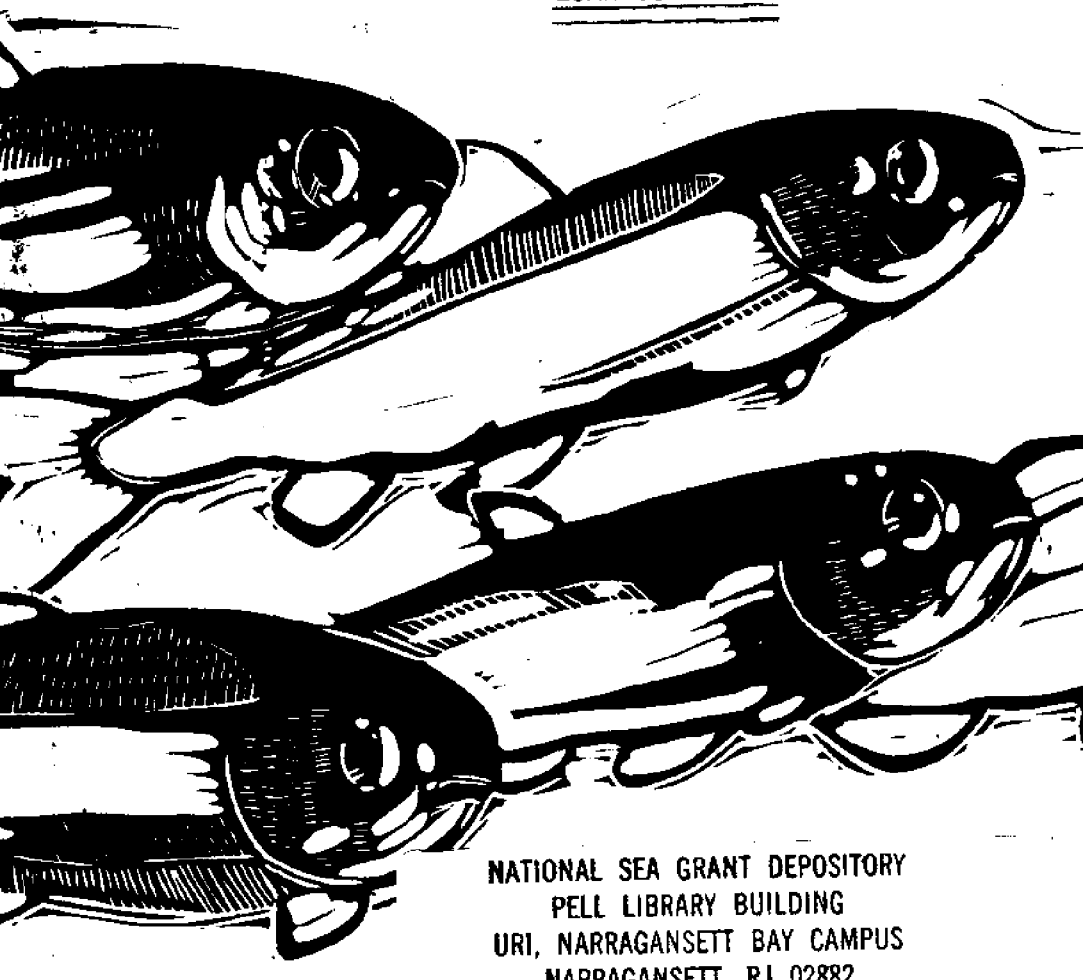
Salmonid Broodstock Maturation

Proceedings of Workshops held at Seattle, Washington
May 20-22, 1980 and March 11, 1981

Terry Nosho, Editor



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Workshop co-sponsors

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Introduction

Terry Nosho

A main objective of Washington Sea Grant's Marine Advisory Program is problem solving. Among many methods, this can be accomplished by 1) timely transfer of research findings through direct communication; 2)developing appropriate advisory publications; or 3) conducting workshops on current problems or issues which require the participation of both research and user groups to find answers. Proceedings of two workshops reported here on the problems connected with maturing salmonid broodstock serve as an example of the latter.

The following scenario provides a background for the workshops. The Marine Advisory Program was made aware that salmon farmers from Alaska to Oregon were experiencing poor survival rates with maturing broodstock with extreme variations in gamete fertility. The situation was scoped in a joint meeting that included representation from industry, University of Washington, and the National Marine Fisheries Service. Telephone calls were also made to the American Salmon Growers Association and regional aquaculture corporations in Alaska. This resulted in a 100% consensus on the need for a workshop. The problems were of sufficient magnitude that the salmon farmers agreed to provide data and hard examples of their experiences. Likewise, contacted researchers agreed to address specific aspects within their area of expertise.

The first workshop was held on May 20-22, 1980. The purpose was to provide a forum for the sharing of experiences and information much of which was not published in the open literature. More specifically, the objectives were to: 1) establish a basis from which research needs could be identified; and 2) provide state-of-the-art information on the central issues. The workshop revolved around panels consisting of researchers and salmon farmers. Panels addressed the following subjects: Experiences with returning and captive broodstock; factors influencing adult survival and maturation; gamete viability/fertility; and research priorities. The latter is reported as recommendations in this report.

Concluding the workshop it was agreed that a follow-up meeting

would be desirable in the next year. This workshop was held on March 11, 1981. The objectives were to: 1) see if salmon farmers had made improvements during the past season and, if so, how it was attained; and 2) report progress on research projects connected with maturing broodstock. Thus, a before and after scenario is reported in these proceedings.

Workshop I

SESSION I
Returning Broodstock

SESSION CHAIRMAN
Ernest O. Salo

PANELISTS

Brian J. Allee
Alex Wertheimer
C. L. Kerns
Dennis Lund
Walter D. Larrick

The Status of Saltwater Maturation of Coho Salmon (*Oncorhynchus kisutch*) at Oregon Aqua-Foods, Inc.

Brian J. Allee, Ph.D.
Weyerhaeuser Co.

Abstract

Saltwater maturation of returning coho salmon (*Oncorhynchus kisutch*) to the Oregon Aqua-Foods facility in Yaquina Bay, Oregon, has not been satisfactory. Data which summarizes results from the 1976 brood year to the 1979 brood suggests that both broodstock survival and egg fertility are below acceptable freshwater performance standards. Relevant parameters appear to be osmotic stress of maturation in the saltwater environment together with disease state and impact of stock. The female component appears to contribute to the variability in egg fertility to a greater degree than the male component, but both are statistically significant. The solution appears to be specific adaptation of coho salmon broodstock over a long time period to saltwater maturation.

Introduction

The operating mode of the Oregon Aqua-Foods ocean ranching program involves release of juveniles and recapture of returning adults at the saltwater facilities in Yaquina and Coos bays in Oregon State. The facility design at Yaquina Bay accommodates both smolts and returning broodstock in the same ponds, but at different times of the year. The operating biological assumption underlying the facility design was that saltwater maturation was feasible. The term saltwater maturation includes both survival of broodstock and production of fertile eggs. The status to date of saltwater maturation of returning coho salmon (*Oncorhynchus kisutch*) is that both broodstock survival and egg fertility are production problems. This paper is an attempt to discuss the magnitude of these problems and some of the research conducted to elucidate the factors controlling these problems.

Methods

In the 1976 brood year, Oregon Aqua-Foods adult coho salmon returned to the saltwater facility at Wright Creek, a tributary of Yaquina Bay. In subsequent brood years, Wright Creek has been phased out as a release/recapture facility so that smolts are released and adults are recaptured in saltwater on Yaquina Bay at a location called South Beach. Adult coho salmon return to South Beach from mid-August to late November. The period of maturation lasts from 50 to 100 days during which time the salinity may vary from 28-32 g/l. Time of spawning varies from year to year as the stocks of coho salmon are an admixture of Oregon coastal and Puget Sound origin. In the 1978 brood year adult coho salmon were transferred by truck in 1,900 liter smolt hauling tanks to Wright Creek, a freshwater stream, in order to complete final maturation and compare to fish which remained at South Beach. In the 1979 brood year some adult coho salmon were transferred to Wright Creek, but the majority were transferred to a ground water brood holding facility at Turner, Oregon. This facility had a constant temperature of $14^{0}C$.

Blood samples of adult coho salmon from the 1979 brood year were taken at the time of entry into South Beach and at time intervals between entry and spawning and at spawning such that comparisons could be made between saltwater and freshwater-held fish. Blood serum was analyzed for levels of sodium, Meg/1, and osmolality, mOs/kg. The former were analyzed using a Sodium/Potassium analyzer Space-Stat 30 by Orion Biomedical Division while the latter were analyzed with a Vapor Pressure Osmometer (5100B) by Wescor, Inc.

A .1 ml solution of Terramycin 100 was injected into the dorsal sinus of 40 female and 40 male coho salmon from the 1979 brood year at the Turner facility. Control groups of similar number were injected with a similar amount of saline. The duration of this test was 14 days.

All fertility data came from three sets of 100 eggs from each of 100 females. The mating design employed involved one male mating with two females.

Results

Broodstock Survival

Survival rates of female coho salmon broodstock from four distinct brood years (1976-1979) indicate no significant improvement in survival has been achieved over time in either saltwater or freshwater environments (Table 1). In the two brood years, 1976 and 1979, where com-

TABLE 1. Average survival of female coho salmon broodstock by brood year and brood holding environment (1976-1979).

Brood Year	Saltwater	Freshwater	
	South Beach	Wright Creek	Turner
1976	14%	95%	
1977	65%		
1978	60%		
1979	13%	56%	61%

parisons between saltwater and freshwater were made, it is clear that survival is higher in freshwater. Moreover, broodstock in the 1979 brood year were transferred by truck to freshwater sites at Wright Creek and Turner from the saltwater recapture site at South Beach and did not return to freshwater on their own volition as they did in the 1976 brood year at Wright Creek. Another significant point is that both brood years of very low survival (1976 and 1979) in saltwater were associated with bacterial infections: *Vibrio anguillarum* in the 1976 brood year and *Furunculosis salmonicida* in the 1979 brood year.

Since Furunculosis was identified from the 1979 brood year freshwater-held broodstock, it seems clear that this is the principle reason for generally poor survival of females in both fresh and saltwater groups. Experimental evidence supports this assumption because when broodstock were injected with Terramycin, a statistically significant improvement in survival was achieved during a 14-day period compared with a control group injected with saline solution (Table 2). It is plausible that the two lowest survival rates of adult broodstock (in 1976 and 1979) were confounded by disease and that a more typical expectation for saltwater survival would be the 60-65% values obtained in 1977 and 1978 brood years (Table 1).

TABLE 2. Efficacy of chemotherapy in coho salmon broodstock held in freshwater.

	Control	Treatment (TM-100)
Adult Mortality	33%	3%
% Infection in Survivors -- Furunculosis	66%	3%
% Egg Survival (Green-Eyed)	29%	45%

The osmoregulatory dynamics of returning coho salmon were characterized by sequentially sampling blood from males and females held in saltwater facilities compared to samples from adults transferred to freshwater facilities with the following results: saltwater-held broodstock exhibited higher serum blood sodium and osmolality levels than a cohort population transferred to freshwater (Figure 1). These differences are statistically significant (probability level $\leq .05$) between freshwater and saltwater and are independent of sex or time of entry from the ocean. Another salient point of comparison is the broad variability in blood sodium and osmolality within the saltwater-held population relative to the narrow variation in the freshwater-held group. The variation found in saltwater-held fish presumably reflects genetic adaptation to osmotic stress experienced during the maturation period.

Definite evidence that osmotic stress (i.e., high serum blood sodium or osmolality) influences broodstock survival in saltwater is not available, but a trend has been identified: mortality (numbers per week) increased precipitously following a statistically significant rise in serum blood osmolality (Figure 2). While this is clearly not a cause and effect relationship, it does suggest a biologically plausible rationale for poor broodstock survival in saltwater.

Early Entry Group: Changes in mean blood sodium and osmolality values over time of female adults held in fresh and saltwater. ± Std. Dev. Noted.

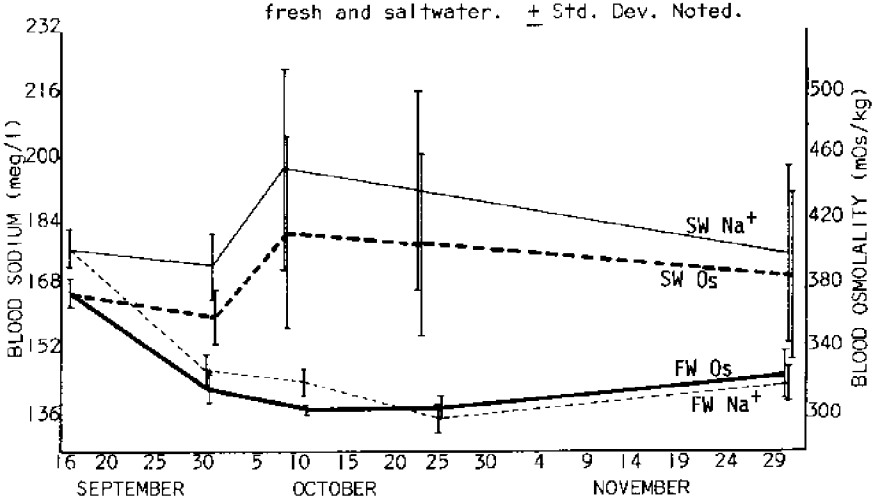


Figure 1. 1979 Brood Year Coho Salmon

Comparison of blood osmolality and mortality in salt water over time.

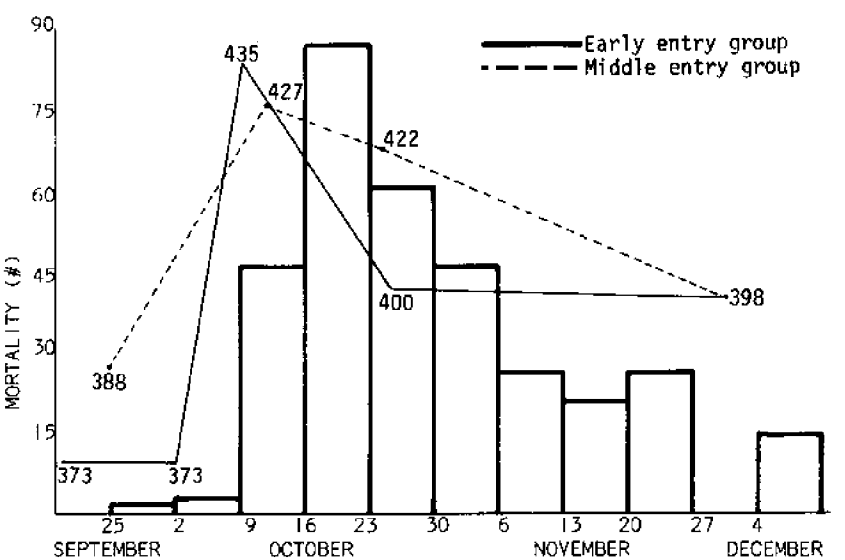


Figure 2. 1979 Brood Year Coho Salmon

Egg Viability

The average egg survival from the green to the eyed stage for production egg taking operations has been summarized in Table 3. These data suggest significantly higher egg survival from freshwater-held broodstock at Wright Creek than from saltwater-held broodstock at South Beach for both 1976 and 1979 brood years. The particularly low egg survival of 15% in the 1978 brood year was due to a lack of fertilization of eggs from the saltwater-held broodstock.

