtaining desirable genetic parameters. In the particular case studied it seems the disadvantages in terms of loss of production are too great and an alternative approach is recommended.

Methods and Implications of Inducing Androgenesis in Salmonids

Jim Parsons and Gary Thorgaard, Washington State University, Pullman

Heterotic effects obtained in progeny generated from crosses between inbred lines have proved to be extremely useful in agriculturally important crop species. Work with crosses between inbred lines of rainbow trout have shown similar success, but are limited due to the long process inherent in the conventional methods for production of inbred lines.

Androgenesis, production of individuals with both chromosome sets from the male parent, could provide an efficient method for the rapid generation of inbred lines. In addition, androgenesis could also be used to restore diploid individuals directly from cryopreserved sperm (sperm banks), and may help in gaining a better understanding of salmonid genetics. Androgenesis involves inactivation of the egg nucleus with radiation, fertilization with normal sperm, and subsequent blockage of the first cell division of the developing haploid embryo to produce a diploid having both chromosome sets from the male parent.

Techniques for successful inactivation of the egg nucleus have been characterized. Current work on blockage of the first cell division shows promise, but appears to be associated with high mortality rates.

Sex Control in Rainbow Trout for Mariculture in British Columbia

I. I. Solar, Ministry of Environment; E. M. Donaldson and G. A. Hunter, Department of Fisheries and Oceans, West Vancouver, British Columbia, Canada

Methods of hormonal sex control and chromosomal manipulation have been considered in the last decade as means of increasing both the quality and the quantity of cultured salmonids. In British Columbia we are studying the application of these techniques to the production of all female and sterile stocks of rainbow trout for aquaculture.

The effect of oral administration of methyltestosterone on sex reversal and sterilization of rainbow trout and the effect of heat shocks on the induction of triploid fish have been investigated. The administration of 17α-methyltestosterone at concentrations between 1 and 100 mg/kg of food for periods up to 120 days following swim-up produced male-female ratios substantially different from the control. No dose-related mortality during the period of treatment was observed.

Heat shocks were applied to rainbow trout eggs for 10 min, starting at different times after fertilization and at temperatures ranging from 24° to 30°C. Analysis of blood samples by flow cytometry revealed triploid induction ranging from 18 to 100% depending on the temperature and timing of the treatment. Survival from fertilization to 60 days and growth of the treated groups were lower than the control.

The Effects of a Broodfish Diet Fortified with Canthaxanthin in Relation to Female Fecundity and Egg Color

Larry Harris, Colorado Department of Natural Resources, Fort Collins

Abstract not available at press time.

Nutrition

The Effect of Dietary Vitamin E on the Distribution of α-Tocopherol in Rainbow Trout *(Salmo gairdneri)* During Ovarian Maturation

J. King, R. W. Hardy, and J. E. Halver, University of Washington, Seattle

Experiments were conducted to determine the effects of the presence or absence of dietary vitamin E on α-tocopherol tissue distribution in rainbow trout during egg maturation. In addition, the influence of dietary Vitamin E on ovarian development, spawning date and egg hatchability were studied. Two groups of 2 + pretreated female rainbow trout (mean initial weight 598g) were fed purified basal diets from July until spawning (January-February). Group 1 was fed a diet containing 90 mg dl-α-tocopheryl acetate/kg diet. Group 2 received α-tocopherol-free diet. At one or two month intervals, five fish from each group were sacrificed for weight measurements and tocopherol analyses. The α-tocopherol levels in plasma, liver, muscle, and eggs were determined using high pressure liquid chromatography.

Analysis of the data showed no differences in growth, prespawning mortality rate, egg development, or egg hatchability between the two dietary treatments. α-tocopherol levels, expressed on a per gram wet basis, significantly differed between treatments in all tissues only

during December. In both treatments, liver α-tocopherol levels decreased most drastically during the early months of the study. The concentration of α-tocopherol in the eggs varied at different samplings, corresponding to the absolute weight changes of the egg. α-tocopherol values in the muscle remained constant during the experimental period. Plasma α-tocopherol values reflected liver levels, but at lower concentrations. These results indicate that the fish had sufficient tissue stores of α-tocopherol at the start of the study to supply the needs of the developing ovaries without additional dietary supplementation.

