Progress Report to the Exxon Valdez Oil Spill Trustee Council for Project 070821: Development of Culture Technology to Support Restoration of Herring in Prince William Sound: Use of *in vitro* studies to validate and optimize restoration actions

Abstract: From April through June 2007 we traveled to and visited the successful herring enhancement program in Akkeshi, Japan, and also conducted studies at the Alaska SeaLife Center and the Seward Shellfish Hatchery to provide a preliminary evaluation of the potential to culture Pacific herring for possible stock supplementation in Prince William Sound. Our objectives were focused on determining: (1) the specific effects of salinity on fertilization and hatching success in PWS herring, (2) the effects of the timing of food availability on larval growth and survival, (3) if calcium sensing receptor proteins (CaSR's) were expressed in the tissues of larval herring and finally (4) establishing a scientific collaboration with the Hokkaido National Fisheries Research Institute for the exchange of technology and methods related to herring stock supplementation in Japan. Each of these objectives were accomplished during this time and taken together, provide the basis for the proposed development of a pilot scale project based at the Alaska SeaLife Center that will permit the testing of stock supplementation methods customized for use in PWS over the next 2 years.

Background: Persistent low abundance of Pacific herring in Prince William Sound has prompted resource managers to consider stock supplementation as a potential mechanism to assist in recovery of the population. As part of this effort, we conducted two preliminary studies in 2007 in support of our more extensive proposal to develop of a pilot-scale release project, and potentially a large-scale stock supplementation program. The results of these preliminary studies are described in this report, as well information related to herring stock enhancement practices in Japan that one of us (TL) obtained during travel there in April 2007.

In response to EVOS Science Panel review comments, we revised our 2007 proposal to address concerns regarding the need to acquire and implement methods for the rearing of larval herring that would provide a basic framework for potential stock supplementation. To accomplish this we constructed a laboratory-scale rearing facility at the Seward Shellfish Hatchery that consisted of culture tanks with a separate room for the production of live feed to conduct rearing studies, and utilized previously installed tanks at the ASLC supplied with flowing seawater and fresh water to conduct studies on the effects of salinity on larval development and survival. Travel to Japan by Tim Linley, Ph.D. was coordinated through the Fisheries Research Agency and Dr. Takahiro Matsubara, Section Chief of the Resources Enhancement Section, Coastal Fisheries and Aquaculture Division, at the Hokkaido National Fisheries Research Institute.

We had originally proposed that our preliminary laboratory studies would focus, in part, on the effects of spawning time on egg quality and survival to hatch. Due to the highly dispersed and irregular timing of herring spawning in PWS in 2007 it was not logistically possible to collect gametes throughout the spawning season. Hence, we obtained gametes and eggs from roe-on-kelp during a single collection in early April. These were used to test, respectively, the effects on salinity on survival to hatch, and the effects of delayed feeding on larval growth and survival. Knowledge of these effects will be important for

identifying suitable areas to support stock supplementation projects due to the wide range of forage and environmental conditions found in PWS.

Additionally, we had suggested that specialized proteins called calcium sensing receptors (CaSR) likely played an important role in allowing herring to adapt in varying degree to a wide range of salinity conditions, and that these regulatory proteins may also serve as molecular targets to control differentiation and growth of the GI tract during larval development. This hypothesis was based on multiple studies by MariCal (Nearing et al. 2002, Nearing et al. *in press*) and others (Radman et al. 2002, Loretz et al. 2004) showing that CaSR's function as salinity sensors in fish, and also coordinate sensing of L-amino acids in various osmoregulatory, sensory and nutrient absorbing tissues (Yamaguchi et al. 2000). Thus, a major objective of our preliminary studies was to determine if, when and where CaSR proteins were expressed in herring early life history. The results of these studies, as well as the information obtained on herring rearing practices in Japan, have been used to revise and refine our proposal for funding in 2008 and 2009.

Objective 1: Determine the effects of salinity on hatching success in PWS herring.
Hypothesis: Hatching success is optimal at intermediate salinity and decreases in full-strength seawater and near freshwater conditions.

