Development of Culture Technology to Support Restoration of Herring in Prince William Sound: Use of *in vitro* studies to validate and optimize restoration actions

Project 090821

Objective 2

Herring Culture Synthesis Report

EXECUTIVE SUMMARY

The decline and persistent low abundance of Pacific herring in Prince William Sound since the early 1990's has prompted interest in evaluating methods of stock supplementation as a potential mechanism to assist in recovery of the population. Studies indicate that the *Exxon Valdez* oil spill likely impacted recruitment early on, but other stressors such as pathogens (e.g. VHSV, ICTH) and shifting predator-prey abundance also appear to have contributed to the decline and may now be limiting recovery. This suggests intervention in the form of artificial propagation may be needed to restore PWS herring to levels that can effectively support the marine food web and provide for sustainable fisheries.

Stock supplementation projects are typically designed to protect young fish through their most vulnerable life stages (egg and larval stages) when the greatest mortality occurs. To be successful, such projects must support favorable conditions for nurturing the production of healthy juvenile fish in large numbers that can be released back into the environment. Thus, detailed knowledge of optimal husbandry conditions, favorable nursery conditions for rearing and release of juvenile fish, as well as the effects of restoration on life stage development are critical building blocks in the formation of successful herring restoration methods. Although extensive research has been conducted on herring life history and ecology, little is known about the husbandry requirements for herring outside of Japan.

The contemporary program for artificial propagation of herring in Japan dates to the early 1980's when the Japan Aquaculture Association was petitioned to develop aquaculture techniques for local herring stocks on the island of Hokkaido. Initial efforts focused on stocks in the Furen and Akkeshi Lakes region in eastern Hokkaido and were subsequently expanded to western Hokkaido and northern Honshu. Local fishermen had a major role in the development of the program through regional fisheries cooperatives and the the Nemuro Jurisdiction Herring Seed Production Management Commission.

The history of and techniques for culturing herring were described in detail by Yamamoto (2001) in a technical report titled Herring Fry Production Techniques, published by the Japan Aquaculture Association Corp. The document provides basic information on the herring culture technique development that occurred at the Japan Aquaculture Association, Akkeshi Station and considerable information regarding the ecology and physiology both for culture of the parent stock and fry production techniques. This document was translated into English (M. Mottet, Alaska-Southeast Bio-Research, 2009) and is available through the Exxon Valdez Oil Spill Trustee Council. The document was published to help advance the establishment of herring aquaculture, not just for the Japan Aquaculture Association, [but] "also for people who are associated with herring aquaculture and who are making efforts to accumulate new information."

The following is a synthesis of this translated publication. It provides stakeholders and others interested in stock supplementation with an overview of the potential for use of these techniques in PWS. Although not complicated, the techniques developed and employed in Japan span much of the herring life cycle and give stakeholders a range of options that can be considered to accommodate specific environmental and ecological conditions within PWS.

HISTORY OF HERRING CULTURE IN JAPAN

Herring research in Hokkaido began in 1870 with studies on fishery catches and was formalized with establishment of the Hokkaido Fisheries Research Laboratories in 1901. Early research at the lab focused on herring life history and was later expanded to collect information on migratory movement, spawning and feeding. These data were important for making predictions on the migratory patterns and fishery forecasting. Additionally, considerable effort was also made to identify and analyze morphological and genetic characteristics of the herring races in the vicinity of Hokkaido and Sakhalin. Propagation projects were initiated by the Akkeshi Fisheries Cooperative in the 1920's and 1930's and focused on developing techniques for artificial hatching and release of larval fish. These were followed by large scale releases of 20-40 billion fry over a 12-year period from 1941-1952 that were largely ineffective in producing adult fish. Subsequent studies clarified the basic culture conditions for rearing herring with experiments on the larval temperature and salinity tolerances, feeding, and phototaxis. By the 1970's experimental culture projects had demonstrated successful rearing of herring through the fry stage, but large scale fry production remained elusive.

Efforts to develop herring nursery production for resource enhancement were started in 1982 and the Akkeshi and Miyako Stations of the Japan Aquaculture Association. Ultimately both of these projects were successful such that current techniques now provide a stable production of 1,000,000 or more herring (>65 mm length) with a 40% survival rate to release. Two fishery councils (Furen Lake, Akkeshi Fishery Cooperative) manage these programs in eastern Hokkaido and have conducted follow-up studies on recovery rates to estimate migratory dispersion. Evidence from Furen Lake and Akkeshi suggests that releases of artificially cultured herring fry have contributed substantially to adult harvests since the onset of the program. Similar activities have taken place at Miyako Bay, Bekkai and in Miyagi and Aomori prefectures.