Crawfish Waste—A Domestic Commercial Source of Astaxanthin

Samuel P. Meyers and Huei-Mei Chen, Louisiana State University, Baton Rouge, LA

Louisiana crawfish *(Procambarus clarkii)* heat-processed waste has been identified as a unique source of naturally-occurring biologically-active pigment based on production and availability of 30 million lbs waste/year with noteworthy pigment concentration (153 μg/g). A pilot plant has been developed for efficient pigment extraction using a vegetable or fish oil for recovery of the oil-soluble pigment. Efforts have included optimization of extraction efficiency and oil recovery, and assessment of process parameters for pending establishment of a commercial plant. Scale-up studies indicate that for every 100 lbs of crawfish waste recovered, approximately 10 lbs of astaxanthin-enriched oil (>600 ppm) can be produced. Initial projections are for a 100-250 metric ton facility. Monitoring of process parameters has allowed increase in pigment concentration to as high as 800-850 ppm. Performance trials with a variety of aquatic species (rainbow trout, coho salmon, American lobster) have demonstrated significant pigment transfer to integument and muscle.

The First Thirty Days of Feeding Salmon and Trout

R. E. Noble, Salmon/Trout Advisory Service, Olympia, Washington

The success or failure of a given population of salmon or trout can be established in the methods of incubating fry and the first 30 days of feeding. The old practice of incubating yolk sack fry without a substrate of some type and/or using too much water flow results in a swim-up fry that must overcome a penalty. Feeding procedure and care is nearly as important as the feed. At a water temperature of 10°C salmonids should gain over 200 percent of their initial starting weight in the first 30 days of feeding. It is cost effective to assign the best fish culturist on staff to care for the fry from the day the eggs hatch to 30 days post ponding. Feed levels should be at 7 to 8% body weight and the food presented at 10-to-15 minute intervals throughout the daylight hours. A start trough having a cubic capacity of approximately 50 feet with water flows of nearly 50 gpm provides the most suitable starting environment. Size variation, disease susceptibility and feeding response along with long term survival rates are all greatly influenced in the first 30 days of feeding. Poor care results in poor fish.

The Effect of Diet Protein Level, Feeding Level and the Holding Water Temperature for Rainbow Trout Broodstock on Their Growth and Reproductive Performance

D. D. Roley, University of Washington, Seattle

Two separate feeding trials were conducted using University of Washington rainbow trout broodstock to determine the effect of dietary protein level, ration size and water temperature on pre-spawning growth and reproductive performance. During the eight months prior to spawning four isocaloric diets with 27, 37, 47 or 56% protein were fed to separate groups of the 1973 brood. Four groups of the 1974 brood were reared under the cross-classified design of two water temperature profiles, cool and warm; and two ration sizes, repletion and half-repletion (as a percent of body weight per day).

The dietary protein requirement for maximum growth was between 37 and 47% of a diet containing 2.8 kcal/g metabolizable energy. Caloric utilization, food conversion and protein efficiency ratio decreased with increasing levels of dietary protein. Dietary protein level did not affect pre-spawning mortality, spawning success or the duration of spawning. Dietary protein level did not have a significant effect on the absolute or relative number of eggs spawned, relative egg size or embryo survival.

Maximum growth was achieved by feeding repletion rations under warm water conditions. Food and protein utilization for growth was optimum with repletion rations in warm water or half-repletion rations in cool water. Feeding repletion rations in warm water increased pre-spawning mortality. Water temperature or feeding level did not affect spawning success, but feeding half-repletion rations increased the duration of spawning and warm water delayed the time of spawning. Relative fecundity was increased by feeding half-repletion rations in warm water. Relative egg size was increased by feeding half-repletion rations in cool water. Warm water and feeding repletion rations had deleterious effects on embryo survival due to their effects on egg size; and ration size had an independent effect on embryo survival.

The Effects of Diet on Quality of Coastal Cutthroat Trout *(Salmo clarki clarki)* Broodstock Adults, Eggs, and Fry

Tim Unterwegner, Oregon Department of Fish and Wildlife, Clackamas, Oregon

Coastal cutthroat trout *(Salmo clarki clarki)* brood stock were fed four different diets to determine the effects of diet on quality of adult brood trout and the resulting eggs and fry. The four test diets were: Spearfish GR-3, Silvercup brood, and two formulations of Oregon Pellets. Ripe females were spawned January-March 1982 as 3-year-olds and January-March 1983 as 4-year-olds. There were significant differences ($P<0.05$) in number of eggs per female, number of eggs per mm fork length, and fry mortalities for fish spawned as 3-year-olds and differences in egg volume, number of eggs, eggs per mm fork length, and fry mortalities for fish spawned as 4-year-olds. The formal feeding trial revealed that, under the conditions of this test, the production formula of Oregon Pellets produced significantly larger fish than either Spearfish GR-3 or Silvercup, better feed conversions than Silvercup, and fewer adult mortalities than Spearfish. Standard formula Oregon Pellets contains cottonseed meal which has been shown to cause liver cancer, so we hesitate to recommend feeding it to brood stock until more research is completed.