TABLE 3. Average egg survival (green-eyed) for coho salmon broodstock (1976-1979).

Brood Year	Saltwater	Freshwater	
		Wright Creek	Turner
1976	54%	88%	
1977	52%		
1978	15%		
1979	68%	74%	56%

Specific research groups of 100 separately spawned females presented in Table 4 show a higher egg fertility in fish transferred to Wright Creek than cohort broodstock which remained at the saltwater facility at South Beach for both 1978 and 1979 brood years. The exception to this involved the fish held at the Turner freshwater facility which showed no statistical differences from those fish held at South Beach. It is speculated that the constant water temperature (14°C) at the Turner facility was a major contributing factor in low egg fertility from fish at that site. It is significant to note the great variability in egg fertility observed among individual females mated to an individual male. A nested hierarchial mating design was employed to determine the relative influence of the male and female component in egg fertility. Data shown in Table 5 indicate that the female accounts for 53% to 56% of the variability in egg fertility in saltwater for the 1978 and 1979 brood years whereas the male accounts for 38% and 35% respectively. These data are remarkably similar between brood years and suggest that both males and females contribute to infertility in saltwater. In contrast to this, data from fish held in freshwater indicate that the female is the major contributor to the variability in egg fertility, and the male is not statistically significant. One might hypothesize that the principle difference between the two broodstock holding environments was osmotic stress during egg maturation. Low fertility in saltwater could be due to exposure of developing eggs and sperm to higher serum blood osmolalities in the adults and therefore higher intracellular osmolalities in the eggs and sperm. Figure 3 shows that eggs from females held in saltwater had statistically higher intracellular egg osmolalities than eggs from females held in freshwater. These data (Figure 3) also suggest that egg intracellular osmolality is lower than blood serum osmolality from the maturing female in either fresh or saltwater environments. This is supporting evidence that an osmoregulatory capability exists in the developing egg independent of the maternal environment. In the final analysis,

TABLE 4. Comparative coho salmon egg fertilities by brood year and brood holding environment.

	% Egg Fertility (7 days)			
	Brood Year 1978		Brood Year 1979	
	$\bar{X}$	Range	$\bar{X}$	Range
Saltwater				
South Beach	56%	0-100%	50%	0-97%
Freshwater				
Wright Creek	77%	0-100%	84%	
Turner (14°C)			56%	0-99%

TABLE 5. Coho salmon broodstock male vs. female influence on egg fertility.

	Brood Year 1978		Brood Year 1979	
	Female	Male	Female	Male
Saltwater				
South Beach	53%	38%	56%	35%
Freshwater				
Wright Creek	94%	0%		
Turner (14°C)			84%	8%

however, there is no correlation between serum blood sodium, osmolality of males and females held in saltwater, or intracellular egg osmolality and egg fertility.

Two other factors which influenced egg fertility include disease and stock. Regarding the former, data from Table 2 indicate that egg survival (green to eyed) was significantly (probability level $\leq .05$) improved in the Terramycin-injected group (45%) when compared to saline-injected controls (29%). Since the control population had a 66% incidence of Furunculosis, the lower egg survival seems to be a function of disease state.

The obvious effect of stock upon egg survival should not be underestimated, as is evident in Table 6. The Alsea hatchery males and females used in this mating design returned to and were held at the freshwater hatchery whereas the Green River and Siletz stocks were transferred as eggs to the Oregon Aqua-Foods hatchery in the fall of 1977 and returned as adults to saltwater in the fall of 1979. It is apparent from this data (Table 6) that the impact on egg viability by stock differences can be quite significant. This also probably explains some of the variability in egg survival between brood years.

Early Entry Group: Changes in mean blood and egg osmolality values of female adults held in fresh and saltwater. ± Std. Dev. Noted.

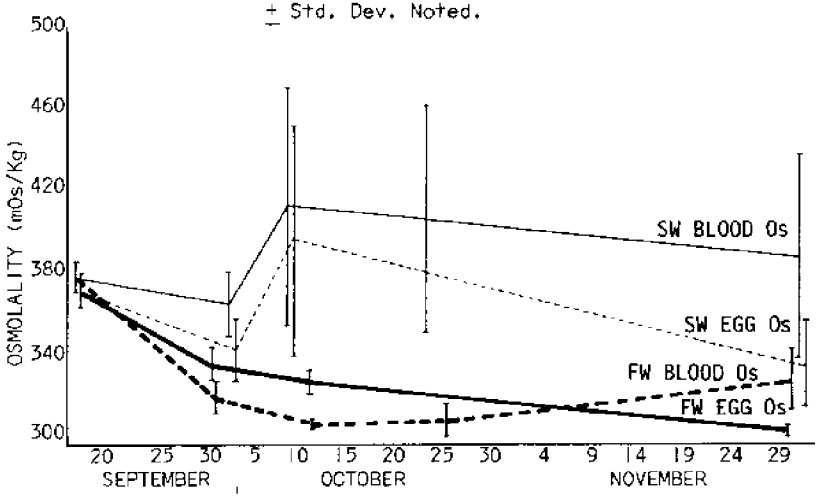


Figure 3. 1979 Brood Year Coho Salmon

Discussion

Successful maturation of coho salmon in saltwater is obviously feasible in that some individuals within a broodstock population are capable of surviving to spawn and produce viable eggs. Specifically, some females had 100% fertile eggs. This process of genetic selection will require several generations within this particular environment in order to produce broodstock performance comparable to that in freshwater.

The specific causes of prespawning mortality in saltwater are not known with certainty, but the osmotic stress of maturation in saltwater is certainly implicated, which appears to lower resistance and precipitates disease. This is particularly true of the 1979 brood year, when it appeared that the incidence of Furunculosis was the primary cause of adult mortality in freshwater and the significantly lower survival in saltwater was no doubt due to the interaction of disease and osmoregulatory stress.

The principle cause of low egg survival (green to eyed stage) in saltwater-held broodstock is lack of fertilization. The hypothesis that osmotic stress in saltwater broodstock produced poor fertility must be rejected as no statistically significant correlation exists between these two variables. In saltwater-held broodstock the female component explains a greater amount of variability in egg fertility than the male, although both are significant contributors. Other factors contributing to lower egg survival (green to eyed stage) include disease state, stock of brood fish, and method of fertilization. The latter factor in our own work has shown a decrease in egg fertility by 17% when fertilization of gametes is delayed by 5 hours.

TABLE 6. Comparative egg survival of three coho salmon stocks (green to eyed) 1979 brood year.

Males	Females		
	Alsea	Green River	Siletz
Alsea	89.4*	54.1	50.9
Green River	81.8	40.4	58.2
Siletz	85.0	56.5	49.5

* Immediate fertilization

There appears to be no simple solution to the successful saltwater maturation of coho salmon broodstock. Some aspects of the problem are better understood based upon research conducted to date; however, the independent variables controlling the dependent variables, broodstock survival and egg fertility in saltwater, have not been defined. Whether some multiple hormonal control system is inhibited or blocked when sexually maturing coho salmon are exposed to saltwater and this delicate mechanism is responsible for poor fertility is open for speculation at present. In my opinion, this is an area of research that should be investigated.

The solution to the problem of saltwater maturation of returning coho salmon is fundamentally long term and will require a concerted effort on the part of industry, university, and state and federal research institutions along the Pacific coast.

Use of Estuarine Netpens for Holding Returning Broodstock

Alex Wertheimer
National Marine Fisheries Service

At the National Marine Fisheries Service Research Station at Little Port Walter, Alaska, we have used estuarine netpens to hold returning brood fish when our freshwater holding capabilities have not been adequate to meet egg-take requirements. As the data shown below demonstrates, the technique has worked well for coho salmon but not for sockeye salmon.

Little Port Walter is located in Southeastern Alaska, on the east coast of Baranof Island. The bay opens onto Chatham Straits. Salinities in Chatham are 31-32 ppt at the surface. Within the bay, however, a distinct halocline occurs, with a low salinity lens of 0-10 ppt at the surface. The depth of the lens varies from a few centimeters to 1 m, depending on the amount of freshwater run-off from the watershed. Below the halocline, salinities are 28-32 ppt. Temperatures during the broodstock holding period average 6-14 C above the halocline, and 8-12 C below the halocline.

The netpens consist of 6-9 mm stretch mesh nylon nets suspended from float frames. A variety of net sizes have been used, ranging from 3.5 m by 3.5 m by 3 m deep to 4 m by 7 m by 5 m deep. A cover net is used to exclude predators. The netpens are anchored in 15-20 m of water off the mouth of the stream to which the brood fish return. Returning coho salmon held in netpens have been captured at weirs in the stream. The sockeye salmon held were captured in the estuary off the mouth of their natal stream. Following capture, the fish were transferred in fiberglass tubs in skiffs to the netpens.

Coho broodstock held in netpens have shown good survival to spawning. In 1977, 123 of 126 females (98%) and 100 of 100 males held were spawned (Table 1). One of the females died in the net; the other two lost were fish judged ripe and killed while still green. We generally spawn 80-90% of the females held in our freshwater holding pond. The higher survival in the netpens is attributed to reduced *Saprolegnia* infestation of the brood fish relative to fish held in freshwater. Survival of the eggs taken from coho ripened in the

estuarine pens in 1977 was 96% to the eyed stage of development (Table 2).

Survival of returning sockeye salmon held in estuarine pens was lower: 28% of the females and 14% of the males held were spawned (Table 1). The holding period was 68-90 days, considerably longer than the 4-35 day period for coho. Viability of the gametes from sockeye that did survive to spawning was good with 95% of the eggs taken surviving to the eyed stage (Table 2). As this is the only time we have ripened sockeye broodstock at Little Port Walter, there are no data from freshwater to compare with these survival rates.

TABLE 1: Survival to spawning of returning coho and sockeye salmon held in estuarine netpens at Little Port Walter, Alaska

Species	Sex	Year	Holding Period (days)	Number Held	Number Spawned	Percent Spawned
Coho	Males	1977	4-35	100	100	100
	Females	1977	4-35	126	123	98
Sockeye	Males	1973	68-90	46	8	17
	Females	1973	68-90	50	14	28

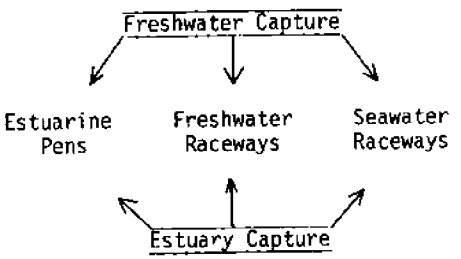
TABLE 2: Survival to the eyed-stage of eggs taken from coho and sockeye salmon ripened in estuarine pens at Little Port Walter.

Species	Year	Number of Eggs Taken	Number of Eyed-Eggs	Percent Survival
Coho	1977	480,800	462,900	96
Sockeye	1973	40,000	37,800	95

The viability of gametes from both sockeye and coho brood fish that survived to spawning indicate there is no physiological barrier to the complete reproductive maturation in the estuarine environment. Two factors may have contributed to the successful maturation of coho at Little Port Walter versus the results of Ore-Aqua with coho in seawater raceways. One of these factors is the presence of the halocline in the Little Port Walter estuary, allowing the brood fish some access to low salinity water; the other factor is that the coho were captured after entering freshwater, then transferred back to the estuary. Access to low-salinity water may be important for osmoregulation during the final stages of maturation; exposure or entry into freshwater could trigger processes for completing maturation. We have planned an experiment this fall at Little Port Walter that will attempt to isolate these factors (Figure 1). Returning coho, pink, and chum salmon will be captured in the estuary by purse seining and at a weir in freshwater. Experimental groups will be held in seawater raceways in which no halocline will be present, in estuarine netpens, and in freshwater raceways. Maturation success will be measured in terms

of the percentage of fish surviving to spawning and the viability of the gametes from fish that do survive.

FIGURE 1. Experimental design to determine the effects of (1) freshwater versus estrary capture and (2) the salinity profile of the holding environment on the maturation and gamete viability or returning broodstock.



Pre-spawning Mortality of Pink Salmon Matured in Salt Water
The Prince William Sound Aquaculture Corporation Experiences 1975-1979

Curtis L. Kerns
Prince William Sound Aquaculture Corporation

It has been the experience of the Prince William Sound Aquaculture Corporation (PWSAC) that significant pre-spawning mortalities of maturing pink salmon *Oncorhynchus gorbuscha* can be expected with close, extended holding in salt water. The stress of the process appears to be non-sex specific and additive. Mortalities build gradually, reaching the maximum rate just before maturation. For each day of close confinement a 1 percent mortality can be expected. The disturbances and handling inherent with extended confinement, coupled with high water temperatures (10C plus) can have severe consequences. Pink salmon allowed to swim unimpeded until shortly before maturation in moderate water temperatures, may, however, be held at high densities for short periods of time with minimal prespawning losses. To minimize mortalities efforts must be made at every stage--capture, maturing, and spawning--to eliminate stressful practices.

Capture

Capture should present scant difficulty, especially where a seine is used. If the netting is secured to a hinged, open-ended transport pen, typically a surface disturbance behind the fish is sufficient to drive them rapidly foreward, clearing the net. Brailing, excessive fish concentrations, and lifting fish above the water are to be avoided.

Transport

Fish are seldom captured at the same location as the holding pens, hence some movement is often required. Transport presents at least one severe problem, excessive water velocities within the pen. Even with very low propelling vessel speeds and fish densities, water velocities within the pen can easily reach an order of magnitude greater than those exterior to the pen. The fish must either maintain very high swimming speeds or suffer the ill effects of piling up to the rear of the pen.

The physiological consequences of both options are familiar to all. Care must be taken in the selection of transport pen construction materials as well. Chicken wire or other such sharp edged materials can cause skin lacerations and exasperate osmoregulatory problems inherent in saltwater maturing. Essentially a closed bow displacement hulled transporter with plastic coated wire on the other four sides is called for.

Confinement

Extended close holding should be avoided. Fish captured in a nearly mature state require a minimum amount of sorting and handling. They become mature at nearly the same time. Extended confinement of fish captured at the same time, perhaps from the same school, results in a lengthy spawning period. Multiple sortings are inevitable. Statistically we may be able to determine many parameters about a population and yet know very little about specific individuals. A system of low density confinement in large water volumes allowing for volitional movement which in effect presorts is highly desirable.

Spawning

When taking large numbers of eggs, procedures must be simplified. Gentle handling coupled with accurate sorting appear to be the key factors. Bleeding is not necessary; a small amount of blood is not harmful. Ovarian fluids, have been demonstrated to aid in fertilization. Ruptured eggs are to be avoided. Dry spawning is efficacious for gametes removed a short period before fertilization occurs. Water hardening within the incubator reduces handling and is biologically quite satisfactory. Fertilization checks should be made on every lot of eggs to assess procedures and personnel performance.

During the past five years, PWSAC has held for spawning in excess of 110,000 pink salmon which produced over 100 million eggs and has resulted in approximately a one million adult fish return. The lessons learned have been reviewed or indicated in this paper.