BIOLOGICAL CHARACTERISTICS OF HERRING AND THE HERRING FISHERY IN JAPAN

Herring are distributed from northern Hokkaido to central Honshu. They grow rapidly and most populations mature at 2-3 years of age at 20-25 cm in length. Life span can reach as much as 18 years but varies widely among populations. Numerous biological and ecological differences among the populations indicate that herring in Japan have considerable genetic diversity. They are broadly defined into 4 distinct ecotypes based on their spawning ecology, the scale and direction of their migratory movements: lake and marsh region type, oceanic region type, widely dispersed oceanic region type, and intermediate region type.

Lake and marsh type herring spawn in lakes and marshes with low salinity, brackish water and utilize aquatic plants such as eelgrass for egg deposition. Larvae and fry live in marshes and lakes and immature fish tend to remain adjacent to coastal areas. Spawning site fidelity is believed to be strong, but these populations have been historically limited in abundance. Ocean region type herring spawn in coastal waters of high salinity where fry remain until moving offshore. Site fidelity of adults is not well known and the resource is not particularly large (annual catches typically < 30,000 tons). The oceanic widely dispersed region type were historically the most abundant stock with catches that

in some years exceeded 1,000,000 tons. These fish also spawn in coastal areas with high salinity (32-34‰), but over a very broad area. They make large scale migrations, mature at a later age (3-5 years) and are long lived. Major populations include Hokkaido-Sakhalin and Okhotsk races. As the name suggests, the intermediate region type herring exhibit characteristics between those of the lake and marsh type and the oceanic type. Spawning occurs in areas with comparatively low salinities, but their migratory range is somewhat large. Age, life span, and growth are also intermediate between the other types. Records indicate fish catches ranging from several thousand tons to 10's of thousands of tons.

Catch records for the modern herring fishery in Japan show rapid growth from the late 1800's and then a subsequent decline over the next 70 to 80 years. The fishery targeted two major stocks; the coastal spring herring stocks that migrated along the western and northeastern coast of Hokkaido, and the spawning aggregations later found in the Sea of Okhotsk and eastern coast of Kamchatka in Russia. At its peak 1897, the former yielded an annual harvest of nearly one million tons, whereas the combined Japanese and Russian catch of the latter had reached roughly half this amount by the early 1960's. In both cases, however, these large catches eventually gave way to marked declines in abundance such that herring catch in Japan today is generally less than 5,000 tons annually. Interestingly, the Hokkaido spring herring stock sustained a harvest over a much longer period than the stocks in eastern Russia. As late as the 1940's to 1950's the Hokkaido coastal fishery harvest exceeded 200,000 tons annually prior to collapsing entirely in the mid- to late-1950's. In contrast, the Okhotsk and western Bering Sea fisheries provided catches exceeding 200,000 – 300,000 tons for a period spanning the mid-1950's to mid-1970's before falling to about 10% of this level in less than 5 years.

Maturation and Spawning

Herring in Japan spawn from February until June depending on the population and area. Studies of cultured fish show that yolk formation begins during April-July with active accumulation taking place August to March, and the completion of egg yolk formation from late March through April. Egg development is synchronous and fertilization can occur up to 30 days after final maturation and ovulation.

Herring that return to the Furen Lake area on eastern Hokkaido arrive between late March and early May. The age structure and maturation schedule for this stock has been studied in detail. The catch is represented by fish ranging in age from 1-5 years, but dominated by fish 2-3 years of age, which are used to obtain eggs for stock enhancement. The gonad index (GSI) of these fish increases steadily from November to February, followed by a rapid increase in March. Eggs become optimally ripe in April. The timing of maturation (ripeness) is inversely related to the age and size of the fish.

Collection of Gametes, Artificial Fertilization and Incubation

Adult fish used in the production of herring fry commonly come from herring that have been landed in the commercial catch and have been bought in the marketplace. Adults are selected on the basis of freshness, lack of body trauma and degree of ripeness. Fertilization rates of >80% can be obtained from female fish landed 6-7 hours earlier if they are refrigerated. Sperm activity in males can be maintained for several days.

To obtain gametes, the ventral part of the parent fish is cut open with a scissors and the gonads are extracted and placed on trays. Immature eggs and testes are excluded. A rubber spatula is used to remove the eggs from the ovaries. The collected eggs are placed in a bowl and the total weight is measured. Testis are cut along their sides and the pieces are placed in a 70-mesh bag and are mashed to squeeze out sperm that is mixed from several males.