Broodstock Management and Husbandry

Water Quality Monitoring for Salmon Culture

B. G. Daoust, Common Sensing, Inc., Bainbridge Island, Washington

Abstract not available at press time.

Some Factors Affecting the Preservation of Salmonid Spermatozoa

A. W. Erdahl, D. A. Erdahl, E. F. Graham, University of Minnesota, St. Paul

Research was undertaken to study various factors influencing the storage of fresh (non-frozen) and frozen semen of several salmonid species. The storage of fresh semen was investigated in regards to storing semen for use within one or two weeks from the time of collection. Frozen semen was investigated as a means of storing semen from year to year. Species studied included: chinook salmon *(Oncorhynchus tschawytscha)*, rainbow trout *(Salmo gairdneri)*, brown trout *(Salmo trutta fario)*, and brook trout *(Salvelinus fontinalis)*.

The diluent used in all studies was formulated according to Erdahl and Graham (1980). Non-frozen semen indicated a decreasing fertility rate with increasing storage time. Using chinook salmon semen at dilution ratios of less than 1:5 (1 part semen to 5 parts extender), fertility remained above 85% at 48 hour storage. Dilution ratios of 1:10 or above yielded fertility levels that decreased after 30 min. storage and were less than 2% fertile at 48 hours. Mean fertility values for brown trout and rainbow trout semen diluted 1:2 with extender decreased from 83 to 59% over 9 days.

Cryoprotective agents investigated included dimethyl sulfoxide (Me_2SO), ethylene glycol and glycerol. With all four species investigated, the addition of Me_2SO to the extender in concentrations less than 7% (vol/vol) resulted in fertility approximating diluted semen without cryoagent (95%). Ethylene glycol resulted in fertility rates 20% below Me_2SO and glycerol showed highly detrimental effects on sperm cells even at very low concentrations.

Frozen semen of brown trout and brook trout diluted 1:2 with 7% Me_2SO as the cryoprotective agent resulted in an average fertility of 54% although the range was from 3 to 98% fertile dependent on trial. Semen frozen in the 0.25 cc straw resulted in fertility averaging 66% compared to semen frozen in the 0.10 cc pellet which averaged 10% fertile eggs over all trials conducted. Frozen semen stored at $-79°C$ and $-196°C$ showed no significant differences in fertility.

Remote Location Hatchery Development

D. E. Pflug, W. Larrick*, L. Fortier, R. W. Beck and Associates, Seattle
* Southern Southeast Regional Aquaculture Association, Alaska

As suitable sites for fish hatcheries become harder to find near population centers, salmon aquaculturists along the coasts of the Pacific Northwest, British Columbia and Alaska will begin to look toward more remote locations as increasingly viable alternatives for future construction. The development and successful operation of aquaculture facilities at remote locations calls for a critical evaluation of site characteristics and operational factors not normally considered in such detail for facilities in more populated areas. Two major considerations involve (1) developing a dependable and efficient power source for the hatchery and residence buildings, and (2) solving the logistical problems associated with transporting fish, hatchery supplies and personnel.

In addition to these major concerns, many other components of a hatchery project must be altered to accommodate remote siting problems. Economic, biological, engineering and construction considerations must all be accounted for and integrated into the overall development

scheme to ensure a successful operation. The economics of becoming self-reliant often prove prohibitive unless innovative engineering methods are applied.

This paper provides some insight into problems associated with developing a remote salmonid hatchery facility. Development is viewed from the perspective of the biologist, the fishery manager and the engineer. A case study is examined and used to illustrate many of the points raised. The author emphasizes the importance of thorough planning and the need to integrate engineers and biologists into a single team to produce a facility capable of operating successfully over a wide range of natural and institutional conditions.

The Aquaculture Data Base: A Useful Bibliographic Retrieval System for Salmonid Literature

Carol B. Rideout, Virginia Institute of Marine Science, Gloucester Point, VA

The Aquaculture Data Base is a centralized source of bibliographic information for literature relevant to aquaculture. All species cultured in water, including salmonids, are covered. The data base is publicly available as File 112 of the DIALOG information retrieval system. Custom bibliographies are easily produced via direct, online searches or by assisted searches. Microfiche or paper copies of non-copyrighted articles from the bibliography may be obtained from the Virginia Institute of Marine Science. Copyright holders have granted permission for many of the other articles to be copied as well. Literature is collected from a wide variety of sources including professional journals and newsletters, proceedings, books, government publications, research reports and un-published papers. Data base holdings are continually expanded at a rate of 100 new entries per month.