PRINCE WILLIAM SOUND AQUACULTURE CORP.

PINK SALMON MATURATION MORTALITIES

1975 - 1979

Year	Salinity $^{o}/_{oo}$	T (^{o}C)	Holding Density (lbs/m^3)	Holding Duration (days)	Mortalities (%)	Comments	Survival Green to Eyed (%)
1975	5	10-14	48.0	1-2	6.02	Remote site egg take Main cause of mortality due to entanglement in netting of lower jaw	63.90
1976	1	7-14	53.2	1-2	2.74	Remote site egg take	77.00
1977	22-30	14-16	133.1	5	6.10	Hatchery egg take	76.00
1978	22-30	10-14	110.5	25-35	31.15	Hatchery egg take	90.44
1979	22-30	12-16	88.7	30-40	41.56	Hatchery egg take	80.00

Sheldon Jackson College Pink Salmon Broodstock Experience

Dennis Lund
Sheldon Jackson College
Sitka, Alaska

Sheldon Jackson College has operated a private pink and chum salmon hatchery at Sitka, Alaska since 1975. Maximum egg take has been 11 million pink salmon and 600,000 chum. The college ripens and spawns all the fish at a freshwater trapping and holding facility 100 meters upstream from the mean low tide level. Pink salmon ripen within 7 days after entering the weir. Fish are sorted daily from the trap. Unripe fish are held in 6' x 16' x 2' deep wire mesh pens, 800 per pen. Subsequent sorting is done at 3-5 day intervals. Fully ripe females could be held up to 11 additional days with no apparent reduction in egg fertility. Fertilization has ranged from 95 to 99% at water temperatures from 10° to 16° C. Prespawning mortality is less than 1%. Females are not bled prior to spawning.

In 1978 chum salmon were seined in seawater ½ mile off Nakwasina River and towed at 1-2 knots, 14 miles to the hatchery. Approximately 25% of the fish were shiney bright with no evidence of water marking. All the chums were transferred to freshwater. Of 543 chums seined, 11% died prior to spawning. The mortalities were mostly males which had been handled excessively in disintangling them from the seine. Females killed with unripe eggs were 1½%. Fertilization was estimated at 95%. Thus, viable egg yield was satisfactory in chums forced prematurely into freshwater even though some fish were held up to 22 days before ripening.

There appears to be little problem with prespawning mortality or fertilization in pink and chum salmon trapped in freshwater and either held in freshwater or moved back to seawater for ripening, provided the fish were fully committed to freshwater at the time of capture. However it is necessary to harvest for sale 80% or more of the fish schooling offshore up to 30 days prior to their natural stream entry. This dictates capture and penning of fish needed for broodstock at that time to avoid over-harvest. This often results in heavy

broodstock mortality and/or poor fertilization. The lack of suitable freshwater holding areas at many Alaskan hatchery sites also often requires estuarine trapping and holding.

Another problem may face salmon ranchers in Alaska in the future. Many good hatchery sites in Southeast Alaska have lake water sources which may become very warm during broodstock handling and spawning. More data is necessary on temperature and salinity thresholds for broodstock ripening, fertilization, and early embryonic development.

An additional concern of Alaskan salmon ranchers is the tendency to select early run fish for broodstock in order to allow maximum harvest of top quality salmon. This problem is particularly acute where broodstock are penned early in the run. The effects of this selection on subsequent run timing and the genetic diversity of the hatchery stock needs further study.

Summary of the 1979 Carroll River Summer Chum Broodstock Collection

Walter D. Larrick and Ward Griffioen
Southern Southeast Alaska Regional Aquaculture Corporation

Southern Southeast Regional Aquaculture Association is in the process of developing its hatchery program through obtaining broodstock from "wild" native Alaskan stocks. In doing so, SSRAA has encountered various situations associated with the collection, spawning, and hatching of "wild" eggs.

Carroll River is the primary source of summer chum eggs for the hatchery's broodstock and production. It is located at the head of Carroll Inlet, which is on Revillagigedo Island in southern Southeast Alaska. Carroll River has an intertidal zone of approximately 2.6 kilometers and a usable river length of 6.4 kilometers. The major species include an average run of 30,000 pink salmon per year and an average run of 10,000 chum salmon per year. The river also supports populations of coho and chinook salmon. The run timing of the chum salmon is mid-July to mid-September. The hatchery's desired egg take goal was 11.5 million eggs or 4,600 adult females from Carroll River.

The remote field operation consisted of six 20' x 20' x 20' floating netpens for holding the adults until maturation and spawning. The netpen floats were anchored as close to the river mouth as possible to obtain a freshwater influence on the adults held in the pens.

The majority of the adult chum salmon were captured through the use of purse seines by commercial seine boats. Other methods used were beach seining on the tidal flats and seining in holding areas in the river. The use of seine boats proved to be the most effective method of capture because of the numbers of adults necessary to supply our egg needs.

Capture of adults began on July 31, 1979, and continued through August 24, 1979, at which time the fish became too dispersed to capture in sufficient numbers. The fish were placed in the pens when captured and sorted according to sex a day later. Periodically, the fish were crowded and sampled for ripeness. Between August 13, 1979 and September 4, 1979 there were ten days on which the fish were spawned.

Chum salmon were held in netpens an average of thirteen days prior to spawning. The salinity ranged from a low of 10 ppt on the surface to 27 ppt five meters down in the pens. The fish seemed to stay in the upper portion of the pens where salinities averaged approximately 20 ppt. The average water temperature of the top three meters was 16.2° C and ranged from 14° C to 18° C (Table 1).

The estimated total chum salmon escapement to Carroll River was 18,000 with SSRAA removing 3,634 adult fish for spawning purposes. A total of 2,434 females were captured and placed in the netpens. Of this number, only 1,660 or 68% were successfully spawned, fertilized and placed into Heath tray incubators at the hatchery. The remaining 774 females died in the netpens or were eliminated because their eggs were unsuitable for fertilization (Table 2). The males held in the netpens experienced similar results, with a 30% mortality before spawning.

A total of 4,374,000 eggs were taken and flown unfertilized to the hatchery where the fertilization process took place. During incubation, a large number of dead eggs were noticed and a subsample was taken to estimate the stage at which the eggs were dying and the percent of fertilized eggs.

The results of this subsample indicated a very low, 45.6%, fertilization rate. The eggs were shocked and picked off at eyed-egg stage, with a 32.6% survival from green egg (Table 3). From all indications, the majority of the eggs were not fertilized. The resultant chum fry were ponded in late December, 1979 and were reared in freshwater at the hatchery until mid-March, 1980. The fry were transferred by barge to remote saltwater netpens for continued rearing. These chum were reared in saltwater until May 6, 1980. The overall survival from green egg to release was 30.7%, with a survival from eyed-egg stage to release of 94% (Table 3).

The factors affecting the netpen mortalities and the large numbers of unfertilized eggs were assumed to be a combination of the following:

(1) Broodstock holding in saltwater
(2) Duration of time in which fish were in captivity
(3) Broodstock holding in temperatures over 15° C
(4) Capture of adults prior to entry of freshwater stream.

Southern Southeast Regional Aquaculture Association would like to extend a special thanks to Dr. Frank Velsen, Department of Fisheries and the Environment, Fisheries and Marine Services, British Columbia, Canada, for his assistance in this project. In addition, SSRAA would like to thank James Wood of the Washington Department of Fisheries and Dr. William McNeil, Oregon Aqua-Foods, Inc., for their thoughts and discussion on the events that took place.

TABLE 1: Summary of physical conditions associated with Carroll River summer chum spawning, 1979

	LOW	HIGH	MEAN
Air Temperature at Floats C°	14°	22°	18°
Water Temperature at Floats C°	14°	18°	16.2°
Salinity of Water in the Netpens	10 ppt	27 ppt	20 ppt
Time from Spawning to Fertilization (hrs)	6.15	8.55	7.06
Temperature of Milt at Fertilization	5°	16°	10.3°
Temperature of Eggs at Fertilization	10.5°	16.5°	13.4°
Water Temperature at Hatchery	10°	12.5°	10.5°

TABLE 2: Summary of Carroll River summer chum spawning data, 1979

Total Females Captured	2,434
Females Spawned	1,660
Females with Bad Eggs	204
Female Netpen Mortality	570
Average Weight of Females	4.45 kg
Average Length of Females	68.4 cm
Total Eggs Taken	4,374,000
Mean Fecundity	2,635

TABLE 3: Summary of survival of Carroll River summer chum broodstock collection

Total Eggs Taken	4,374,000
Survival to Eyed-Egg Stage	1,426,000
Estimated Percent Fertilization	45.6%
Percent Survival to Eyed-Egg Stage	32.6%
Percent Survival from Eyed-Egg to Swim-Up	96.9%
Percent Survival From Swim-Up to Saltwater Rearing	97.5%
Percent Survival from Saltwater Rearing to Release	99.5%
Percent Survival from Eyed-Egg Stage to Release	94.0%
Total Number Released and Percent Survival	1,343,000 30.7%

Workshop I

SESSION II
Captive Broodstock

SESSION CHAIRMAN
Lee Harrell

PANELISTS

Carlin McCauley
Paul L. Hickey
James L. Mighell
William Waknitz
James Gearheard

DOMSEA Farms
Coho Broodstock Program

Carlin McAuley
DOMSEA Farms

Being a private enterprise, Domsea Farms has a real need for developing a domestic broodstock. The two main reasons for doing so are: 1) to provide a stock of coho specifically suited for survival and growth in intensive net pen culture and 2) establish a secure and reliable source of eggs. To accomplish this goal Domsea entered into a cooperative venture with the University of Washington in the Fall of 1977 to develop such a broodstock.

Two successive year classes of F1 generation brood coho were spawned in December of 1977 and 1978 using a nested design (1 male to 2 females). From this, 40 families ('77 and '78 year class) were ponded and reared in 15^{0}C water for 5 months. To allow for more rapid genetic selection the brood program was set up to run on a 2 year spawning cycle by accelerating growth in the freshwater phase to produce zero age smolts. At the end of this 5 month period each crop was sample weighted, graded ($\leq$10gms stayed in freshwater), cold branded for family identification, and transferred to saltwater (July 1978 and 1979). After 4 and 8 months in saltwater the broods were again inventoried and sample weighted. A selection index was devised and the top 15 families isolated, during the 8 month inventory. From these, members of the top 10 families were mated utilizing a rotation line crossing scheme.

At present we have completed the odd year cycle and are 3/4 of the way through the even year. The progeny (F3's) of the odd year class (ponded 3 months ago) were inventoried in April 1980 and at that point showed a 40% increase in size over the parental generation, based on comparable temperature unit accumulations.

While this program has run along fairly smoothly it

has had some definite problems, especially in the later stages of adult maturation. During the 17 months that the '77 brood were in saltwater mortality was generally 1%/month, until the last 5 months prior to spawning when it increased to 10-15%/month. The initial rise in mortality was attributed to furunculosis and vibrio; the tail-end to normal pre-spawn mortality. What was responsible for the high mortality in between was not determined. In the end only 23% of the broods transferred to saltwater survived to maturity. This number would have been higher (37% survival) but for the fact that a substantial number of small non-smolts were included in the transfer to saltwater.

Another problem that became apparent at spawning time (it was expected) was the low (20%) percentage of females in the population. This is bad news from a commercial operation standpoint as the large number of excess males takes up a lot of valuable pen space.

The final blow came after spawning. Despite good looking spawners and eggs, a large percentage of the eggs turned up blank. At eye-up only 45% of the 700,000 eggs taken were viable. This was further reduced to 30% at ponding time. Poor fertilization was suspected as the cause.

In addition to the problems, there have been some plusses. Despite the high adult mortality and low percentage of females, the first completed cycle produced 700,000 eggs. Two to three million eggs are projected for this year's take. The spawning adults were 50% larger ($\bar{x}$= 3 lbs) than any of their predecessors, and fecundity was 50% higher.

From our experiences over the past 2½ years, it has become obvious that there is much to be learned about the husbandry of coho salmon broodstock, especially maturing adults. We don't know, for instance, how densely they can be reared in net pens; or what special nutritional requirements they have nor when to satisfy these requirements. We don't even know for sure if they can be successfully matured and spawned in a saltwater environment. We do know, however, that these are areas that require attention in order to establish and secure a viable, successful domesticated broodstock.

Broodstock Selection Program At Aquasea Farms, Inc.

Paul L. Hickey
Aquasea Farms, Inc.

Aquasea Farms, Inc. began raising coho salmon commercially in 1976. By the fall of 1978, the company had set aside 415 of the largest fish for broodstock.

The first fish were spawned on December 1, 1978. During the next month and a half we spawned 229 females (55.2%), and 186 males (44.8%) at the saltwater site. All eggs were treated with erythromycin for 45 minutes during the water-hardening process. The water-hardened eggs were shipped to 2 private hatcheries that were 5 and 7 hours distance from the pen site.

Initial egg mortality was between 20 and 25% at each hatchery. Survival to hatching averaged about 21%. We attributed the low survival to the transport and handling of the eggs, and to holding the broodstock in saltwater up to the spawning date. At one of the hatcheries, eight families were kept separate as potential broodstock. Egg survival to hatching among these groups ranged from 3.5 to 99%. All of the smolts from this year class have been introduced into saltwater as yearlings.

In October of 1979, about two weeks before spawning was to begin, a massive *Chaetoserous* bloom swept through the pen site killing an estimated 21% of the total number of fish, including 40% of the broodstock. The average size of the broodstock at this time was 4.2 pounds. In order to replenish the depleted egg supply, 100 of the largest females from among the production fish were incorporated into the broodstock pool. These fish averaged 1.7 pounds each.

Between November, 1979 and December, 1980, we spawned 201 females (52.8%) and 180 males (47.2%) at the saltwater site. Erythromycin was again used to disinfect the eggs during the water-hardening process. Transport time to the company's small hatchery on Orcas Island was between 1 and 3 hours. The eggs from each female were kept separate so that family performance could be monitored. Initial egg mortality was about 2%, while mortality after shocking varied from 90% to less than 1 %.

An unfortunate circumstance occurred during incubation. The thermostat controlling the upper temperature limit on the water heater malfunctioned one night, and the water temperature rose to about 58°F for an undetermined amount of time. For the next few days, the eggs were checked for mortalities. Very few were found. At hatching, we found that eggs which had accumulated at least 330 temperature units before the shock occurred, hatched normally. A large number of eggs that had not accumulated this number of T.U.'s hatched either head first, or ruptured prior to hatching, releasing a thin stream of yolk.

Broodstock Selection Program

The broodstock selection program at Aquasea Farms, Inc. is based on a scoring system that measures the performance of potential broodstock families for each of four traits: percent hatch, egg size, spawning date, and saltwater growth to 1 pound. The first three traits are used to select 16 families while the fish are still in the incubation trays. These families are kept separate until the fish can be branded. The last trait is included in the selection index at the time the fish reach harvest size to determine which families are ultimately to be kept as broodstock.

Broodstock Diet

Our broodstock are fed the same diet that is fed to the production fish approaching harvest size. During the last three months before spawning begins, we feed a supplemental diet of arctic krill.