Eggs are fertilized using the dry method. About 50 ml of sperm solution is added to a bowl holding 600-800 g of eggs, which are then thoroughly mixed with a feather. After mixing seawater is added to the bowl then rinsed. Eggs are attached to either hatching screens or hemp palm brushes. Hatching screens are compact and effective for observing and counting eggs. Dead eggs can also be easily removed. Hemp palm brushes, in contrast, have a much greater surface area, eggs attached rapidly when placed in a mixing bowl with seawater, and egg loss is minimal. This is now the standard method used at the Akkeshi Station.

Egg Development

The effects of temperature and salinity on egg development have been well documented at the Akkeshi Station. Incubation temperature averages ~ 10ºC, and hatching begins at ~ 100 Dº (degree days) and is completed by 150 Dº. Hatching success was ≥ 75% when temperature remained below 17 ºC. Data from multiple studies have been used to generate a formula for the relationship between water temperature and the number of days for half of the eggs to hatch. Evidence also suggests that the cumulative temperature units to hatch may be stock dependent. For example, at Akkeshi Station the number of days until hatching was about 130 ºD when the water temperature was below about 10ºC, whereas only 90-110 ºD were required for hatch at 10ºC in the Hokkaido-Sakkalin race. Herring eggs develop normally at a wide range of salinities, although the range of tolerance appears to be stock specific. Stocks considered lake and marsh region type typically have a lower range of salinity tolerance than those of oceanic region type.

Egg Incubation

Various methods have been employed for egg incubation. Initially, specialized egg culture water tanks were used for incubation in conjunction with horizontal hatching screens. Eggs were supplied with filtered sea water that was exchanged every 24 hours and supplemented with aeration using 13 vinyl tubes to provide good water circulation. Larger tanks were subsequently used with hatching screens made of wood to created natural floatation. These screens were anchored to the tank bottom and positioned randomly to maintain high water flow across the eggs from air tubes located in each corner of the tank. This approach was also used with hemp palm brushes that were suspended across the top of the culture tank but without touching the tank bottom. Eggs are disinfected with an iodophore (50 ppm) 3-4 days after collection and again at the eyed egg stage.

DEVELOPMENT OF LARVAE AND FRY

Basic information on the herring developmental process is important for the production

of herring fry. Descriptions of the developmental process of the principle exterior morphological features, the skeleton, fins, gastrointestinal tract, muscles, otoliths, etc. include systematic observations of changes in body parts relative to duration of the developmental process, as well as the associated morphology of developmental steps, physiology, and life history characteristics.

Developmental process and basic observations

Considerable information on herring development and the seed production process through the larval and fry periods has been collected at the Akkeshi Station and published by multiple authors. Herring growth stages resemble that of young sardines, including a white fry stag during which there are large changes in body form. These stages have been divided into the early stage larval period from hatching to a body length of 10 mm, late stage larval stage I from 10 to 18 mm; and late stage larval period II from a body length of 18-30 mm.

Skeletal Formation

Skeletal ossification of vertebrae begins at a body length of about 18 mm and proceeds rapidly until completion at a length of 21 mm, whereas all facial bones are ossified at a body length of 40 mm. Fin ray ossification begins at a body length of 14 mm and is largely completed when the dorsal and pelvic fins ossify at a body length of 30 mm. This time period coincides with the development of swimming behavior. In culture tanks, schooling behaviors began at 21 mm, and the completion of the fin rays was related to this swimming behavior. Scales begin to appear in the area of the pelvic fins at a length of 36 mm, and gradually spread widely to support areas of the whole body. By 50 mm the entire body was covered with scales and by 58 mm in length the formation of the scales was nearly completed.

Development of internal organs

Yolk absorption in larval herring at Akkeshi Station occurs rapidly and has essentially disappeared within four days after hatching at which time the total length of the larval fish is about 9.8 mm. Similar results were obtained at the Miyako Station, and both reported large annual variations in the size of the egg yolk at hatching.

The gastrointestinal tract during the larval period is a simple tube with a constriction that differentiates into the pyloric cecum. The tube persists until fish reach a length of 27.0-29.0 mm, at which time the cecum has the same Y-type shape that occurs in mature fish. Other organs such as the pancreas and liver appear along the dorsal edge of the yolk and provide backup functions for the digestive tract until the constriction of the gastrointestinal tract. The development of the gastrointestinal tract in the early to late stage larvae have been divided in 5 stages: 1) Rod-shaped gastrointestinal tract, 0-1 day after hatching; 2) Basic formation of the stomach, total length 8.6mm; 3) Formation of the intestinal mucosal epithelium, total length 11 mm; 4) gastrointestinal tract rotation (pyloric cecum development), body length 20.0 mm; and 5) Formation of the mucosal epithelium in the stomach, total length 22.0mm. Immediately after hatching the air bladder also appears on the dorsal side of the gastrointestinal tract and connected to it by an air duct. At a total length of 26-32 mm, gas is introduced and the bladder opens.