The number of unique, useful salmonid bibliographies that may be created through searches of the Aquaculture Data Base is practically unlimited. By August 1983, 310 of approximately 10,000 documents described in the data base concerned the culture of coho salmon, *Oncorhynchus kisutch*; 221 were about chinook salmon, *Oncorhynchus tshawytscha*; 109 about sockeye salmon, *Oncorhynchus nerka*; 197 on Atlantic salmon, *Salmo salar*; and 743 concerned culture of the trout *Salmo gairdneri*.

The Timing of Ovulation and Stripping and Their Effects on the Rates of Fertilization, Eying, Hatching and Swim-up in the Rainbow Trout, *(Salmo gairdneri)*

J. R. C. Springate, N. Bromage, and J. Elliott, University of Aston, Birmingham, United Kingdom

Britain's rainbow trout farmers with only 40% of their eggs surviving as 5g. fry, need 70-100 million eggs p.a. to satisfy the annual requirements of the table fish producers. Although the eggs are ovulated under conditions of intensive culture they are not oviposited and remain in the body cavity until they are artificially stripped. During this time a process of ripening occurs and thus the timing of stripping in relation to ovulation is likely to have profound effects on the subsequent viability of the egg. Correct timing of stripping may enable significant improvements to be made in the numbers of eggs which can be fertilized.

In this study eight female rainbow trout, maintained in 10°C water, were examined daily until mature ova could be manually stripped from them (day 0). From this day onward approximately 100 eggs were stripped from each fish every other day for three weeks. Eggs from each stripping were subdivided for fertilization, eying, hatching and swim-up rate determinations, as well as for wet and dry weight and other analyses. Each batch of eggs was fertilized with the milt of two males. Blood samples were taken at each stripping for vitellogenin and steroid analyses.

The results show that the four developmental stages are closely correlated, and that poor fertilization rates are followed by reduced success at each of the subsequent developmental hurdles. The timing of stripping and fertilization after the eggs have been released into the abdominal cavity is an all important determinant of egg and fry survivals. Maximum productivity is shown to be achieved when the eggs are fertilized 5 days after they are ovulated. Ways in which this timing can be exploited commercially are discussed.

Environment and Genetics Shapes Evolution?

Abe Vanderhorst, Salmon Troller, Nanaimo, British Columbia, Canada

Can it be said that Environment plus Genetics will shape the Evolution of a species and thus that the "Oregon Problem" will continue and be felt elsewhere? In the troll fishery on the West Coast of Vancouver Island a difference in size between marked hatchery coho and unmarked and marked "wild coho" was noted.

For at least four years running (1979 to 1982 inclusive) adipose fin clipped coho salmon were always found to be much more prevalent when fishing on small coho than when fishing on large coho regardless of time of season. Work noting the relative size of all tagged fish caught and finding their origin was done. It appeared that hatchery fish from more recently constructed

hatcheries (mainly Canadian) were larger than fish from older hatcheries (mainly American).

Research and other literature were obtained to find if this had been appreciated by anyone else. Hatchery techniques of attempting to get excellent immediate returns were looked at. The creation by hatcheries of large numbers of jacks with the culling out of these jacks is suspected and questioned.

Overall with the manipulation of the salmon's early life environment by hatcheries such as the use of rapid growth feeds and temperatures, the salmon are evolving into a lesser species.

TUESDAY EVENING

1 November 1983
7:30 to 9:00
Idylwood/Marymoor

ROUNDTABLE DISCUSSIONS

The roundtable discussions will be based on the benefits and limitations of current research results applied to salmonid production. Research areas in salmonid reproduction requiring further identification and definition will be discussed. Specific topics include:

- Precocious maturation
- Sex reversal
- Dietary requirements of captive broodstocks
- Genetic studies in reproduction
- Temperature and photoperiod effects on maturation

Questions that have been raised include:

Induced ovulation, sex control, and environmental manipulation. What are the effects on the growth, physiological development, migratory behavior, and reproductive status of resultant progeny? Are one or a few generations of monitoring effects sufficient justification for the production-scale application? Should the criteria for production-scale application be adjusted for captive *vs.* ocean-going broodstock?

Genetics and reproduction. (1) Gamete banks–Have we sufficiently demonstrated that gamete banks are an effective means of preserving the genetic uniqueness of endangered strains? How can we be reasonably assured that genome changes in captive broodstock supplying progeny for enhancement are minimized? (2) Chromosomal manipulation–What may be the implication of co-mingling of polyploid individuals and gynogenetic females with "normal" diploid fish?

Nutrition. Diet is a crucial aspect of the production of salmonid broodstocks, yet the dietary requirements for larger, maturing salmonids are not fully known. What are the effects of different diets on maturation, development, disease and growth? How do dietary deficiencies affect other physiological or endocrinological research results?