Observation on Non-Native Broodstock Reared in Netpens In Puget Sound, Washington

James L. Mighell
National Marine Fisheries Service

Introduction

In the early part of the 1970's a resurgence of interest in salmonid aquaculture occurred on the Pacific Coast of North America. Pen rearing techniques were developed largely through the efforts of biologists at the NWAFC in Seattle. Nearly all species of Pacific salmon native to the Pacific Coast as well as steelhead and rainbow trout were reared in nylon open-mesh netpens for production of pan-sized salmon to be sold in the fresh and frozen fish market. As the supply of eggs and fish for rearing in netpens became periodically uncertain, there was interest in holding some fish to maturation to provide a source of eggs. Captive broodstocks have been only partly successful due to a variety of factors affecting survival and egg viability.

Almost all of the broodstock trials have been conducted with native stocks such as coho and chinook salmon and rainbow (steelhead) trout, with coho salmon being the most successful. However, beginning in 1971 the National Marine Fisheries Service conducted pilot tests on the rearing of several exotic (non-native) broodstocks. The species tested were: masu (cherry) salmon, *O. masou*; chinook x masu hybrid; pink x masu hybrid: and Atlantic salmon, *Salmo salar*.

Methods and Materials

The four species were reared from egg to smolt in freshwater in Heath incubators and 4' diameter circular fiberglass fish tanks. Dechlorinated city water (Seattle) was provided at a depth of about 1 foot, with oxygen at or near saturation value and total ammonia nitrogen at less than 1 ppm. pH was between 6.4 and 6.9. Saltwater rearing was in netpens with mesh sizes between ¼" and 1" and volumes of 200 ft^3 -- 2560 ft^3. Saltwater rearing was at Clam Bay, near

Manchester, Washington on Puget Sound. The fish were fed a basic diet of Oregon Moist Pellet formulation (OMP) throughout their life span; some stocks were fed supplemental rations of shrimp, whole herring, krill, or added oil at various times as noted later. All feeding was by hand at a modified demand basis; that is, 4 times daily until active feeding response ceased. An exception was that Atlantic salmon were fed fixed rations of about 0.5-1.0% of body wt/day during the last 10 months of the 30-month saltwater rearing period.

Results

General results of the trial brood rearing are summarized in Table 1. Masu salmon and Atlantic salmon "smolted" only at an age of 1+ years, while the two hybrids of masu smolted readily at an age of about 7 months. Size of the smolts was 15 grams or over for the yearling smolts and only 6 grams or over for the sub-yearling smolts. Despite their small size at seawater entry, the sub-yearling smolts had excellent growth rates and rapidly caught up to coho salmon smolts that entered saltwater at a much larger size. At maturity, however, there were great differences in sizes between the various species, partly due to the length of saltwater rearing. Masu salmon were a maximum size of only about 1.25 lbs after 1.5 years in saltwater, while Atlantic salmon reached a size of nearly 20 lbs after 3.5 years of saltwater rearing.

The two hybrids of masu salmon, chinook female x masu male and pink female x masu male, were held to maturity in netpens to assess their survival, longevity, and gamete viability. The chinook x masu hybrid matured at 4, 5, or 6 years of age. About 80% reached an age of 4 years. Their average weight was 1.3-2.3 kg (3-5 lbs) at 4 years. Their gamete production was practically nil, being essentially infertile. Sperm production was nearly nonexistent, and only about 3-10 eggs were produced by females. The fish exhibited only slight secondary sexual coloration changes associated with spawning.

The pink x masu hybrid, though, lived to an age of 3 years (females) while the males lived to 2 years of age; a few of undetermined sex lived to start their fourth year of life before being lost to poachers. Their low survival (15%) was mostly the result of an epizootic of kidney disease (BKD). The fish ranged from about 0.9-2.3 kg (2-5 lbs) at maturity. Males spawned at 2 years of age and had normal quantities of sperm. An attempt to backcross with pink eggs was unsuccessful. Females matured at 3 years of age and produced an average of about 600 eggs each. A backcross with pink sperm was again unsuccessful.

Atlantic salmon first matured after 2.5 years in saltwater at an average size of about 2.7-5.4 kg (6-12 lbs). About 40-60% of the available females matured at 4 years of age and produced an average of about 5,200 eggs. Their diet was variable, some lots received only OMP while others received additions of soybean or salmon oil, and/or shrimp, or krill. Survival from smolt to maturity was generally over 80%.

The masu salmon females averaged 31.2 cm in fork length and 380.7 g in weight, and produced an average of 369 eggs. The egg survival to hatching ranged from 23.4-99.1%, with an average of 85.3%. Regression analysis showed a positive correlation ($r = .657$) at the 95% confidence level for size vs. egg production.

Regression analysis of Atlantic salmon egg production data showed a positive correlation at the 99.9% level ($r = .842$) for the 1978 brood year (saltwater reared), 90% level ($r = .407$) for the 1979 brood (saltwater reared), and 99.9% level ($r = .751$) for the 1979 brood held to maturity in freshwater.

The 1978 spawning stock, fed a ration of .75-1.25% of their body weight per day with no supplemental feeding other than a 4% addition of soy oil and replacement Vitamin C (.04%) produced yellow colored eggs ranging in numbers from 2,500-8,600 per female ($\bar{X}$ = 5,623). The 1979 spawning stock, fed at .50-.75% of their body weight with supplemental (10%) feeding of krill (*Euphausia superba*) and 4% additional of salmon oil and Vitamin C, produced orange to deep orange eggs ranging in numbers from 2,300-10,700 ($\bar{X}$ = 4,773). Also, in 1979, 5 year old females produced an average of 6,079 eggs. The 5 year olds received no supplements, and consequently the eggs were yellow in color.

A small number of 4 year old females held in freshwater and fed OMP only, produced yellow colored eggs numbering 400-2,300 per female. Those fish ranged from about 40-52 cm in length and 0.4-1.6 kg (1-4 lbs) in weight.

Survival of the 1978 spawned eggs was highest from adults returned to freshwater before spawning. The time of exposure to freshwater ranged from 18 hrs to 1.5 weeks ($\bar{X}$ = ± 64 hrs). Over 60% of the freshwater held spawning pairs produced eggs that had a survival rate of 80% ($\bar{X}$ = 80.5%) or better to eyeing, while 88% of the spawning pairs that were spawned directly from saltwater had less than 80% ($\bar{X}$ = 50.4%) survival. In 1979, all females were brought to freshwater before spawning and their eyed egg survival rates were generally above 80% with most above 90% ($\bar{X}$ = 88.0%). The higher overall survival of the 1979 eggs was attributed to the addition of krill in the diet. With one exception, egg survival values lower than 80% were from females that had not received krill.

TABLE 1. Summary of results of rearing four broodstock species in saltwater netpens in Puget Sound.

SPECIES	SMOLT AGE	SMOLT SIZE	S.W. REARING TO MATURITY	DIET	SURVIVAL TO MATURITY	SIZE AT MATURITY	APPROXIMATE FECUNDITY	SPAWNING MONTH
Cherry (Masu) Salmon (*O. masou*)	1 + year	15 + g	1 + year	OMP	90%	.75 lbs	300	Sept.
Chinook x Masu Hybrid (*O. tchawytscha* x *O. masou*)	6-7 months	6 + g	2 + year	OMP + Shrimp	80%	3-5 lbs	10	Nov.
Pink x Masu Hybrid (*O. gorbuscha* x *O. masou*)	6-7 months	6 + g	2 + year	OMP + Shrimp	15%	2-5 lbs	600	Oct.
Atlantic Salmon (*Salmo salar*)	1 + year	15 + g	2 + year	OMP OMP + Shrimp OMP + Salmon OMP + Krill	80-90%	6-12 lbs	5200	Oct. Nov. Dec.

Broodstock Programs at Manchester Fisheries Laboratory

William Waknitz
National Marine Fisheries Service

A captive broodstock program presents additional problems to those inherent in rearing salmonids for release or for commercial sale. Prominent among these are methods for accurate identification of individuals, disease control for adult and maturing fish, and nutrition of adult fish. Moreover, it seems that the means necessary to mitigate these problems may differ for fish reared in freshwater and those reared in saltwater. Certainly, each species presently used in captive broodstock programs responds differently to and has different requirements concerning disease control and nutrition. In fact, there are often pronounced differences between races within a species. Unfortunately, the requirements for brood fish are not fully understood, especially for those reared in saltwater.

At the Manchester Research Station, salmonids of many indigenous and exotic species have been reared to maturity under a variety of experimental pursuits. However, limited space and an emphasis on problems associated with young fish have, for the most part, precluded detailed analysis of the requirements of adult fish. Nonetheless, our experience has resulted in not only partial solutions to some of the questions, but has also pointed the direction for research necessary to consistently rear fish that will produce desirable, viable gametes.

Identification of Individuals

Popular marking systems, primarily the wire-coded tag, although accurate and readable, require the death of the fish for evaluation and do not provide for the identification of individual fish. The external tags and marks tested at the station, while not requiring sacrificing of the fish for enumeration, have been unacceptable usually because of tag loss or poor readability of marks. In addition, existing external identification systems often result in the

unintentional death of the fish, by abrasion wounds (tags) or burns (marks). Because fish held in netpens are trapped in the zone of maximum photosynthetic activity, algae eventually encrusts external tags, increasing both the severity of wounds and the amount of energy required for normal swimming.

Decreased growth rates and an increase in mortality usually occur subsequent to fouling of the tags. Therefore, the need exists for a tag or mark that is readable, retainable, and that neither requires nor results in the death of the fish. Permanent individual tags or marks will benefit many areas of research because a relative few individually tagged fish will allow the same statistical conclusions as many unmarked groups of fish. Individually tagged fish will reduce numbers of fish necessary in some areas of genetic research, thereby reducing costs associated with rearing space, feed, data evaluation and personnel. When the entire adult life history is known for each fish, great precision is available concerning the selection of desirable characteristics to pass on to future generations, including growth profiles, disease history, response to known stress and other criteria where they are pertinent.

Disease Control

In the past, *Vibrio anguillarum* was the bacterial pathogen that most commonly dictated the success or failure of a marine netpen rearing program. The development of efficacious vaccines and efficient means of delivery, along with timely treatment should the disease appear, have reduced vibriosis to nuisance status. However, many diseases remain for which no adequate prevention or treatment exists. In both freshwater and saltwater, Bacterial Kidney Disease (BKD) is a serious problem. In one group of maturing pink salmon, all of the fish succumbed to BKD before any eggs or milt could be taken. Presently a rare stock of spring chinook salmon is being reared in saltwater at the station to provide an egg supply for the future. After very few deaths, the mortality rate increased dramatically during the summer of 1979 and all of the dead fish had BKD. The entire group of fish was then intraperitoneally injected with a solution consisting of a booster vibrio vaccine and a combination of anti-bacterial agents. At approximate 6 month intervals the fish have been reinjected, and to date no BKD has been isolated from the few fish to die. Periodic injections, while not economically feasible for thousands of production fish, seem to be advantageous for brood fish which often require many years of maintenance.

In general, the genus *Salmo* seems to be more resistant to diseases in saltwater than *Oncorhynchus*. Thus, as a result of our previous experience, the relative disease resistance of salmonid species commonly cultured in saltwater is as follows: 1) Atlantic salmon; 2) rainbow and cutthroat trout; 3) coho and masu salmon; 4) chinook salmon; 5) sockeye salmon; and 6) chum and pink salmon.

Nutrition

The nutritional requirements of cultured adult salmonids are poorly understood. Indeed, the literature is very limited concerning broodstock rations. Experience at the Manchester station, while by no means complete, has provided empirically derived conclusions that may be valuable for others. Some of our findings follow.

Fish that have been fully reared in captivity for many generations

perform much better (growth, survival, fecundity, and egg viability) on commercial diets than do fish from wild parents or parents from traditional release and return hatchery programs. Both the Donaldson rainbow and our Atlantic salmon perform relatively well on standard diets: both have been fully cultured for over 25 years. Conversely, attempts to rear progeny of wild parents to maturity on commercial diets have met with little success. In one case, the viability of eggs from wild-stock chinook salmon was less than 10%. In another, all possible crosses were made between cultured coho salmon and a cohort group which had returned to the station. Viability was normal except where a cultured female was used, in which case viability was poor. Diet was thought to be the difference between released and captive females--there were no differences between released and captive males. Further, for captive brood fish reared at Manchester, approximately 75% of the prespawning mortalities have been females. Presently available diets do not seem adequate to both produce hardy eggs and to minimize the depletive effects of maturation. Thus it seems that if time (years) is not limiting, it would be possible to develop brood fish that perform well on presently available commercial diets. However, most culturists do not have the blessing of time and it is therefore imperative that brood diets be developed so that virtually any desirable race of salmonid species, wild, endangered, or hatchery--can be successfully reared to maturity with excellent egg viability. Some degree of success has been achieved by feeding natural foods (krill, shrimp and crab wastes, herring, anchovies, and salmon eggs) to captive brood fish. However, such food requires freezer storage, is seldom pasteurized, and does not lend itself to the addition of antibiotics or vitamin/mineral supplements. Pelleted rations for brood salmonids will lessen storage problems, reduce disease transmission through pasteurization, and easily facilitate the addition of supplemental ingredients. Diets nutritionally equal to wild forage will allow the culture of many valuable stocks with minimal sacrifices to their genetic character. The development of nutritionally adequate brood diets, targeted to specific species and environments, is at least as important as any other area of research concerning cultured salmonids.

Captive Broodstock Problems at Washington Department of Game Hatcheries

James Gearheard
Washington Department of Game

The Washington Department of Game maintains eleven broodstocks on eight stations to provide eggs for a program of stocking the waters of the state. The annual egg take is near thirty million. Stocks are maintained to provide variability in timing of maturation and to provide fish for variations in requirements of the types of waters stocked.

Rainbow trout broodstocks are held at four stations, South Tacoma, Goldendale, Spokane and Tokul Creek. The entire needs of the Department are provided by these stations. Many problems have been encountered while carrying out this program. At South Tacoma the most serious problem is that of a below normal egg survival to eye-up. Loss here runs from 18 to 30%. A water temperature of 56°F. is the probable cause for this difficulty. At Spokane and Goldendale the major problem is post spawning and chronic mortality of the older brood fish. Oral and injected antibiotics have not been the answer to this problem. Mortalities ranging from 13 to 72% have occurred in these hatcheries. The most viable appearing solutions to this problem are release of the older broodfish after spawning, water hardening of brood replacement eggs in 2 ppm of erythromycin 200, and improvement of the broodstock diet.

Cutthroat broodstocks, both resident and anadromous, are held at Tokul Creek, Beaver Creek, Ford and in saltwater at Manchester. Problems encountered with these stocks parallel those of rainbow stocks. Additionally, there appears to be a problem of low gamete output by the males. Also at Ford Hatchery, a serious problem of egg loss to eying amounting to 50% to 80% annually occurs. At the Manchester saltwater site the problems associated with saltwater captivity occur. These include acclimation to saltwater, vibrio disease and external parasites. We feel that a more specific diet for cutthroat trout might help solve some of our problems.