Muscle development and swimming behavior

Swimming behavior occurs when 40-60% of the body lateral muscles are developed. Initial formation takes place during yolk absorption, followed by differentiation into small and large type muscle fibers. Up to a length of about 17 mm, the main development is an increase in the diameter of muscle fibers, and there was a small increase in the number of muscle fibers. At a body length of from 20 mm to 28 mm, there is a small increase in the diameter of the large type muscle fibers. Small type muscle fibers appear shortly afterward, which initiates a rapid increase in the number of muscle fibers. At a body length of 28 mm and larger, the rate of muscle fiber creation begins to slow. Thus, distinct changes in the development of the body lateral muscle occur between body lengths of about 18 mm and 30 mm, which coincide with an increase in swimming speed from 6.9 cm/sec (at a body length 11 mm) to 47.6 cm/sec (at a body length 46 mm). Presumably, this provides the capability to swim in dense schools, allows active feeding and escapement behavior.

Otolith development

Herring otoliths can be observed during embryonic development and at hatch the average diameter is 23 μm. Size increases rapidly thereafter reaching 42.3μm in diameter at 10 days, 90.4 μm at 20 days and 162 μm at 30 days post-hatch. The otolith constricts and becomes arrow shaped when the fish reach 60 mm. Daily growth rings can be identified with a light microscope until 60 days post-spawning.

Changes in body composition

Protein, nucleic acids, fats, and glycogen all increase during early development, but their proportions relative to body weight change considerably. Major changes occur at body lengths of 18-19 mm and about 30 mm. Triglycerides and phospholipids increased little until ~ 18 mm.
Protein/DNA ratio also increases at this time suggesting a period expanding cell size. This is followed by a decline in the protein/DNA ratio and higher RNA/DNA at about 22 mm suggesting active growth cell divisions and an increase in the number of cells. This is also the period during which body organs form. The phospholipid/body-weight ratio specific was constant supporting the view that neutral lipids are indicators of energy storage. Fat consumption is high during the metamorphosis period from 18 mm to 30 mm in length, suggesting that during the period of peak morphological changes the triglycerides are used as a source of energy. Once fry reach 30 mm in body length or more, there is a rapid increase in storage of triglyceride and glycogen indicating that the digestive system has the ability to accumulate energy.

Developmental stages related to feeding, nutrition and behavior

During the period from hatching until yolk absorption of the egg, the body length increases from 8.5 mm to 10 mm at which time there is a change from internal to external nutrition. Although many of the fundamental structures forming the organs are in place, numerous other character traits have not yet been expressed. For example, immediately after hatching, the larval fish lie recumbent on the sea bottom, swimming occasionally

before sinking back to the bottom. They later demonstrate strong phototaxis during the daytime and swim into the surface layers when the surface is calm. There is no nocturnal swimming at this time and herring larvae drift to the bottom with the currents. The larval fish are effectively controlled by the physical environment.

After the yolk has been absorbed and during the period of growth from 10 mm to 18 mm there are no major changes in the basic structure of the external morphology and skeleton. At this time the growth of the body is principally in length. The gastrointestinal tract is rudimentary and has the minimal organ differentiation necessary for feeding.The principle foods are eggs of calanoid copepods or nauplius larvae. Protein uptake by the intestinal epithelium cells is by pinocytotic absorption and there is little energy accumulation. Although movement remains largely passive, there is an improved ability to feed.

Between 18 mm to 30 mm in body length there is a marked change in metabolism and a metamorphosis. This is also the period of active differentiation in organ weights and tissue organization that leads to completion of the skeleton, fin, and gastrointestinal systems, etc. Energy demand is high and is supplied primarily by use of triglycerides. Sense organs such as the inner ear and lateral line develop, as do the lamella of the gills and air bladder. Active schooling behavior also begins. At this stage the larval fish are found primarily in mid and deep water layers. Feeding behavior is further refined.

Above 30 mm length organ and tissue differentiation shifts to growth. Sense organs of the visual system and lateral line continue to develop, gills surfaces expand and there is specialization, etc. of the circulatory system and respiratory organs. At 40 mm and larger black pigment cells enlarge and scale formation begins. By 60 mm herring have the characteristic appearance of the adult fish, and at 90 mm their appearance is the same as that of mature fish.