DISCUSSION LEADERS

Brian Allee, Prince William Sound Regional Aquaculture Corporation, Cordova, AK

Rolland Billard, Laboratoire de Physiologie des Poisson, Institut National de la Recherche Agronomique, Jouy-en-Josas, France

Edward Brauer, Domsea Farms, Inc., Bremerton, WA

L. W. Crim, Memorial University of Newfoundland, St. John's, Canada

Walton W. Dickhoff, University of Washington, Seattle, WA

Graham Gall, University of California-Davis, Davis, CA

Ronald W. Hardy, University of Washington and National Marine Fisheries Service

Lee Harrell, National Marine Fisheries Service, Manchester, WA

William K. Hershberger, University of Washington, Seattle, WA

David Higgs, Department of Fisheries and Oceans, West Vancouver, B.C., Canada

Bill Hopley, Washington Department of Fisheries, Olympia, WA

George A. Hunter, Department of Fisheries and Oceans, West Vancouver, B.C., Canada

Robert N. Iwamoto, University of Washington, Seattle, Wa

James Lannan, Oregon State University, Newport, Or

Conrad Mahnken, National Marine Fisheries Service, Manchester, WA

William McNeil, Oregon Aqua Foods, Springfield, Or

F. K. Sandercock, Department of Fisheries and Oceans, Vancouver, B.C., Canada

Stacia Sower, University of New Hampshire, Durham, NH

Gary H. Thorgaard, Washington State University, Pullman, WA

J. E. Thorpe, Department of Agriculture and Fisheries for Scotland, Pitlochry, Perthshire,

Fred Utter, National Marine Fisheries Service, Seattle, WA

WEDNESDAY MORNING

2 November 1983
8:15 to 12:15
Idylwood/Marymoor

ENDOCRINOLOGY

Chairman
Edward M. Donaldson
Fisheries Research Branch
Department of Fisheries and Oceans
West Vancouver, British Columbia, Canada

8:30 **Sex Control in Pacific Salmon: Implications for Aquaculture and Resource Enhancement**
Edward M. Donaldson and George Hunter, Department of Fisheries and Oceans, West Vancouver, Canada

The recent development of hormonal and hormonal/genetic techniques for sex control in Pacific salmon has widened the range of management strategies available to salmon culturists and fisheries managers.

For salmonids there are two basic strategies: the production of female stocks and the production of sterile stocks. For the aquaculturist the production of females eliminates problems associated with precocious maturity in males and reduces the space required for holding broodstock. For resource enhancement the female has a higher value where the ovary is valued separately, precocious maturation is eliminated and a larger egg take can be obtained from a given hatchery escapement. This latter aspect is particularly relevant to the enhancement of endangered stocks such as the chinook.

Sterile production is also of benefit to the aquaculturist as sterile fish by definition do not undergo sexual maturation and the associated loss of market acceptance. Sterile fish also present significant options for fisheries management. Specifically we have shown that sterile fish do not undergo an anadromous migration but remain in the fishery and continue to grow. Thus the production of sterile fish is a means of redistributing fish from fisheries of low value to fisheries of high value, i.e., from terminal harvest to ocean harvest.

8:50 **Reproductive Activity of a Twice-Annually Spawning Strain of Rainbow Trout**
Katsumi Aida, Show W. Lou, Isao Hanyu, University of Tokyo; Kiyoshi Sakai, Minoru Nomura, Tokyo University of Fisheries; Mikio Tanaka, Shiro Tazaki, Fisheries Laboratory of Saitama Prefecture, Japan

A strain of rainbow trout which breeds twice a year was found at Kumagaya Branch of Fisheries laboratory of Saitama Prefecture. At present after several selections, almost all females of the strain ovulate in the ordinary breeding season from November to January and 60–70% of the females ovulate again in another breeding season from April to July. It is still uncertain why this strain can breed at intervals of about six months under almost constant temperatures and natural photoperiods. In this investigation, we studied the endocrine background of reproductive activity of this strain.

Ninety four females which ovulate in November, were tagged. Blood and a piece of ovary were repeatedly sampled for a year. Ovulation occurred from April to July (62.3%) out of 77 females and from November to January in 59 (96.7%) out of 61 females which survived.

In 48 females which ovulated from April to July, plasma estradiol-17β (E_2) began to increase in January, attaining highest values from March to May. From January to March plasma GTH of these fish was significantly higher than that of the females which failed in vitellogenesis in spring. On the other hand, in the non-vitellogenic females, plasma E_2 and GTH showed small increase from January to February, but decreased in March. Lengthening day length in spring probably inhibited GTH secretion in this group, but not in the fish which had already started the vitellogenesis by the critical period in spring.