An eastern brook trout program is also maintained at our Ford Hatchery. The major problem with this program has also been mortality among the older fish. We have begun a program of only taking eggs at

two years of age and then releasing the spawned-out adults. This provides a solution to post spawning mortality, saves raceways, and provides smaller eggs thus reducing the size of fry which formerly were growing larger than necessary for spring planting.

In summary, some of the problems our agency encounters are serious mortalities among adults after spawning, egg losses, and low sperm output by cutthroat. Solutions may lie in diet improvement and in not attempting to hold broodstock beyond an age of four years.

Workshop I

SESSION III
Factors Influencing Adult Survival and Maturation

SESSION CHAIRMAN
William Hershberger

PANELISTS

Edward Donaldson
Donald W. MacQuarrie
Ronald W. Hardy
Graham A. E. Gall
Stacia Sower

Ovarian Maturation and Induced Spawning in Pacific Salmonids in Fresh and Salt Water

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Ovarian development in salmonids is regulated by circannual environmental changes, especially photoperiod. These cycles are detected by the sensory and central nervous systems and result in changes in the rate of release of hypothalamic hormones. In teleosts there is good evidence for a gonadotropin releasing hormone (GRH) and some evidence for a gonadotropin release inhibiting hormone (GRIH). These hormones control the production and release of glycoprotein gonadotropin in the pituitary gland. This gonadotropin is responsible, directly or via promotion of steroidogenesis, for the stimulation of ovarian development, oocyte final maturation and ovulation. There is also evidence for a low glycoprotein gonadotropin which is responsible for stimulating the uptake of vitellogenin into the oocyte by pinocytosis.

Ovarian maturation and ovulation in salmonids can be accelerated by intervention with exogenous hormones at several points in the hypothalamic, hypophyseal, ovarian axis.

1. Injection of antiestrogens such as tamoxifen results in blockage of the estrogen receptors in the hypothalamus and or pituitary and interrupts the estrogen negative feedback system. This results in a stimulation of gonadotropin release.
2. Injection of the mammalian GRH, LHRH or its potent analogs results in stimulation of the production and release of endogenous gonadotropin.
3. Direct ovarian stimulation by administration of exogenous salmon gonadotropin can be achieved by injection of GTH purified from pituitary glands collected from mature salmon or by injection of an extract of fresh, frozen, or lyophilized salmon pituitary glands or an extract of an acetone powder of salmon pituitary glands.
4. Final maturation i.e. germinal vesicle breakdown in the salmonid oocyte can be induced directly by injection of a progestogen such as 17α 20β dihydroprogesterone.

All of the above techniques have been used to induce ovulation in coho salmon in fresh water. Success rates have ranged from 40% ovulation in coho spawned 40 days prior to normal egg take to 100% in coho spawned close to normal egg take. Salmon pituitary preparations have also been used to spawn chinook salmon, steelhead, cutthroat and rainbow trout under hatchery conditions and chum salmon in the laboratory.

Oocyte growth and development is incomplete in broodstock which return to the hatchery early. Further studies will be required to investigate techniques for accelerating the completion of oocyte growth prior to the induction of final maturation and ovulation.

Photoperiod Induced Off-Season Spawning of Coho and Pink Salmon

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J. R. Markert
W. E. Vanstone
Department of Fisheries and Ocean

Coho Salmon Studies[1]

Three groups of coho salmon were reared under artificially controlled photoperiod regimes. One group was exposed to a normal first, second and third year photoperiod cycle. The other two groups were maintained on a normal first but modified second year cycle designed to advance or delay spawning by 120 days. The third cycle was of normal duration but shifted out of phase to maintain the time differences that had been established during the second altered photoperiod cycle.

Sexually mature adult coho salmon with viable gametes were produced 78-90 days prior to the normal spawning period and 148 days after the normal period. Mean egg fecundity ranged from 645 for the 120 day advanced group through 792 (normal) to 803 for the 120 day delayed group. Mortality to swim-up in the fry stage was 8.5 p. 100 in the normal group, 16 p. 100 for the 120 day advanced group and 52 p. 100 for the 120 day delayed group.

Nineteen percent of the 120 day advanced fish, 17 p. 100 of 120 day delayed fish and 6 p. 100 of the normal fish did not respond to light treatment. They retained their marine colouration and were histologically identified as immature females.

Some of the progeny of the 120 day delayed group reared on a normal photoperiod spawned in December, 1979. Survival from egg fertilization to swim-up was 83 p. 100.

Pink Salmon Studies[2]

Four groups of pink salmon, which had been reared under artificial light, became sexually mature and produced viable gametes: 59 days prior to, and 19-32 days, 115 days and 220 days after their expected date of reaching sexual maturity. Altered times of sexual maturation were obtained by accelerating, leaving unchanged, or decelerating the

rate of change of photoperiod which each group of fish would normally receive during its first year of life. All groups of fish were exposed to a normal rate of change in photoperiod during their final year of life. Mean fecundity was reduced from the 800-2000 ova observed in wild stocks, and ranged from 629 for the 59-day advanced fish, to 862 for the 115-day delayed fish. Egg mortality during the period from fertilization to eyeing was much greater in the three groups of fish subjected to accelerated or decelerated rates of change in photoperiod than in the fish subjected to the normal rate of change in photoperiod. Some of the progeny of the 220-day delayed fish, which were reared under artificial light with the normal rate of change in photoperiod set 220 days out-of-phase, became sexually mature 2 years after they had begun life as fertilized eggs.

Of those females that became sexually mature 73.9 p. 100 of their ova survived to the swim-up stage.

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1) Ann. Biol. Anim. Bioch. Biophys., 1978, 18 (4), 1051-1058.
2) Aquaculture, 18 (1979) 289-302.

Nutrition and Broodstock
A Summary

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Published research and unpublished observations indicate that the diet received by captive broodfish influences egg production by affecting the size of the female at spawning. Diet influences egg quality by altering the chemical composition of the material deposited in the yolk. Diet may also influence physical characteristics of the eggs (weight, volume) but physical characteristics do not seem to affect egg survival as much as does chemical composition of the eggs.

Before diets can be formulated specifically to enhance the quality of eggs from broodfish, more information must be obtained on the nutritional needs of broodfish. Several approaches should be used. First, eggs from both captive and wild maturing salmon should be analyzed from the time wild fish can be captured in the fishery as they return in early summer to offshore areas until the time they spawn. Analysis of the chemical composition of developing and maturing eggs may indicate differences between wild and captive fish and thus indicate nutrients that may be deficient in captive broodstock diets. Second, analysis should be made of captive broodstock diets to insure that adventitious toxins that may accumulate in developing eggs and lower egg survival are not contributing to egg viability problems. Finally, a more thorough understanding of the sequence of nutrient deposition in developing eggs must be achieved so that the temporal aspects of broodstock diet supplementation can be addressed.

Factors That May Influence Sexual Maturation in Salmon

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There is a paucity of genetic information concerning sexual maturation in salmonid species; therefore, any comments made in this regard must be considered only conjecture. It is known that a number of hormonal reactions or changes take place as the fish migrate through the saline gradient into fresh water and that most of these changes irreversibly affect osmoregulation. Changes in both thyroxin and cortical steroids of renal origin appears to be important in allowing the fish to make the transition. In addition, there is a large increase in the level of gonadotropin, which plays a significant role in the hydration of eggs. It seems reasonable to assume that the availability of isotonic water is critical to the normal development of both eggs and sperm.

An assessment of whether low hatchability results from the low quality of ova or from ineffective fertilization will be critical to understanding and developing a solution to this problem. Fertilization may be influenced by the availability of isotonic water, which is required for activation during spermatogenesis. The quality of ova may also be reduced by insufficient egg hydration, which could result in initial egg resorption and the spawning of atretic eggs.

With this brief general background, it seems reasonable to postulate the existence of genetic adaptations to the time that different stocks take to migrate through the saline gradient as well as the time spent in fresh water prior to spawning. The time required in fresh water may be shorter for stocks that undergo a reasonably short-distance migration than for stocks that undergo migration over long distances before spawning. In either case, there is a need for sufficient time for hydration of the ovaries. From studies with rainbow trout, it is also known that there may be differences among families in the time of the season in which they spawn. Some full sisters spawn early in the season, whereas other families of full sibs spawn as much as several weeks later. The first step in assessing

the nature of sexual maturation seems to be a detailed analysis of egg development. This analysis will require the use of histological techniques that are sufficient to assess or identify abnormal gametogenesis.

Sexual Maturation of Coho Salmon (*Oncorhynchus kisutch*) Induced Ovulation and Serum Hormone and Ion Levels of Salmon In Fresh versus Salt Water

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Salmon gonadotropin, pituitary extract, and LH-RH were used to induce ovulation in coho salmon held in fresh or saltwater. Female coho salmon were injected in mid-October with one of the following treatments: (1) control (no injection), (2) control (saline injected), (3) partially purified coho salmon gonadotropin (SG-G100) followed three days later by LH-RH ethyl amide (LH-RH), (4) Syndell purified chum salmon gonadotropin (S-GTH) followed three days later by LH-RH, and (5) chum pituitary extract (SPE) followed three days later by SPE. In freshwater at day 17 after the first treatment, 82% of the salmon treated with SG-G100 plus LH-RH had ovulated compared to 50% for S-GTH plus LH-RH-treated fish, 42.2% for SPE-treated fish and 0.0% for control fish. In saltwater at day 17, 40% of the SG-G100 plus LH-RH-treated fish had ovulated compared to 36.4% for S-GTH plus LH-RH-treated fish, 13.0% for SPE-treated fish and 5.3% for control fish. At day 17, there was no mortality in the freshwater salmon compared to 47% in saltwater. The most effective treatments were those with SG-G100 plus LH-RH and S-GTH plus LH-RH, particularly in freshwater. Osmoregulatory difficulties or ensuing mortality seemed to have diminished the responses seen in the saltwater groups.

Serum was sampled from adults in salt and freshwater from the middle of September until the beginning of December, 1979, at which time the fish had ovulated. In early October, serum sodium, osmolality, progesterone, and estradiol levels of salmon in saltwater were elevated compared to fish in freshwater. Thyroxine levels in saltwater fish were elevated at the time of ovulation compared with freshwater fish. Increased levels of progesterone and estradiol in saltwater may be attributable to dehydration of the fish and hemoconcentration, as suggested by elevated sodium and osmolality. This was also concomitant with the highest mortality in saltwater. Coho salmon remaining in saltwater during the final stages of sexual maturation appear to experience hormonal differences compared to salmon maturing in freshwater.

Workshop I

SESSION IV
Gamete Viability and Fertility

SESSION CHAIRMAN
Ernest L. Brannon

PANELISTS
W. Craig Clarke
John Jensen
J. Stoss
Dennis Roley

Influence of Nutritional Factors on Fertility in Coho Salmon (*Oncorhynchus kisutch*)

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Coho salmon held in netpens at the Experimental Fish Farm in Nanaimo reach maturity about 18 months after introduction to seawater However, the time of ovulation and the rate of fertilization in these fish are highly variable. Initial experiments indicated that variability in fertility could be related to the females. An obvious defect in the eggs of pen-reared females, apart from their small size, was the pale colour.

In order to examine the influence of nutritional factors on fertility, a number of diets were tested. In the fall of 1978, an experiment was set up to compare regular Oregon Moist Pellet (OMP) with 1. modified OMP (20% of fish meal replaced with euphausid shrimp mix), 2. Ewos Broodstock diet (Ritchie-Smith, Abbotsford, B.C.), 3. Tess Broodstock diet (Skretting Co., Stavanger, Norway) 4. West Van. Broodstock diet (Formulated by Dr. Higgs, West Vancouver Laboratory). The latter three were dry diets, having 10% moisture or less. The diets were administered from September through to spawning in mid-December. There were no significant differences in growth rate, total egg volume nor in percent fertilization. Fertilization averaged about 50% with individual variation from 8% to 91%.

The following year, two groups of coho were selected in January at a mean weight of 220 g. They were fed either OMP or the West Van. Broodstock diet until December. Growth rates and total egg volume were similar in the two groups. Fertilization rates again were highly variable, ranging up to 93% in both the OMP and dry diet groups. Egg colour was improved noticeably by inclusion of euphausid shrimp mix in the West Van. dry diet.

Some Spawning and Incubation Conditions That Affect Salmonid Gamete Viability and Embryo Development

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Introduction

The viability of salmonid broodstock gametes is known to be influenced by environmental, nutritional, and genetic factors. These factors may operate directly or indirectly either on the spawning adults or on the gametes they produce. Investigations of mortality, development and growth of eggs or alevins, relative to such factors, often are accompanied by high variability in the measured responses. A substantial portion of the uncontrolled variance in such instances may result from the operation of variables either unrecognized or assumed to be of minor importance. Some of these variables are associated with aspects of gamete manipulation in the spawning technique, or with water quality in the incubation environment.

To illustrate the significance of such variables I shall present some information on (1) the effects of temperature, gamete storage time, and mechanical shock on egg mortality. I will also present data from three ongoing projects on (2) the effects of calcium levels on rates of waterhardening and mechanical shock sensitivity of newly fertilized eggs, (3) the combined effect of calcium level and gas supersaturation on steelhead eggs and alevins, and (4) the combined effects of un-ionized ammonia and dissolved oxygen concentrations on salmonid embryos and alevins.

Spawning Technique

Temperature is an important factor affecting gamete viability. A comparison of chinook gametes stored at different temperatures for 24 h (Jensen 1977) indicated that milt could be stored safely at temperatures between 0.5 and 15°C with no significant ($\alpha = .05$) loss in viability. Viability of chinook eggs stored unfertilized, however, was significantly reduced at temperatures greater than 5°C. The viability of the stored eggs was quite low overall, probably as a result of the prior temperature

experience of the gametes, the adults having been held at 12.2°C. An indirect comparison of the effect of prior temperature experience with unfertilized coho eggs, taken from adults in 5.8°C water and stored at similar temperatures, indicated that higher egg survival was achieved when adults were previously held at lower temperatures (Jensen 1977). In addition, it also appeared that the optimum storage temperature was lower (near 0°C) for eggs from adults held at 5.8°C than the optimum storage temperature (near 5°C) for eggs from adults held at 12.2°C.

Another important factor affecting gamete viability is gamete storage time. For most spawning techniques this may not be a problem. However, if gametes must be stored for several hours or more, this storage "time delay" should be standardized. Time delay becomes even more important once gametes are combined. For example, coho egg mortality rose from 4.4% to 10.6% for a 1-h delay and to 41.6% for a 2-h delay when inseminated but unactivated eggs (i.e. eggs and sperm mixed but not exposed to freshwater) were stored (Jensen 1977). The threshold for significant (α = .05) mortality increase for these data was between 1 and 2 h.

Once eggs have been activated with water another factor resulting in mortality is mechanical shock associated with the spawning technique. Smirnov (1975) showed that maximum shock sensitivity of coho eggs occurred 15 min after activation. However the threshold in time delay for significant egg sensitivity to mechanical shock to develop was not determined.