Throughout the period of fry development there is a characteristic increase in power capacity. Muscle fibers for basic movements are substantially completed and there is an improvement in the glucose metabolism system and enzymatic activity. With an increase in feeding opportunities and a complete functioning of the digestive system the variety of food items consumed increases. Stored energy is accumulated through triglyceride and glycogen metabolism, which strengthens their resistance to starvation, and allows for active swimming, feeding and movement into offshore waters.

NURSERY PRODUCTION

The annual schedule for producing herring fry at Akkeshi Station runs from mid March to mid July. Eggs are collected in early April and fry production begins when the larvae hatch in early May. In mid July when fry reach a length of 40 mm they are collected and counted. At a length of 50 mm they are transferred to sea cages for additional rearing before release.

Hatching and stocking

The hatching mechanism for herring eggs is similar to other fish species. Just prior to hatch, proteolytic enzymes are released from hatching glands on the head that dissolve

some of the egg membrane. Active movement inside the egg also promotes hatching since the egg membrane is elastic and has thinned. Generally, the larvae hatch from the egg head first. The amount of time for hatching after the head protrudes is typically several seconds and the larvae immediately begin to swim.

Before stocking the water temperature in the culture tanks is set at 10ºC and adequately aerated to minimize the potential for gas supersaturation. This aeration also helps to establish a weak current. The eggs are stocked in the fry production tank prior to hatching after reaching an accumulated water temperature of 70 Dº. Netting is placed over the discharge water of the culture tank so it can be examined for the presence of hatched larvae. The fish are counted using a volumetric method. The movement of eggs to the fry culture tank is performed rapidly to avoid temperature changes. Hatching commonly takes place at night and once completed the attachment substrates are removed from the culture tanks, generally at an accumulated water temperature of 150 Dº.

Hatching success in production tests at Akkeshi Station averaged 39% for Furen Lake production and 37% for Akkeshi. Hatching rates at other facilities are comparatively higher at 60-90%. Experiments with carefully selected brood stock at Akkeshi have yielded hatching rates of 60-80%, suggesting the broodstock quality (freshness) is important for hatching success.

To estimate the number of hatched larvae in the culture tank samples are collected from water column at night since larvae distribution during the day is uneven. Sampling begins 2 hours after sunset. The culture tanks are strongly aerated to mix the water and a polyvinyl chloride tube (diameter 50 mm) is used to sample the water column in 30 different locations. The number of fry in each tank is estimated volumetrically from the samples.

Rearing facilities and environment

The culture tanks at Akkeshi Station are rectangular and have a $50m^3$ capacity (7.8 x 4.8 x 1.4 m). The sides are treated with epoxy resin and the color is dark green. Water temperature is controlled with a boiler in the side of the tank that supplies heat to a titanium heat exchanger. At the start of the production season the water temperature is raised ~ 1ºC per day above the ambient level (mean 8.0ºC, range of 4.3-12.5ºC) until reaching 13ºC. Filtered, aeratered sea water from Akkeshi Bay is used for culture. Water from each tank is discharged through a hose and strainer fitted with a mesh screen that increases in diameter over the rearing period. The strainers are washed daily to remove debris. Daily water exchange (1/2 of the total rearing volume) begins 5 days after hatching. The daily exchange increases to a rate of once per day after 10 days, twice per day at 20 days, 3 times per day after 40 days, and 4 times per day after 50 days. Aeration is provided with air stones in the center of the tank and air tubes are installed in each corner. This configuration produces a slow circulating current that is increased as the herring grow.

Herring larvae exhibit phototaxis and school together in the presence of light. Light also increases the phytoplankton in the culture water, and for these reason, methods (shading screens, green water) are used to disperse the light in the culture facility at Akkeshi. A commercial concentrate of a freshwater species of *Chlorella* (1.0 ℓ per tank per day) is added to the water to provide color contrast.

Tank cleaning begins 5 days after hatching and is performed using an automatic bottom cleaning device once daily until the fry are harvested. This is supplemented with hand cleaning during the last half of culture. An important factor when cleaning is to avoid startling the larvae and fry that school on the bottom with *Artemia* that also settle there. Hence, cleaning is avoided until well after feeding. During cleaning, dead fish that have also accumulated in the drainage are retrieved with a net to volumetrically estimate mortality.

Feeding sequence and schedule

The standard feeding sequence is rotifers, *Artemia*, and formula feed. After hatching, rotifers are fed from day 1 to day 15; *Artemia* are fed from day 10 to 45; and formula feed from day 20 until landing. This schedule was developed from a series of experiments that were performed to determine the effects of feeding the various feed types on survival to specific life stages.