In almost all females, synchronous increase of plasma E_2 was induced in July, probably by shortening day length. E_2 attained highest values in October and ovulation synchronously occurred in December.

Now in Japan, the spawning of rainbow trout mainly occurs from September to November as a result of the repeated selection of early spawners. The selection is probably responsible for this twice-annually spawning strain of rainbow trout.

9:05 **Endocrine and Ovarian Changes in Three Strains of Rainbow Trout Subjected to Both Constant and Seasonally Changing Photoperiods**
J. A. K. Elliott, N. Bromage, J. Springate, University of Aston, Birmingham, United Kingdom

Although it is ultimately the environment and more particularly the photoperiod that is involved in the overall control of reproduction in trout, it is now well recognised that it is changes in the hormonal milieu that directly control the sequence and timing of the different phases of

ovarian development and maturation such that spawning occurs at the most propitious time of year. However, few studies have investigated whether the spawning separation shown by different strains of salmonids are due to a response to photoperiod cues of different length or to a modification in the endocrine control. The present study investigates this question by comparing the endocrinological and histological development of three strains of rainbow trout subjected to three types of seasonal and constant photoperiod regime.

The three strains used in this study were: Caribou (November spawning), Grampian (December) and Whitebrook (January). Over a period of 28 months the same groups of fish were subjected to the following three photoperiod cycles:

(1) Constant long days (18L:6D), followed by a direct switch to short days on June 21st

(2) A compressed 6 month seasonal photoperiod

(3) A normal 12 month seasonal photoperiod, 6 months out of phase

Groups of fish from each strain were blood sampled regularly and serum assayed for estrone, estradiol-17β, testosterone, vitellogenin and calcium. Fish from the same stocks were sacrificed for histological analysis of the ovary and for determinations of GSI, HSI and mean oocyte diameter. The photoperiod was provided by flourescent lighting controlled by a 24-hour timeclock and water temperature (10°C) and feeding (0.5% bw) kept constant.

Under all photoperiod cycles female fish of the three strains showed similar sequences of endocrine changes and ovarian development. However, the spawning identity of the three strains was maintained under all regimes, although the timing of the speed of attainment of the various serum profiles was clearly modified to account for the perceived differences in spawning time. Initial increases in serum levels of estrone and estradiol-17β were followed by increases in vitellogenin, calcium and testosterone. Similar, although much reduced, changes were observed in immature fish of one strain at a time of year suggestive of a practice or dummy run, one year before the first spawning, as a rehearsal for full reproductive development.

These data demonstrate that different strains maintain their separate spawning times by responding differentially to photoperiodic cues rather than by any modification of their endocrine control. Collectively the responses suggest that the different strains respond to different numbers of daily photoperiodic stimulations, rather than photoperiodic cues of different lengths.

9:20 **Is the Poor Survival of Lake Erie Coho Salmon Eggs Due to Nutritional Deficiency of the Eggs or to Endocrine Dysfunction in the Adult?**

J. F. Leatherland and P. F. Morrison, University of Guelph; and R. A. Sonstegard, McMaster University, Hamilton, Ontario, Canada

Coho salmon (*Oncorhynchus kisutch*) were introduced into the Great Lakes in the early 1960's from stock originating in either Oregon (Lakes Michigan, Ontario, Superior and Erie) or British Columbia (Lake Ontario). In addition to the thyroid neoplasia exhibited to varying degrees by Great Lakes coho salmon, considerable interlake variation was noted in the survival of eggs to the fry stage. In order to determine whether this apparent interlake difference in survival could be attributed to differences in water conditions, husbandry methods, etc. at the different hatcheries, coho salmon eggs were collected during the peak migration of salmon from Lakes Ontario (Oregon and British Columbia stock), Erie and Michigan. They were fertilized, hardened in the local river water and transported to the University of Guelph to be reared under standardized conditions. Survival to hatch was 79%, 86%, 78%, and 24% for eggs from Lake Ontario Oregon stock, Lake Ontario British Columbia stock, Lake Michigan and Lake Erie salmon, respectively. These data generally agree with the survival values obtained by the different hatcheries, and suggest that the low survival of eggs from Lake Erie salmon is intrinsic and not due to hatchery factors. This is supported by the observation that eggs from small Lake Erie females showed a lower survival (9%) than eggs taken from larger females (26%). Total lipid, triglyceride, phospholipid and protein content were similar in eggs from the four sources of salmon, suggesting that the poor survival of the eggs was not related to nutritional deficiency. However, differences in serum gonadotropin, 17β-estradiol and testosterone levels in salmon from different Great Lakes suggest that the high mortality of the Lake Erie eggs between fertilization and hatch may be related either to a dysfunction in steroid secretion or an enhanced catabolism of gonadal steroids.