An example of the rapid increase in egg sensitivity to mechanical shock occurred at a hatchery in British Columbia, Canada. An erythromycin phosphate rinse was incorporated into the standard spawning procedure in an attempt to reduce bacterial kidney disease (Jensen et al. MS). The result was an increase in egg to fry mortality to 35%. The erythromycin was thought to be the cause but an examination of the timing of the altered spawning technique showed that a significant delay of 6-13 min had occurred from egg activation to the time when the eggs were poured into incubator trays. This information prompted several experiments verifying the suspicion that mechanical shock and not erythromycin was the factor causing mortality. The erythromycin rinse was used again the following year, but "activation-to-tray" delay was kept to only a few minutes. No unusual egg mortalities resulted.

Another ongoing project involves our efforts to understand the factor or combinations of factors that cause white spot (coagulated yolk) disease. Numerous variables, including gas supersaturation, soft water, and high temperature reportedly are associated with white spot disease (MacKinnon 1969; Wedemeyer et al. 1976; Wood 1979). Currently we are conducting an experiment to determine the combined effects of gas supersaturation at 100, 105, and 110% total gas pressure; calcium levels at 10 and 100 mg·L^{-1} as $CaCO_3$; and temperatures of 8, 10, and 12°C on steelhead egg and alevin survival. Preliminary information suggests that these combinations of treatments do not cause white spot disease. However, we have noted the formation of a large gas bubble in the mouth cavity of the alevins about 5-7 days after hatching. This response has occurred at all three temperatures but only in the soft water (10 mg·L^{-1} as $CaCO_3$) and higher gas supersaturation level (110% total gas pressure). The alevins at this early stage of development were unable to expel the bubble and exhibited abnormal swimming behavior. Only a few mortalities resulted from this phenomenon but observations are continuing to detect possible sublethal effects associated with this response.

The final topic illustrates how egg loading density may affect such responses as egg and alevin mortality, hatching rate, and alevin growth. For the past few years we have studied the lethal and sublethal

effects of elevated ammonia levels and reduced levels of dissolved oxygen on sockeye eggs and alevins. In air-saturated water at 10°C and pH 8.3 the LC50 for sockeye eggs from fertilization to hatching was 100 $\mu g \cdot L^{-1}$ un-ionized NH_3 (Rankin 1979; Jensen, unpublished data). Yet at pH 7.5 and under similar conditions, no significant egg mortalities occurred at 200 $\mu g \cdot L^{-1}$ un-ionized NH_3 (Jensen, unpublished data). This suggests that pH has an influence on egg survival as a "main effect" apart from its interacting influence on the fraction of un-ionized NH_3 present in the water. Alevin mortality was noted with certain combinations of high un-ionized ammonia and low dissolved oxygen levels (Table 1), indicating significant interaction between these two variables. In addition, similar levels of un-ionized ammonia and dissolved oxygen both combined to cause delayed hatching and alevin growth reduction.

Table 1. Mean alevin mortality, time to 50% hatch, and embryo to yolk dry weight ratios when sockeye eggs were exposed to combinations[a] of un-ionized ammonia (NH_3) and dissolved oxygen (D.O.) levels at 10°C and pH = 7.5.

NH_3 $\mu g \cdot L^{-1}$	D.O. $mg \cdot L^{-1}$	Alevin mortality %	Time to 50% hatch °C - days	Embryo ÷ yolk ratio[b] mg ÷ mg
10	10.9	0.6	649	.294
10	3.4	1.9	660	.163
191	10.9	1.4	649	.264
191	3.4	38.1	713	.117
100	3.0	54.7	714	.114

[a]These treatment combinations are part of a 13-point orthogonal contrast experimental design tabulated to illustrate the interaction between NH_3 and D.O.

[b]At 675 °C - days.

Summary

Three important factors involved in salmonid spawning technique are temperature, gamete storage time, and mechanical shock. Exposure of adults and gametes to higher temperatures was shown to reduce egg viability. Storage of inseminated but unactivated eggs for greater than 1 h resulted in increased mortalities. Mechanical shock sensitivity of coho eggs increased significantly between 6 and 13 min after activation, while chum egg sensitivity increased between 1 and 5 min after activation.

In the incubation environment, a higher calcium level resulted in a more rapid increase and higher internal egg pressure in chum eggs. No differences were detected in egg shock sensitivity associated with calcium level. The combination of soft water and gas supersaturation levels tested did not cause white spot disease but did produce bubbles in the mouth cavities of newly hatched steelhead alevins. Finally, the combinations of lowered dissolved oxygen and elevated un-ionized ammonia levels resulted in mortality, delayed hatching, and reduced growth rates of sockeye embryos.

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Gamete Storage in Domestic Rainbow Trout (*Salmo gairdneri*)

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Short-term Preservation above Freezing Point

Sperm Storage

The following storage conditions for semen proved to be suitable: $0^{o}C$, moisture saturated oxygen-atmosphere, addition of 125 IU/µg penicillin/streptomycin per ml semen, semen sample height 5 mm. Undiluted sperm pooled from several males fertilized 77% of eggs after 34 days storage (control with fresh semen 83%). Simplifying the technique by storing antibiotic containing sperm in oxygen filled and sealed plastic bags, 81% of the eggs were fertilized after 20 days storage (control 100%).

Egg Storage

$0^{o}C$ moisture saturated air, 125 IU/µg penicillin/streptomycin per g egg + coelomic fluid weight, pooling eggs from several females and allowing not more than 4 layers of eggs in the storage container provided over 90% fertilization after 10 days and still about 70% after 20 days storage (100% day 0).

Freezing of sperm

Composition of diluent: 101.3 mMol NaCl, 23.1 mMol KCl, 5.4 mMol $CaCl_2$, 1.3 mMol $MgSO_4$, 200 mMol Tris (hydroxymethyl) amino methane, citric acid to pH 8.0, 0.4% bovine serum albumine, 0.75% promine D (soybean protein - Central soya International, Inc.) and 10% dimethylsulfoxide (DMSO), 3 parts diluent + 1 part semen, freezing pellets (size 0.05 ml) on dry ice ($-79^{o}C$), storage in liquid nitrogen ($-196^{o}C$). Thawing in 120 mMol $NaHCo_3$ solution ($9^{o}C$, 1 pellet per ml), fertility of thawed semen ranged between 0-20% below comparable results with fresh semen.

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SESSION V
Panel Summaries and Research Priority Recommendations

SESSION CHAIRMAN
Brian J. Allee

Recommendations

Terry Nosho
William Hershberger

Reported here is a compilation of material gathered from the sessions Panel Summaries and Research Priorities. The points listed represent a consensus resulting from those two sessions. The listing is not in priority sequence. Please note that many specific questions were asked and suggestions made during the course of the workshop. It was impossible to list all of these in this section. However, an attempt was made to at least include a general flavor of this important aspect of the workshop.

1. Broodstock must be subjected to three or four successive generations of exposure to the aquaculture system before measurements of any factor concerned with maturation and survival should be considered "normal."

Experience with other animals has shown that this length of time is necessary for adaptation to a new environment, especially with somewhat artificial conditions. Measurements prior to this time may lead to incorrect conclusions because the biological system is rather labile during adaptation.

2. The use of captive broodstock for ocean ranching systems as well as netpen systems should be studied.

Successful development of such a system would assist two areas of current concern to ocean ranching. First, this would provide a solution to the uncertain return rate and, thus, gamete supply for production. Second, such a stock could be used as a nucleus for genetic selection and improvement.

Studies should focus on reducing adult prespawning mortality from osmoregulatory, disease, and nutritional standpoints. More specifically, objectives might be to 1) improve egg viability; 2) develop techniques to identify fully matured broodstock; 3) develop an optimum broodstock ration for each species and determine the most effective time to apply

the diet during brood maturation. Presently, no information is available on the nutrient requirements of maturing adult fish.

3. Coho salmon broodstock should be subjected to freshwater treatment during the final maturation of sex products.

Based on the results presented at this workshop it has become apparent that with the present "state-of-the-art," improved gamete production and decreased prespawning mortality can be acheived by subjecting maturing adults to fresh water. The details of this procedure have yet to be defined, but some freshwater exposure is desirable.

4. A new histological evaluation (gross and detailed) of normal salmonid reproduction is needed, including all stages from vitellogenesis through final maturation.

Two factors were mentioned in conjunction with this recommendation. First, it is well recognized that salmonid ova are some of the most difficult to work with histologically; this undoubtedly partially explains why more complete information is not currently available. Second, a valuable asset to the aquaculturist would be the publication of this information in a form that could serve as a reference to assess the state of broodstock maturation.

Several areas were recommended for study in relation to the characterization of the maturation cycle.

a. Evaluation of the reproductive stages should also be conducted in relation to various treatments that are utilized in aquaculture, e.g., penculture, return to salt water, and holding in salt water. This could provide some indication of the stage(s) at which primary effects on ovulation and final maturation are expressed.

b. In addition to histological examination, chemical constituent analysis of gametes should be conducted to determine the dynamics of deposition of critical constitutive nutrients. This may provide a measure of the necessary nutrients and their levels for a balanced diet.

c. Concomitant with gamete production a survey of reproductive and osmoregulatory hormones should be conducted to determine the critical control components involved in ovulation and final maturation.

5. Further studies are needed to optimize and standardize the techniques that are utilized for induced spawning of salmonids.

This procedure is currently successful and can be used to temporally advance reproduction. However, more work is needed to determine the "best" materials and the time and sequence of treatment.

6. Studies are needed to determine the feasibility and efficacy of sex control by hormonal and/or genetic means.

This approach has promise in exercising some control of the sex distribution of broodstock and in utilizing the most productive sex of fish in the commercial crop. However, the actual incorporation of this technique into a production scheme needs to be evaluated.

7. The use of environmental control, i.e., photoperiod, in the maturation of broodstock was discussed but did not receive a consensus opinion. Evidence presented during the workshop showed that time of

final maturation could be adjusted with photoperiod control. The major use would seem to be with captive broodstock, but there were questions concerning the difficulty of effective light control in floating marine netpens and the utility of a large alteration in spawning date with the current necessity of meeting a fairly rigid saltwater entry date.

8. An empirical study of salinity and holding environment for returning broodstock was considered to be a top priority for the coming season. It was agreed that the NMFS Little Port Walter study design would provide a basis for developing a standard protocol with a standardized sampling design for those wishing to participate (private companies, university and federal laboratories, and state research people). This then would provide direction in finding solutions to the problems connected with saltwater maturation. A committee was formed to develop the protocol.

9. A follow-up workshop after the 1980-81 egg-taking season is desirable. Such a workshop would further information transfer and stimulate candid discussions on issues pertaining to broodstock maturation.

Workshop II

FOLLOW-UP SESSION

SESSION CHAIRMAN
Terry Nosho

PANELISTS

Carlin McAuley
Edward M. Donaldson
Stacia A. Sower
Joachim Stoss
Brian J. Allee
Alex Wertheimer
Mike McDowell
Ward Griffioen

1981 Followup

DOMSEA Farms Coho Broodstock Program—Update

Carlin McAuley

As a matter of quick review, Domsea began development of a coho broodstock in 1977 with the help of the Univeristy of Washington's College of Fisheries. A breeding program was set up in December 1977 and 1978 utilizing F1 generation spawners. Progeny from these crosses were reared under accelerated conditions in freshwater to ensure a 2 year life cycle. All maturation and spawning through December 1979 was carried out at our saltwater facilities. For three successive years we have experienced declining egg viability, culminating last year with a 45% survival through the eyed stage. Compare this with our normal survival of 85-95% in eggs taken at state hatcheries and it becomes obvious that something is amiss. In addition to this we have also suffered heavy adult losses during the 3-6 months prior to spawning. In last years adults, for example, our losses escalated from the normal 1%/month to 20% in July and 34% in August. Disease was the major contributing factor, most of which was furunculosis.

To alleviate this problem, an erythromycin injection program was conducted in early September (3 months prior to spawn) with dramatic results. We reduced our monthly losses from 34% in August to just 7% in September, and continued at this reduced level through spawning.

To help solve the poor gamete viability problem, two appraoches were taken - nutritional and environmental. For the nutrition end the broods were divided into 5 dietary groups beginning 5 months prior to spawn: 1) control diet (normal Domsea formulation), 2) increased Vitamin C, 3) increased Vitamin E., 4) increased fat and 5) increased Vitamin C and E and fat. The diets were fed right up to cessation of feeding activity, about the end of October. Results tend to indicate that no beneficial effect on gamete viability was produced, although the high Vitamin E diet actually had a detrimental effect.

The second approach, environmental (transfer to freshwater), proved to be the most effective. Initially two small test groups were transferred to freshwater, one in September and one in October. These tests were made to evaluate the best time to transfer based on adult survival,

time of maturation, and egg viability. No differences were noted between the groups for the three criteria tested. Two other differences were observed, however; the October group had less problem with fungus build-up, and distinguishing males from females (at time of transfer) was much easier in the October group.

The majority of the broodstock were transferred to freshwater between October 15 and November 15, and placed into declining temperature creek water. A small group was left in saltwater to serve as a control. As a result of the transfer program, the overall timing of maturation was advanced 2 - 3 weeks over the saltwater held broods.

Hatchery performance was also much better in the freshwater group - 82% survival to eyed stage versus 64% in the saltwater group. Compared to last years 45% survival, this represents a big turn around.

Domsea will repeat the freshwater transfer and spawn program again this fall to reaffirm our conviction that this is the best course of action to take to improve the yield from our broodstock program.

1981 Followup

Pre-spawning Mortality of Pink Salmon Matured in Fresh Water

The Prince William Sound Aquaculture Corporation Experiences 1980

Curt Kerns

Introduction

For the past 5 years (1975-1979) the Prince William Sound Aquaculture Corporation (PWSAC) has held pink salmon in salt water (20-28 o/oo) pens (3m x 6m x 2m D) prior to spawning. The broodstock transplanted were predominately intertidal donor stocks from streams 25 miles distant in 1975 and 90 miles away in 1976. While in excess of 110 million eggs were taken over the 5 year period resulting in a production of 2.25 million adult fish (1979 brood year fry still at large), high pre-spawning mortalities (1% per day) were evident.

The numbers of adult pink salmon produced by PWSAC's Port San Juan hatchery has approximately tripled every year since the first return (44,000 in 1977 - 1.5 million in 1980). To avoid straying of fish to nearby streams even longer holding periods (5 days in 1977 and 40 days in 1979) have been required. Concomitant with the extension of close confinement came higher pre-spawning losses which resulted in increasingly significant foregone revenues to the corporation. Labor requirements were far greater for sorting mature from immature fish and for removing moribund adults than for the actual artificial spawning which represented a further economic inefficiency. Observations in 1979 of the behavior of non-captive broodstock spawning in the hatchery creek (Larsen) led to extensive changes in the method of maturing broodstock in 1980.