Live animal feeds

At Akkeshi Station the rotifers are batch cultured continuously in a constant temperature tank for 40 days prior to starting fry culture. The culture tanks are stocked at a density of 100 rotifers/mℓ that increases >500 rotifers/mℓ after 14 days. *Artemia* are hatched at a water temperature of 22°C and for 48 hours. Polyunsaturated fatty acids such as DHA and EPA and added during *Artemia* to enhance nutritional value. The feeding rate for *Artemia* is based on the time required for digestion and evacuation from the gut, which at a temperature of 8.6ºC-9.6ºC requires 7-8 hours to digest and an additional 5-7 hours for total evacuation. Feed consumption (number of prey consumed per feeding event) increases exponentially with fish length.

Formula feed

Formula feeding (commercial diet) begins 20 days post-hatch (18 mm). The diet is blended with live feed over a period of 5 to 7 days to make the transition. Feed is supplied using a combination of automatic feeders and hand feeding.

Growth

Fry and larvae are sampled ($n \geq 20$) and measured at intervals of 5 to 10 days to monitor growth. The average growth at Akkeshi Station from hatch through 55 days of feeding is approximately 0.6 mm per day. From 55 days onward the growth rate increases to an average of 0.8-0.9 mm per day. Individual variation in the growth in length also increases over time and is particularly related to the conversion from live food to formula feed. Temperature also has a direct effect on growth rate. At a water temperature of 10ºC growth was 0.37-0.39 mm/day, and at a temperature of 14ºC the growth rate was 0.69-0.70 mm/day. Above 14ºC growth either stayed constant or declined slightly. Using these data in conjunction with energy costs to heat the water, the optimal temperature for culture from both a growth and economic perspective is approximately 13ºC.

Survival and production results

Survival of cultured herring at Akkeshi Station varies widely over time. There is almost no mortality during the first 10 days after hatching, but from 10-20 days post-hatch total mortality rises to nearly 30%. Thereafter mortality declines slowly until ~ 60 days post-hatch when the cumulative loss reaches about 60%. Somewhat differing survival trends have been observed at Miyako and Haboro Stations. At these facilities mortality either as a large single peak near the beginning of the culture period (Miyako) or is low but relatively constant over time (Haboro), presumably because of differing culture methods.

The major causes of mortality during culture are believed to be due five factors: 1) source of eggs; 2) vulnerability of specific developmental stages; 3) failure to transition between dietary changes in feeds; 4) inadequate nutrition; and 5) Alizarin complexone (ALC) labeling treatments. Egg size, for example, may affect early survival. The high mortality that occurs between 10 and 30 mm length corresponds to the developmental stages of rapid organogenesis suggesting that there may be inadequate energy for metamorphosis. High mortality is also linked to nutritional deficiency, such as that which occurs during the transition from live to artificial feed. ALC labeling produces a rapid change in the physical environment hence prudent care is needed during the marking operation. To mitigate these effects, emphasis is placed on providing adequate nutritional enhancement of the live food and feeding to satiation, matching the amount of water exchanges with thorough bottom cleaning to maintain good water quality, and eliminating environmental stressors such as excess illumination. The cumulative effect of improvement in culture methods and conditions has resulted in nearly five-fold increase in survival from egg to fry at Akkeshi Station.

Otolith marking

Two fluorescent marking methods have been employed to label otoliths: alizarin complexone (ALC) and tetracycline hydrochloride (TC). ALC was tested on herring larvae at 20 and 40 days post-hatch. There was no difference in staining at ALC concentrations between 20 ppm and 40 ppm. However, no mark was observed at 10 ppm and below, and massive mortalities occurred at 80 ppm. A concentration of 20 ppm is now standard for marking during a 24-hour labeling treatment. Herring were tested with TC at 47 days post-hatch (17-18 mm in total length, water temperature 9ºC). The concentration of the TC was 200 ppm and the time period was 24 hours. Distinct fluorescence could be observed.

Oral administration of both ALC and TC has also been tested. Fingerlings (80 mm total length) were fed a formula feed with ALC added at 0.5-5% for a period of 10 days, but produced no discernible mark. TC was fed to herring fry (average total length of 60 mm) for a 5-day period. The method used was to absorb TC into the formula feed. TC was dissolved in distilled water until the water was a transparent orange color (the objective was 10 g of TC per 25 ml of distilled water). The solution was sprayed onto commercial formula feed where it spread over and was absorbed into the feed. (The amount added was 1% of the formula feed). Before use, the feed was air dried under refrigeration. The food was hand feed four times a day to satiation. At a concentration of 5% of the formula, there was a detectable but weak fluorescence produced. At 10% fluorescence was clearly

observed.