9:35 **Contribution of Ocean Released Sterile Coho to the Commercial and Sports Fishery**

George Hunter and Edward M. Donaldson, Department of Fisheries and Oceans, West Vancouver, Canada

This experiment was designed to determine whether sterile coho salmon released from a production hatchery would remain in the ocean at the time of the normal anadromous migration, remain accessible to the commercial and recreational fisheries and continue to grow beyond the normal time of death.

Approximately 39,000 coho, administered a sterilizing androgen treatment in the spring of 1979 were marked with coded wire tags and released in the spring of 1980. Contribution to both the recreational and commercial fisheries from 1980-1983 has been monitored via the Head Recovery Program. No sterile fish have returned to the hatchery. These fish have however remained in coastal waters and have contributed to both fisheries at rates comparable to normal production fish. Further, the fish have continued to grow throughout their prolonged ocean residence. Data will be presented on the spatial and temporal distribution of the catch.

9:50 **Coffee Break**

BROODSTOCK MANAGEMENT AND HUSBANDRY

Chairman
Lauren R. Donaldson
University of Washington
Seattle, Washington

10:15 **Broodstock Management and Husbandry: The Challenge**
Lauren R. Donaldson, University of Washington, Seattle

For millions of years the salmonid fishes have been reproducing successfully in the temperate waters of the northern hemisphere. The more than sixty species and countless number of racial stocks should be more than adequate proof that the fish "know how to do their thing."

In my collection of books on early fish culture is a delightful little volume, *Practical Trout Culture*, by J.H. Slack, M.D., published in 1872, the same year the first salmon hatchery was put into operation on the McCloud River, California. Dr. Slack records the early attempts to understand the reproduction of fishes. One story is particularly intriguing. It involves a Frenchman named Joseph Remy.

> Remy was a fisherman who gained his livelihood by the capture of trout in the streams of the Vorges Mountains. He noticed with regret the rapid disappearance of his favorite fishes, and being, though uneducated and ignorant, active, energetic, and persevering, devoted himself for several years to the study of their habits, especially during the spawning season. The excessive drought during the summer of 1842 favored his investigations. It was, of course, impossible for one man to keep a constant eye upon a school of fishes; nature would demand rest. Remy therefore associated with him a tavern-keeper named Gekin, who alternated with him in his observations. So earnestly were these pursued, that in one instance, during the full of the moon, a school of trout was kept constantly in view for four consecutive days and nights. The result was the discovery of the process of reproduction, which they at once put into practice. The results of their observations were kept secret until 1848 when they were reported on by a Dr. Haxo. Remy became at once a celebrity; he was invited to Paris, and the fisherman, but a few months previous utterly unknown, was an honored guest at the table of the president of the Republic.

Here we are assembled, 141 years after Remy made his great discovery, trying to understand the mystery of salmonid reproduction. Either Remy's claim to fame was greatly exaggerated or salmonid reproduction is much more complicated than many had assumed.

In this, the final session of this symposium on Broodstock Management and Husbandry, we must try to take advantage, not only of "discoveries" of Remy, but of the thousands who have worked in this area—including the papers and informative posters of this symposium—to produce more "bigger and better" fish.

10:35 **Successful Development of a Pink Salmon Broodstock Husbandry Program and Facility Design at Port San Juan in Prince William Sound, Alaska**
Brian Allee, Prince William Sound Regional Aquaculture Cooperative, Cordova, Alaska

The historical development (1975–1980) of the Port San Juan pink salmon hatchery was described by Kerns in 1980. The initial design involved broodstock being held in floating net pens in saltwater at salinities of 20–30 gms/l. This approach resulted in significant prespawning mortality and low egg viability.

Modifications each succeeding year have resulted in increased survival during maturation and improved egg viability. The present system incorporates volitional immigration from saltwater to freshwater during the maturation process. Data will be presented which summarizes maturation survival, egg viability and environmental parameters for eight specific brood years. Based upon this experience, a maturation program and facility design will be proposed for a 200 million pink and 100-million chum salmon egg capacity hatchery in Prince William Sound.

10:50 **Maturation Success of Pink and Coho Salmon Held Under Three Salinity Regimes**
Alex C. Wertheimer, Auke Bay Laboratory, National Marine Fisheries Service, Auke Bay, Alaska

Adult pink and coho salmon were held in freshwater, seawater (28–32 ppt), and estuarine (24–31 ppt, with a lower salinity surface lens) environments to determine the effects of these salinity regimes on survival to spawning and gamete viability. The salmon were captured at first entry to freshwater or in the estuary and held until they were judged fully mature or had died. Gamete viability was determined by individual matings between and within holding environments. Blood and gonadal fluid samples were taken at spawning for osmolality and potassium analysis.