1980 Egg Take

The philosophy that PWSAC implemented in 1980 called for 1) restraining fish only as much as absolutely required and 2) allowing individual fish to volitionally select the time to present themselves for spawning. Holding was broken down into 4 stanzas: 1) Corral. As soon as possible (immediately to 4 days) after capture via a conventional salmon seine, the returning adults were placed behind a

150 fathom long, 2 strip deep herring seine that enclosed the saltwater delta of Larsen Creek. The corral enclosed an area that ranged from approximately 1/5 ha at low tide to 3.6 ha at high tide, with a maximum depth of 3m at mean low low water. 2) Freshwater plume. At low tide the discharge of Larsen Creek was through a channel 4-6m wide and some 100 m long. During high tide the channel banks would typically be submerged. 3) Freshwater pool. At the base of the barrier falls we constructed a 25m x 25m x 2m deep pool using rock-filled gabbions for the dam face. Gabbions are water permeable so sand bags were used to help seal the dam face. The pond bottom and inside wall of the gabbions were further sealed with 4 mil nylon-reinforced Visqueen which also acted to make the rock and gravel of the pool floor unavailable for spawning. The pool was connected to the freshwater plume via 3 sections of aluminum steep pass inclined at a 1:3 ratio. 4) Collector boxes. Two 3.7m x 2.5m x 1.2m deep aluminum raceway sections were connected to the pool. One, the "upstream collector," was placed at an elevation 30cm above that of the surface of the pool. Fed by the incubator discharge water, the upstream collector passed into the pool via a 30cm wide and high steep pass. The "downstream Collector" was located at an elevation just slightly (5cm) below that of the pool. Water ran via an additional 30cm steep pass into the box. Adjacent to the collector boxes were 2 - 14.8m x 3.7m x 1.2m deep raceways used in the event immature fish found their way into the collector boxes.

Results

Observed behavior of the fish once placed into the corral was quite predictable. The bright and nearly bright fish swam randomly about the enclosure. More mature fish moved into the freshwater plume and then up the steep pass into the pool. After an estimated residency period of approximately four days, the fish would move into either collector box. While the downstream collector box seldom ever contained immature fish, the upstream collector captured by far and away the largest number of fish. The fish in the upstream collector box averaged 90% plus mature except during a freshet when the ratio dropped to as low as 50%

Of the 104,150 fish placed into the corral, we spawned 66,380 females and 31,412 males. Pre-spawning mortalities were estimated to be 8.03% and felt to be mainly due to seagulls pecking the fish's eyes out while they were in shallow water. Some 4.0% of the females were discarded due to having green or prewater hardened eggs. Surplus males (4,124) were discarded. Spawning and incubator loading followed previous PWSAC practices. Pink eggs taken amounted to 94.7 million over 26 days. Green to eyed survivals were 94.24% with many of the mortalities occuring in the lots taken during the first and last days' spawning. During the peak two week spawning period survivals regularly hit 98% for the days' efforts.

1981 Followup

Summary of the 1980 Summer Chum and Fall Chum Broodstock Collection

Ward Griffioen and Walt Larrick

Summer Chum Egg Take at Carroll River

Saltwater environmental and physical conditions were much the same as described in our 1980 report for most of the netpen maturation program. A freshwater weir, adult holding pen and egg-take facility were installed upstream above the tidal influence to eliminate saltwater maturation problems such as were experienced during 1979.

Due to severe flooding we were unable to contain the majority of our captured females and large numbers escaped upstream. Also we were forced to take the largest portion of our eggs from chum matured in saltwater netpens. A total of 5.6 million eggs were taken with an overall survival of 61.1 percent to eyed stage.

Less handling of the adults because of less frequent checking for fish ripeness and a floating anaesthetic box installed during part of the egg-take aided our low adult mortalities of less than 5 percent. Carbon dioxide was bubbled in from a compressed air bottle through micropore tubing in the bottom of the selection box, reducing stress both on the crew and the fish when checking for ripeness.

Due to slower maturation of males in saltwater and our lack of knowledge of sperm quality at this time, we only used sperm taken from freshwater males at the start of the egg take. In accordance with Dr. Stoss's suggestion at last year's workshop sperm was transported in plastic bags with compressed oxygen. Motility checks were carried out back, at the hatchery, for all bags of sperm and all egg shipments tested continually for fertility 10 hours after activation with water. The following information was gained on sperm and gamete quality by a series of experiments:

1) *Males tested.* Ten individual freshwater males and saltwater males were used to fertilize pooled gametes from saltwater. After dead eggs were picked replicated fertility tests subjected to statistical analysis showed no significant differences among the two groups of males.

2) *Females tested.* Pooled freshwater and saltwater sperm was used to fertilize gametes from 10 individual females taken from fresh and saltwater. A high statistical degree of variation was found between egg mortality records of duplicated groups of individual female chums which were matured in saltwater netpens. Survival of eggs from individual females ranged from a high of 98.1 percent to a low of 11.4 percent.

Data collected on the physical characteristics of the individual female or egg condition did not reveal any physical characteristics for future identification of females with low fertility. We concluded that our problem of low fertility was related to egg viability of individual females. In effect the average egg survival of the saltwater females tested in the experiment very closely represented the overall egg survival of the 61.1 percent of the total egg take.

3) *Captured fish.* Egg survival from experimental female chum collected from the spawning grounds was statistically compared with eggs from the same number of females that had been held in freshwater holding pens for up to 10 days, and after the eggs had been fertilized with pooled milt. We found first of all that the mean survival of the eggs was not significantly different within their groups. But the freshwater females held in pens until mature showed a mean decrease of fertility of 6 percent. (88.1% from 94.7%)

Fall Chum Egg Take at Disappearance Creek and Lagoon Creek

A total of 25 million fall chum eggs were taken by SSRAA's personnel for both our own hatchery at Whitman Lake and the A.D.F.& G. facility at Beaver Falls. Seven of the 25 million eggs were taken from females that had been matured in saltwater netpens at Lagoon Creek. The overall survival of 16.7 million eggs taken to our hatchery was 94.5 percent to eyed stage. Survival of eggs taken from matured and spawned chum from saltwater netpens was 93.6 percent to eyed stage. (general egg take procedures were as described earlier in this report).

Liquid carbon dioxide used as a general anaesthetic for adult testing limited our netpen mortalities to a minimum of one percent.

Both saltwater and freshwater males, as available, were used for sperm with equal success.

1981 Followup

Maturation Studies of the 1980 Brood Year Coho Salmon (*Oncorhynchus kisutch*) at Oregon Aqua-Foods, Inc.

Brian J. Allee and Bruce K. Suzumoto

Introduction

This paper is an effort to extend the research described in the first University of Washington Sea Grant workshop on Salmonid Broodstock Maturation to include the 1980 brood year coho salmon studies.

Results

Adult Survival

Maturing coho salmon of the 1980 brood year held in ambient hatchery water (McKenzie River) exhibited statistically higher survival (97%) than their cohorts held in tempered hatchery water at a constant 14°C (66%), or in ambient estuarine water of 28-32 g/l (61%) (Table I). It is of particular significance to note that the tempered hatchery water held fish in 1980 essentially replicated, in terms of adult survival (66%), the adult survival of coho held in a constant ground water facility of the same temperature in 1979 (61%) (Table I). Coho salmon broodstock survival in ambient estuarine water in 1980 (61%) is essentially equivalent to survivals in 1977 (65%) and 1978 (60%) held under similar salinities (Table I). The lower survivals experienced in 1976 and 1979 in the ambient estuarine environment were due to *Vibrio anguillarum* and *Aeromonas salmonicida*, respectively.

Efficacy of Oxytetracycline Treatment

In 1979, therapeutic treatment with oxytetracycline increased adult survival from 67% to 97% during an acute disease outbreak of *A. salmonicida* (Table II). Coho adults prophylactically treated with oxytetracycline and held in hatchery tempered water had higher survival rates than non-treated fish. Fish held in ambient freshwater did not exhibit a significant difference in survival when prophylactically

TABLE I: Comparative survival of female coho salmon broodstock from 1976, 1977, 1978, 1979 and 1980 brood year by holding environment

	Percent Survival of Female Coho Salmon by Brood Year				
	1976	1977	1978	1979	1980
FRESHWATER					
Ambient Stream Water	95	--	--	56	--
Ground Water (14°C)	--	--	--	61	--
Ambient Hatchery Water	--	--	--	--	97
Tempered Hatchery Water (14°C)	--	--	--	--	66
SALTWATER					
Ambient Estuarine Water	14	65	60	13	61

TABLE II: Efficacy of oxytetracycline in coho salmon broodstock held in freshwater from 1979 and 1980 brood years.

	Percent Survival of Coho Salmon Broodstock By Brood Year	
	1979	1980
AMBIENT GROUND WATER (14°C)		
Saline Injectd	67	
Oxytetracycline Injected	97	
AMBIENT HATCHERY WATER		
Saline Injected		99
Oxytetracycline Injected		97
TEMPERED HATCHERY WATER (14°C)		
Saline Injected		62
Oxytetracycline Injected		75

treated. It appears that oxytetracycline can enhance survival in potentially stressful conditions.

Egg Fertility

Fertilities from coho broodstock held in ambient hatchery water with a declining temperature regime were 94%, which was clearly the highest performance to date independent of brood year or holding environment (Table III). Fertilities from fish held in tempered hatchery water and ambient estuarine water were higher than comparable environmental regimes in 1979, primarily due to prophylactic injection of oxytetracycline in all broodstock (Table III). The egg fertilities of

TABLE III: Comparative coho salmon egg fertilities from 1978, 1979, and 1980 brood year holding environment.

	Percent Egg Fertility at 80 CTU's					
	1978 $\bar{x}$	Brood Year Range	1979 $\bar{x}$	Brood Year Range	1980 $\bar{x}$	Brood Year Range
FRESHWATER						
Ambient Stream Water	77	0-100	84	--	--	--
Ground Water (14°C)			56	0-99	--	--
Ambient Hatchery Water					94	24-100
Tempered Hatchery Water (14°C)					80	0-100
SALTWATER						
Ambient Estuarine Water	56	0-100	50	0-97	76	0-99

coho broodstock held in ambient estuarine water were potentially lower than expected, due to a four hour delay from fertilization and water hardening to incubation.

Induced Ovulation

In 1980, salmon gonadotropins were administered to coho adults in an effort to accelerate maturation and ovulation. Thirty-five days after injection, all hormone treated groups had greater percent ovulation and significantly lower mean days to ovulation than the saline injected groups (Table IV). Average egg fertility did not differ significantly between hormone and control groups after 35 days; whereas, when the experiment was terminated 65 days after injection, hormone treated groups exhibited fewer mean days to ovulation, but had a lower percent fertility than saline controls. Because the hormone treated adults were checked for ripeness approximately twice as much as the control fish, the depression in fertility could possibly be attributed to greater handling stress.

Discussion

An ambient declining freshwater environment appears to be the best broodstock holding condition for the stocks of coho salmon which have been evaluated in this study. Saltwater maturation of coho salmon in a pumped system, exposing brood fish to 28-32 o/l salinity, is not recommended due to lowered adult survival primarily, and lower fertility of eggs relative to a freshwater control. There does appear to be an opportunity for genetic selection of saltwater coho salmon broodstock, but this will clearly be a long range solution. A constant temperature regime of 14°C in freshwater, as a broodstock holding environment for coho salmon, will produce deleterious effects on adult survival and egg fertility based on the conditions encountered in this study.

TABLE IV: Comparative maturation time and egg fertility for coho salmon broodstock between induced ovulation groups and control groups by brood holding environment.

	Days to Spawning		Percent Fertility	
	35 days	65 Days	35 Days	65 Days
FRESHWATER				
Ambient Hatchery Water				
Hormone Injected	18.6 (43%)	39.6 (92%)	87	75
Saline Injected	28.3 (21%)	48.7 (97%)	91	93
Tempered Hatchery Water (14°C)				
Hormone Injected	15.0 (30%)	32.9 (51%)	79	76
Saline Injected	32.2 (21%)	45.6 (66%)	81	80
SALTWATER				
Ambient Estuarine Water				
Hormone Injected	13.1 (30%)	31.0 (53%)	67	72
Saline Injected	26.1 (27%)	41.4 (61%)	67	76

1981 Followup

Maturation Success of Coho Salmon and Pink Salmon Held under Different Salinity Regimes

Alex Wertheimer

During last year's workshop, presentations were given detailing a tremendous variability in the maturation success of returning Pacific salmon broodstock held in seawater. Two of the possible factors contributing to the differential success rates identified were: 1) capture of returning adults in freshwater versus capture in the estuary and 2) the presence of a low-salinity lens in estuarine netpens, versus the uniform salinity of seawater raceways. In order to isolate these factors, I applied the experimental design outlined last year (Figure 1, preceeding article) to pink salmon and coho salmon returning to the National Marine Fisheries Service (NMFS) Little Port Walter research station in 1980. The data and analysis presented here should be considered provisional and subject to revision.

Adult pink salmon and coho salmon returning to Sashin Creek at Little Port Walter were captured either in the estuary with a small purse seine or in freshwater at the Sashin Creek weir. Fish from each capture group were divided into three types of holding units: 1) 800 ft^3 vertical floating raceways receiving single pass freshwater; 2) 800 ft^3 vertical floating raceways receiving single pass pumped seawater; and 3) 2,700 ft^3 netpens of 0.25 mm webbing susnended in the estuary with water exchange by tidal action. Salinities in the netpens and seawater raceways are shown in Table 1. Approximately 60 pink salmon and 50 coho salmon from each capture group were held in each type of holding unit.

The fish were routinely checked for ripeness and were spawned when judged mature. Holding time from capture to spawning varied to some degree with capture location. For pink salmon, all fish captured in freshwater were fully ripe in 12-14 days, versus 14-22 days for fish captured in the estuary. For coho salmon, fish captured in freshwater were ripe in 15-28 days, versus 26-40 days for fish captured in the estuary. Gametes from fish held in freshwater raceways were crossed with gametes from indiviauals of the opposite sex from each of the three

TABLE 1: Salinities (0/00) in seawater raceways and netpens used for holding pink salmon and coho salmon adults to maturity.

Species	Seawater Raceways Mean	Seawater Raceways Range	Netpens Surface Lens Mean	Netpens Surface Lens Range	Netpens Below Halocline Mean	Netpens Below Halocline Range
Pink Salmon	30.7	30.1-31.7	18.6	9.9-23.9	29.9	25.8-31.0
Coho Salmon	30.4	28.2-31.2	10.9	4.5-19.3	29.1	24.3-30.7

holding units; gametes from fish held in netpens or seawater raceways were crossed with gametes of individuals of the opposite sex from the same holding unit and from the freshwater holding unit. The individual matings were incubated separately in partitioned Heath incubator trays. Successful maturation of the fish was measured in two ways: 1) percentage of fish held that survived to spawning and 2) gamete viability, defined as the percentage of eggs from each mating that were fertilized and survived to the eyed stage. Blood and gonadal fluid samples were taken from each fish at spawning for determination of Na^+, K^+, Cl^-, and total osmolality.

Pink salmon adults captured in freshwater and held in seawater raceways had a significantly lower ($P < 0.05$) survival rate to spawning than those held in estuarine netpens or freshwater raceways (Table 2). There was virtually no difference, however, in survival among the three holding units for pink salmon captured in the estuary (Table 2). Gamete viability of pink salmon held in seawater was lower for fish captured in freshwater than for fish captured in the estuary (Table 3). Gamete viability of pink salmon held in seawater was lower than that of estuarine netpen or freshwater raceway fish, regardless of capture method. There were no significant differences in gamete viability among freshwater and estuarine netpen fish for either capture group.

TABLE 2: Survival rate to maturity of pink and coho salmon adults held in freshwater raceways, netpens, and seawater raceways.