Otoliths collected from marked adults are read using a fluorescence microscope with red, G-excitation filter for ALC tags and a yellow B-excitation filter for TC. TC marking is affected by ultraviolet light, causing activity to decrease rapidly, thus TC marked otoliths are read within several days after recovery.

HANDLING AND TRANSPORTING HERRING FRY

Herring become tolerant to handling when the average body length exceeds 40 mm (60 days post-hatch). The ideal body length for handling is believed to be 50 mm when scales, bones and skeletal muscles are fully developed. Fry are collected from the culture tanks using a round haul net and hand dip nets, then transferred to transport tanks outfitted with small mesh netting that contain seawater. The number of fry collected is determined by a weight method in 5 liters of water. Oxygen is added to the culture tank during seining. Historically, mortality during fry collection and transfer has averaged ~ 3%.

During the transport period of 1.5 to 2.0 hours fish density is 20,000 fry/m^3. The seawater in the transport tank is exchanged at least once during the transfer period using a submersible pump. Supplemental oxygen is also use and all tanks are covered to prevent water spilling. Fry are transported overland from the hatchery to ships for transport to the nursery culture facility. A truck with a hoist is used to lift the transport tanks on to the transfer vessels. Upon arrival at the nursery culture facility (net pens) the fry are siphoned from the tanks into the cages.

NURSERY CULTURE

Generally, nursery culture is used to habituate fry to the natural environment. If the fish are larger in size they recover better from the effects of transport, are more adept at catching natural food, and to learn the characteristics necessary for their mode of life. This period also provides time during which the fry can accumulate adequate energy reserves so they can tolerate low food conditions, and improve swimming ability to catch prey and avoid predators.

A significant feature of local herring populations is that they exhibit a homing pattern for spawning. Although herring homing migrations for spawning are not as pronounced as they are in salmon species, most of the released fish return to the nursery culture area for spawning from where they were released. The mechanism by which the herring imprint on environmental characteristics is not clearly understood.

The herring nursery facilities in the marine areas on the east side of Hokkaido are located in Furen Lake, at Nokke and Akkeshi harbors. Tidal currents at these locations are comparatively slow. Locations are selected where the water is deep enough that the small-mesh nets do not touch bottom. Water temperature can vary widely in these locations during the culture period from mid-July to early August (e.g. 13.5ºC to 22.2ºC at Furen Lake), but generally remain within the range of tolerance for the species. Rainfall also has a marked effect on salinity and has resulted in rearing morality of as

much as 10%.

Culture facilities and operations

The net pens used for nursery culture at Furen Lake and Akkeshi Bay measure 4 x 4 x 2.5 m with four small mesh (4mm) nets (actual volume 32 m^3). The cage is anchored and a walkway extends over the net. Each cage is outfitted with an automatic feeder and bird netting.

The standard number of fry that are stocked in each cage during nursery culture is 40,000. However, higher rearing density was shown to yield equally high survival to release (99.6%), but with reduced growth. Further research is necessary to determine the optimal culture density during the nursery culture period.

During cage rearing herring are fed a commercial diet similar to that used for ayu (sweetfish) or salmon. The ration is ~ 10% of the body weight per day. Automated feeding is supplemented hand feeding twice daily, and natural prey such as calanoid copepods is also available. Currently, the general period for nursery culture is 14 days during which 50 mm fry grow to a total length of 70 mm and body weight increases from ~ 0.7 g to 2.7 g. Survival to release is typically 90% or more.

Natural foods play an important role in the diet and growth of herring during nursery culture. At Akkeshi Station calanoid copepods were the principal planktonic organisms, as well as polychaetes, crustacean larvae, rotifers, and barnacle larvae. Also, in the period from mid June to late July, species of rotifers were comparatively abundant. At Furen Lake, the planktonic species were roughly similar to those occurring along the coast. Results of gastrointestinal tract analyses show that despite adequate amounts of formula feed available to the herring in cages 7 days after being moved offshore at Akkeshi, the volume percentage of natural food made up 56.0%. In contrast, other sites (e.g. Furen Lake) are less than 1%. The differences appear to relate to currents at the nursery culture sites for transport of plankton.