Maturation success, in terms of both survival to spawning and gamete viability, was significantly reduced for both species of fish held in seawater relative to those held in freshwater. The presence of the low salinity lens in the estuarine holding environment eliminated or moderated the detrimental effects of seawater maturation. For pink salmon, there was no difference in survival or gamete viability between fish held in freshwater and estuarine environments. For coho salmon, fish held in the estuarine and seawater environments had significantly lower survival and gamete viability than those held in freshwater, but fish held in the estuarine environment had significantly higher survival to spawning then those held in seawater. Both maternal and paternal effects contributed to reduced gamete viability.

Higher average levels of blood and gonadal fluid osmolality and potassium were associated with the groups of brood fish that had lower average gamete viability. However, correlations between gamete viability of individual matings and osmolality or potassium levels of the parents were poor.

11:05 **Development of an Arctic Charr *(Salvelinus alpinus)* Broodstock**
M. H. Papst and G. E. Hopky, Department of Fisheries and Oceans, Winnipeg, Manitoba, Canada

The results of preliminary experiments in the development of a hatchery brood stock of arctic charr, *Salvelinus alpinus*, are described. The successful culture of arctic charr in a pilot commercial freshwater system and the difficulties in obtaining spawn from the wild have made the development of such a brood stock essential. The alternate use of warm (13°C) and cold (6.0°C) water, brought a group of charr to maturity in four years, compared to ten to eighteen years for natural stocks. Mature females were significantly smaller than those observed in the wild. Mature males were larger than females but smaller then mature males observed in the wild. The observed 1,769 eggs per female was lower than the mean 4,781 observed in the wild stock and the mean egg diameter of 4.9 mm was less than the 5.1 mm reported for the wild stock. The observed maturity index of 11.4% was only slightly less than the 12.8% reported for mature females in the natural stock. Mortality in eggs prior to eyeing was 75% in the cultured group, compared with 12% for the parental stock obtained as eggs from the wild and incubated under the same conditions. Time to hatching was similar for both groups. Examination of dead eggs revealed a significant number of unfertilized eggs in some of the spawned groups, while others exhibited the majority of mortalities in the cleavage and embryo stages. Survival and growth of eleutheroembryo, alevin and fry were comparable with those of stocks obtained from the wild. The occurrence of precocious maturity among intensively reared stocks was observed. Factors contributing to the poor survival of hatchery spawned arctic charr eggs and current culture experiments are discussed.

11:20 **Breeding Guidelines for Abating Inadvertent Genetic Change in Trout Hatchery Brood Stocks**
S. R. Phelps, W. B. Schill, R. C. Simon, U.S. Fish and Wildlife Service, Kearneysville, West Virginia

The importance of maintaining genetic variation in brood stocks is becoming well recognized as a key factor for successful salmonid husbandry. However, our research indicates that the loss of genetic variation in brood stocks is often rapid, resulting in inbreeding levels correlated with a decrease in fish performance in only a few generations. This rapid loss of genetic variation is mainly inadvertent and is caused by genetic drift. Inadvertent loss of genetic variation has to be minimized so that the available genetic variation can be used to produce selective gain and/or remain in the brood stock.

Recent recommendations of the numbers of brood stock fish to use for breeding to obtain an acceptable rate of genetic drift in brood stocks are based on idealized breeding conditions and are not directly applicable to fish culture operations. We have used population genetic data from trout hatchery brood stocks, test mating, and computer simulations to determine practical brood

stock spawning procedures and developed guidelines compatible with fish culture operations to minimize inadvertent loss of genetic variation.

The number of breeding adults needed to maintain an acceptable rate of genetic change depends on the type of breeding methods used and the amount of genetic variation in the brood stock. We found two breeding methods, pair mating and an egg pooling procedure, to be effective in reducing the loss of genetic variation. Population genetic data from several trout strains have indicated genetic differences between various lots taken throughout the spawning season and also year to year variation. Thus, fish for the next brood stock generation have to be taken throughout the spawning cycle. Crossing generations will reduce year to year variability. By taking the necessary steps to minimize inadvertent genetic change in a brood stock, the fish culturist need not be concerned with inbreeding from mating closely related individuals.

SYMPOSIUM WRAP UP

11:35 **Final Comments**

Robert Iwamoto, University of Washington, Seattle, and Stacia Sower, University of New Hampshire, Durham