Species	Holding Unit	Freshwater Capture Male	Freshwater Capture Female	Freshwater Capture Total	Estuary Capture Male	Estuary Capture Female	Estuary Capture Total
Pink Salmon	Freshwater	100	100	100	100	100	100
	Netpen	100	93.5	96.7	100	100	100
	Seawater	72.0	81.2	77.2	96.7	100	98.3
Coho Salmon	Freshwater	100	96.0	97.9	100	100	100
	Netpen	79.2	92.0	85.7	87.0	92.6	90.0
	Seawater	65.0	53.6	58.3	47.6	51.7	50.0

For both capture groups, coho salmon broodstock held in freshwater had a significantly higher ($P < 0.05$) survival rate than estuarine netpen fish, and netpen fish had a significantly higher ($P < 0.05$) survival rate than fish held in seawater (Table 2). The coho salmon held in the

TABLE 3: Viability of gametes from pink salmon adults held under various salinity regimes. Vaibility was determined by the percentage of eggs within individual pairings surviving to the eyed-stage of development. n = number of pairings, $\bar{x}$ = mean percent survival.

MALE COMPONENT	FEMALE COMPONENT: Freshwater n	$\bar{x}$	range	Netpen n	$\bar{x}$	range	Seawater n	$\bar{x}$	range
Freshwater Capture									
Freshwater	16	94.0	72.6-99.5	16	92.0	4.6-99.8	16	79.8	0-99.6
Netpen	16	92.9	3.9-99.9	16	92.5	3.9-99.9	----------		
Seawater	16	87.1	6.8-100	----------			15	73.8	0.5-98.7
Estuary Capture									
Freshwater	15	94.8	55.3-99.6	14	97.1	89.5-99.5	15	92.6	66.4-98.4
Netpen	13	94.8	61.2-99.8	15	96.7	84.0-99.2	----------		
Seawater	14	91.1	52.8-99.8	----------			15	91.3	70.6-98.7

TABLE 4: Viability of gametes from coho salmon adults held under various salinity regimes. Viability was determined by the percentage of eggs within individual pairing surviving to the eyed-stage of development. n= number of pairings, $\bar{x}$ = mean percent survival.

MALE COMPONENT	FEMALE COMPONENT: Freshwater n	$\bar{x}$	range	Netpen n	$\bar{x}$	range	Seawater n	$\bar{x}$	range
Freshwater Capture									
Freshwater	16	97.0	85.8-99.7	15	93.9	80.6-99.6	15	90.1	66.2-98.9
Netpen	15	96.4	90.1-99.9	15	92.8	74.6-99.0	----------		
Seawater	10	97.0	93.3-99.3	----------			10	95.3	81.6-98.7
Estuary Capture									
Freshwater	17	97.5	88.0-99.7	15	93.5	70.3-98.7	14	88.6	48.9-98.5
Netpen	15	93.9	78.9-99.0	15	89.8	68.4-98.0	----------		
Seawater	10	98.0	96.0-99.6	----------			10	87.3	57.8-98.3

hyperosmotic environments also had lower gamete viability than fish held in freshwater (Table 4). There was little difference, however, between the gamete viability of coho salmon held in estuarine netpens and those held in seawater raceways.

I have not completed the analysis of blood and gonadal fluid samples for ion levels; however, I have assessed total osmolality for these physiological parameters. Pink salmon held in seawater raceways and coho salmon held in netpens and seawater raceways had very variable levels of both blood and fluid osmolality. For both pink salmon and coho salmon, there were significant correlations ($P < 0.005$) between

gamete viability and either female blood or ovarian fluid osmolality. For both species, ovarian fluid was the most highly correlated and explained 13% of the observed variation in gamete viability for pink salmon and 16% for coho salmon.

Capture location did have some effect on survival and gamete viability of pink salmon held in seawater. The most important effect of capture location, however, may be the reduced holding time for fish captured in freshwater. The higher gamete viability of pink salmon held in estuarine netpens versus that of fish held in seawater raceways, and the high survival rates to spawning of coho salmon in estuarine netpens versus those held in seawater raceways, clearly indicate that the low-salinity lens is an important factor contributing to the maturation success of brood fish held in estuarine netpens. Although the maturation success of coho salmon in the estuarine netpens was somewhat less than that of fish stock held in freshwater, maturation in netpens was still adequate to recommend this technique as an alternative in coastal areas where freshwater holding facilities for broodstock are limited.

The relatively high average gamete viabilities of fish surviving to spawning in seawater indicate that successful maturation and osmoregulation in a hyperosmotic environment are not incompatible. The wide range of blood and fluid osmolality of fish matured in saline environments is indicative of great variability in the capability of individaul fish to respond to the osmoregulatory stress of maturation under hyperosmotic conditions. This potential of high gamete viability and the individual variation in osmoregulatory competence suggest that selective breeding for seawater maturing broodstock could be successful.

1981 Followup

Effects of Holding Adult Chum Salmon in Fresh Water on Gamete Quality

Mike McDowell

Purported problems with egg-to-fry survival in pink and chum salmon ocean ranches in Alaska, as well as the need to review standard hatchery practices, prompted questions regarding factors affecting gamete quality in Pacific salmon. The objectives of the current study are to determine if the length of time adult chum salmon are held in freshwater or the time of their arrival within a run affects their fertility, the occurrence of monsters and the sex ratio of their offspring. Investigations at Big Beef Creek Research Station, under the auspices of Sea Grant, have addressed these questions.

The data from studies in 1979-1980 indicate length of holding as long as fish remain alive, or time of arrival within a run have no biologically significant influence on fertility, the occurrence of monsters or the sex ratio of offspring. Analysis of fertility data by three factor analysis of variance indicates holding females has a significant effect of fertility. However, the lowest mean fertility of any treatment group was 91.1 percent and because deviation was non-linear it was felt this indicated handling as the main source of variation. Similar analysis of monstrosity and sex ratio data shows none of the treatments had any significant effect on either of these factors. The occurrence of monsters remained below 2.4 percent and almost all deformities appeared to arise from physical restrictions in rearing compartments rather than genetic deficiencies. Sex ratios of offspring were not significantly deviant from 1:1. The results indicate almost no significant deviations caused by treatment effects and in the case of female holding period affecting fertility handling is the probable cause of variation.

The studies will be continued in 1981-1982 in a manner more sensitive to measuring individual deviation as well as verifying the results mentioned above.

1981 Followup

Control of Sex Ratio in Pacific Salmon Broodstock

Edward M. Donaldson and George A. Hinter

The presence of the normal 1:1 male to female sex ratio in salmonid broodstock is disadvantageous to the maximization of egg take in sea ranching and aquaculture. It would be advantageous for both captive broodstock aquaculture and sea ranching to have a higher proportion of females. In the pen rearing of broodstock, minimizing the number of males held would reduce the cost of egg production. In sea ranching, the maximization of the proportion of females in the anadromous migration would provide two options to the hatchery manager. He could either reduce the spawning escapement and still obtain the same number of eggs or alternatively he could maintain the current escapement and obtain a larger egg take. Increasing the proportion of females also has the benefits of reducing the numbers of precocious males (jacks) and increasing the landed value of the catch (roe production).

There are two techniques being developed for increasing the proportion of female Pacific salmon. The direct technique involves the estrogen treatment of alevins and fry and results in the differentiation of all female groups of salmon, half of which are genotypic females and half of which are genotypic males. The indirect treatment involves the androgen treatment of alevins and fry to cause genotypic females to differentiate as males. These males then produce "female" milt which is expected to result in the production of groups of 100% normal genotypic females when used to fertilize normal eggs.

To date, we have produced all female groups of adult coho by the direct method. These fish have been successfully spawned and produced viable off-spring with a high hatching rate. This technique has also been successfully applied to chinook and pinks. Experiments are currently underway on the testing of the indirect technique for coho and chinook.

In addition to the above studies on the production of all female groups of Pacific salmon similar methodologies have been successfully applied to the production of sterile coho, chinook and pink adults.

1981 Followup

Hormone Induced Ovulation in Coho Salmon, Steelhead Trout Hybrids, and Atlantic Salmon

Stacia A. Sower, Walton W. Dickhoff, and Robert Iwamoto

Early ovulation (spawning) of coho salmon (*Oncorhynchus kisutch*) can be induced by treating fish with partially purified coho salmon pituitary gonadotrophin (SG-G 100) and an analogue of the brain hormone, luteinizing hormone releasing hormone (LH-RHa; D-Ala^{6}-des Gly^{10} LHRH ethylamide). Ovulation of salmon has been advanced by as much as six weeks using doses of 0.1 mg/kg SG-G 100 and doses of 50 µg/kg LH-RHa. Presumably, the ovarian response to hormone treatment is mediated through the hypothalamic-pituitary gonadal axis. The response of the ovary to hormonal treatment is dependent on both the stage of gonad maturation and the dosage of hormone administered. One of the objectives of this work is to determine the precise endocrine or neuroendocrine mechanism of hormonal induction of ovulation. Another aim of our studies is to establish the minimal effective dose (the most cost-effective technique) for hormonal induction of ovulation in salmonids. Obtaining eggs at an earlier time as a result of induced ovulation allows additional time for freshwater growth of fish so that salmon smolts can be released at a larger size which should optimize their success in the seawater phase of their life cycle.

Coho salmon were injected with 0.1 mg/kg SG-G 100 followed by injection of one of three doses (50 µg/kg, 5 µg/kg or 0.5 µg/kg) of LH-RHa three days later. Fish were tagged with Peterson disc tags and held in the spawning pond at the College of Fisheries, University of Washington, Seattle. The fish were checked for degree of maturation (ripeness) approximately every other day. Injections were initiated on October 24, 1980; 38 female and 5 male coho salmon were anesthetized with 2-phenoxyethanol, weighed to the nearest 0.1 kg and injected intraperitoneally with hormone or 0.6% saline. The three dosage schedules of gonadotrophin and LH-RHa were equally effective in inducing over 75% ovualtion by day 12, compared to 12.5% spontaneous ovulation of control fish. Maximal ovulation of control fish occurred 3 to 4 weeks later than that of injected fish. Thus, although these dosages of LH-RHa are the lowest that have been shown effective for

induction of ovulation in salmon, a minimal effective dose has not yet been established.

In a parallel series of tests, Atlantic salmon (*Salmo salar*) held at Northwest and Alaska Fisheries Research Center, National Marine Fisheries Service, Seattle, Washington, were injected with coho SG-G 100 (0.1 mg/kg) followed three days later by LH-RHa (60 µg/kg). The hormone treatment induced ovulation at an earlier date than it occurred in controls; however, ovulation in treated fish was prolonged compared to control fish. Several possible reasons may be offered to explain this prolonged period of ovulation; 1) the injections may have been given at the improper maturational stage, or 2) coho gonadotropin may be species specific. To further analyze the effects of an *Oncorhynchid* gonadotropin injected into a species of *Salmo*, steelhead trout hybrids (*Salmo gairdneri*), held at College of Fisheries, Seward Park, Seattle, Washington, were injected according to the same treatment protocol as for the Atlantic salmon. SG-G 100 followed by LH-RHa was effective in inducing 100% ovulation by day 10. In contrast, in control fish 100% ovulation was not achieved until day 26. These data indicate that the use of gonadotropin from coho salmon can be an effective primer for inducing ovulation with LH-RHa in a *Salmo* species.

1981 Followup

Salmonid Gamete Storage

Joachim Stoss

In continuation of the work on gamete storage, attempts were conducted to freeze salmonid eggs. Fertilized but not water hardened eggs from steelhead trout (*Salmo gairdneri*) and coho salmon (*O. kisutch*) were used for the studies. Although no successful freezing and storage at temperatures of -196^{0} C was achieved, some important information was gained:

- Exposure of eggs to an artificial media containing 1 Mol DMSO causes osmotic shock and reduced hatchability. This effect was eliminated by gradually equilibrating to DMSO.

- DMSO concentrations of 2 Mol and higher were not tolerated by coho eggs.

- Immediate survival of eggs after ice formation (-4.6^{0}C) in an artificial media containing 1 Mol DMSO is high ($\wedge$ 80%) but hatching is reduced.

- Freezing at 0.3^{0}C/min, 1 Mol DMSO, was not successful when -20^{0}C and -30^{0}C was reached.

1981 Followup

Mechanical Shock Sensitivity of Coho Eggs

J. O. T. Jensen

Research into the well-known but poorly understood problem of mechanical shock sensitivity of salmonid eggs is continuing. Using coho eggs, activated and incubated at 10°C, a series of shock tests were conducted with a device that allowed for repeated standard shock intensities ranging from 0 to 13,700 ergs (drop height of 0 to 50 cm). The data has been analyzed to yield median tolerance limits (TLm) from one minute after water activation to two days prior to 50% hatch.

The TLm estimates indicate that three levels of shock sensitivity occur for coho eggs. Sensitivity rose rapidly, reaching the first level of sensitivity between five to ten minutes after water activation. Sensitivity then increased between 45 minutes and two hours to a second higher level of sensitivity for the period of two to 72 hours from water activation. A further increase to the highest level of sensitivity occurred on day four and remained at this level of high sensitivity until day 14. At this time (blastopore closure) a reduction in sensitivity commenced until day 20 (just eyed), when no egg mortalities occurred at the maximum shock intensity of 13,700 ergs. Eggs remained resistant to shock (no mortalities at the maximum shock intensity) until day 43, two days prior to 50% hatch.

These results indicate that if eggs are to be handled after water activation this should be done as soon as possible, without the customary one to two hour delay to allow the eggs to "water-harden." Further tests with other species and other water quality conditions are planned.

Summary

Terry Nosho

This section amalgamates findings, conclusions, and recommendations for the two workshops. Papers in the preceding section are of sufficient length to serve as abstracted presentations.

1. Reports provided by salmon farmers demonstrated that they all experienced great improvements in adult survival and gamete viability. These improvements were primarily attributed to: 1) improvements in fish culture techniques; and 2) the use of fresh water to trigger the maturation process.

2. The problem of maturing broodfish in salt water still remains. A partial solution might be stock selection. It appears that some stocks are more adaptable to saltwater maturation. However, the long-term benefit may come from defining the mechanism involved in maturation. Further research should be carried out on these aspects because of the potential cost savings that might accrue to the private sector.

3. The NMFS estuarine netpens situated in a halocline environment appear to be a practical and valid method for maturing broodfish, particularly in Southeast Alaska where freshwater lensing occurs.

4. Basic science is becoming more and more important in fish culture. Some examples of this as reported in the workshops are as follows:

1. *Sex control.* Potential benefits might be a reduction in the required spawning escapement, increased egg take, reduction of the proportion of precocious males, or increase in landed value via roe sales.

2. *Induced ovulation.* The major benefit would be providing for an earlier egg take. Subsequently, a longer growth period during

rearing would produce larger fry. Also, it may become possible to salvage eggs from diseased or stressed fish before they die.

3. *Gamete storage*. Benefits that might accrue relate to the possibilities of fertilizing different lots of eggs that vary by area and/or time of return. Applications might be very similar to those used in the dairy and cattle industries.

5. In general, recommendations arising from the 1980 workshop are still valid. Of particular interest is the histological evaluation of normal salmonid development including vitellogenesis through final maturation (recommendation 4).

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José M. Ridelman

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