RELEVANCE TO PWS HERRING RECOVERY

Large scale herring fry production for stock enhancement has occurred in Japan since 1982. The development and refinement of the techniques for herring culture has enabled communities and organizations throughout Hokkaido and northern Honshu to establish programs that have helped stabilize and restore local herring populations at levels that support sustainable fisheries. Similar methods may be suitable for use in rebuilding herring in PWS, provided that critical biological and ecological characteristics of the population can be defined so as to maximize growth, survival and recruitment of released fish to the spawning stock. The critical components include: (1) the degree to which adult herring exhibit homing or site fidelity to the area of origin, (2) whether large numbers of cultured fish can be effectively mass marked (and recovered) to determine their contribution to the spawning population, (3) the susceptibility of cultured fish to marine pathogens, and (4) production costs for large scale enhancement.

In Japan, studies on herring propagation have focused on populations that repeatedly

spawn at the same location. These populations have comparatively restricted ranges and at maturation return to coastal areas to spawn. Since they repeatedly return to spawn in limited spawning areas, it is comparatively easy to determine their abundance size of the resource and to obtain results from released fish. The herring stock(s) in PWS exhibit similar characteristics. Their natural range (PWS, near shore Gulf of Alaska) is somewhat restricted. Although inter-annual spawner abundance in specific areas in PWS can vary widely, spawner activity in specific areas (e.g. Port Gravina, Sheep Bay, St. Matthews) has been consistent in recent years. Mark – recapture studies at Furen Lake, Akkeshi and Miyako have clearly demonstrated that cultured herring originating from local stocks to the bays from which they were released, suggesting the potential for similar results with PWS herring. Identifying suitable areas with limited spawning abundance but consistent inter-annual spawning activity would be a key step in testing the hypothesis that cultured PWS herring return to their area (bay) of release.

Mass marking of cultured herring in Japan has been based on either immersion or oral administration of two fluorescing compound to label otoliths: alizarin complexone (ALC) and tetracycline hydrochloride (TC). Both have been shown to be effective, although there is ~ 10 fold difference in cost between the two compounds ($0.018 per fish for ALC, $0.0013 per fish for TC, see Reference Data Table Calculation 3). Regardless of the method, mass marking techniques for herring have been developed and used routinely in Japanese herring stock enhancement programs to assess the contribution of cultured fish to the spawning populations. Alternative methods (e.g. thermal otolith marking, otolith marking using trace elements / isotopes) may be prove technically or economically more effective, but the important point is that identification of cultured fish in the spawning stock is not a major impediment to supplementation.

In contrast to observations on both natural origin and cultured herring in Japan, pathogens have a marked effect on the health of herring in PWS. VHS has been reported in some marine fish species in Japan, but not in herring. If large scale production methods similar to those used in Japan are adopted for use in PWS, then techniques to mitigate the risks associated with VHSV and Ichthyophonus must be developed. Although techniques are available for early (hatchery) life stage production (e.g. depuration of hatchery water supply and discharge), this is not the case for the later stages of production associated with cage culture. Additionally, the transport of juvenile herring from shore-based facilities to seawater net pens is inherently stressful, which will exacerbate the risk to pathogen exposure. Development of pathogen specific vaccines or methods to boost innate immune response may be needed. Similarly, adjusting the timing, location and duration of cage culture may further aid in reducing disease risk.

The Japanese herring supplementation program is based on an approach that is technically intensive and hence has significant upfront capital costs as well as high operating costs. Yamamoto provides estimates for both total capital and operating costs related to the production of juvenile herring. In 2001 the estimate to produce ~ 1 million herring for release (at 70-80 mm length) was approximately 19 yen per fish (in $US today ~ $0.19 per fish). This reduces to ~ $0.08 per fish for 3 million herring due to the economy of scale. Assuming a similar approach with similar costs for PWS, a pilot project to evaluate the contribution of e.g. 1 million juvenile herring release would cost at a minimum of ~ $200,000 annually. This figure is almost an order of magnitude less than the lower estimated provided by Hay (2007) for a pilot scale release of 1 million fish in PWS, although Hay's estimate also includes substantial funds for strategic planning,

research, discretionary needs etc. Factoring in different assumptions for major expenditures such as facility costs (i.e. all up front or amortized) and labor, the actual cost for such a project is likely to fall somewhere between these estimates.

The Japanese herring program provides an excellent model for stakeholders to consider with regard to rebuilding the herring resource in PWS. Much of the methodology for successful juvenile production has been developed and refined over a period of 25 years. The stock enhancement / supplementation programs that have been built on this technology have clearly contributed fish to the spawning populations throughout Hokkaido and northern Honshu. Whether these programs will be ultimately self-sustaining (i.e. do the contributions to the spawning stock result in additional recruitment in the future, or are these simply put-and-take fisheries) remains to be seen. Nevertheless, the technical framework certainly exists for a pilot scale project to evaluate the utility of stock supplementation for PWS.