Methods and Materials

Eggs and sperm were collected from 30 spawning adult herring (15 of each sex) in Matthews Bay in Prince William Sound on April 2, 2007. The gametes were transferred to the Alaska SeaLife Center in a cooler where they were divided into three groups (5 fish of each sex) for fertilization and incubation in seawater at 32 ‰, 16 ‰ and 8 ‰. Unfertilized eggs from 5 females were pooled into three groups and were spread onto 9 (n=3 for each group) 250 cm^2 glass plates etched with a grid for counting.

The sperm from 5 males was also pooled into 3 groups and was placed into a small bucket filled with seawater corresponding to 32 ‰, 16 ‰ or 8 ‰ salinity. The three glass plates (eggs from 5 females) were placed into each of the buckets and allowed to sit for 10 to 15 minutes. Glass plates were then removed and placed into one of three larger (300 L) tanks supplied with flowing seawater and freshwater to produce corresponding salinities of 32 ‰, 16 ‰ or 8 ‰ at a flow of 5.0 $L \bullet min^{-1}$.

Salinity, water temperature and pH were monitored throughout the trial. Sub-samples of eggs were taken for histological examination and to document development on April 6, April 14, April 20, April 28 and May 10. Counts were conducted on certain grid cells three days after fertilization and at the end of the trial to estimate survival. The grid cells used for counting were not used for sampling.

Results and Discussion

The mean (± SD) salinity, temperature and pH for each of the three treatment groups are summarized in Table 1. Eggs from each of the groups that were fertilized on April 2 began to hatch on May 3 and completed hatching on May 10. Each of the treatment groups developed at approximately the same rate despite wide differences in environmental salinity.

Treatment Group	Temperature	Salinity	pH
32 ‰	4.1 ± (0.2)	31.7 ± (0.3)	8.1 ± (0.02)
16 ‰	4.1 ± (0.1)	15.5± (0.5)	8.2 ± (0.01)
8 ‰	4.7 ± (0.2)	8.1± (1.2)	8.2 ± (0.04)

Table 1. The mean (± S.D.) salinity, temperature and pH for the three incubation treatment groups from April 3 to May 10, 2007.

The photographs shown in Figure 1 are a composite illustration of the development of the three treatment groups. At three days post-fertilization (DPF) all 3 treatment groups exhibited evidence of fertilization with nearly all eggs showing development (Figure 2A). By eleven DPF developing embryos were distinguishable (Figure 2B) and by 14-16 days eyes were beginning to form (Figure 2C). Eyes were pigmented by approximately 25 DPF (Figure 2D), followed by the start of hatching at 30 DPF (Figure 2E).

Although eggs in each treatment group were successfully fertilized, many of the eggs incubated in at 32 ‰ and 8 ‰ failed to develop. The number of fertilized eggs within selected grids on each of 3 hatch plates (i.e. egg count at start), and the resulting number of eggs that failed to hatch (egg count at end) and the estimated survival for the three treatment groups are reported in Table 2. Survival for eggs incubated at 16 ‰ (47.5 %) was significantly higher ($P < 0.01$) than for eggs incubated at either 32 ‰ (8.9 %) or 8 ‰ (28.6 %). There was also a significant difference ($P = 0.03$) in survival between the eggs incubated in 32 ‰ and 8 ‰.

Salinity Group	Egg Count @ Start	Egg Count @ End	Estimated Survival
32 ‰	1 – 80 2 – 103 3 - 164	1 – 67 2 – 97 3 - 152	1 – 16.3 % 2 – 5.8 % 3 – 7.3 %
	347	**316**	**8.9 %**
16 ‰	1 – 120 2 – 60 3 - 62	1 – 68 2 – 26 3 - 33	1 – 43.3 % 2 – 56.7 % 3 – 46.8 %
	242	**127**	**47.5 %**
8 ‰	1 – 79 2 – 17 3 - 14	1 – 61 2 – 13 3 - 10	1 – 22.8 % 2 – 23.5 % 3 – 28.6 %
	110	**84**	**28.6%**

Table 2. Egg counts on labeled hatch plates and estimated survival at the beginning and end of the salinity tolerance trial.

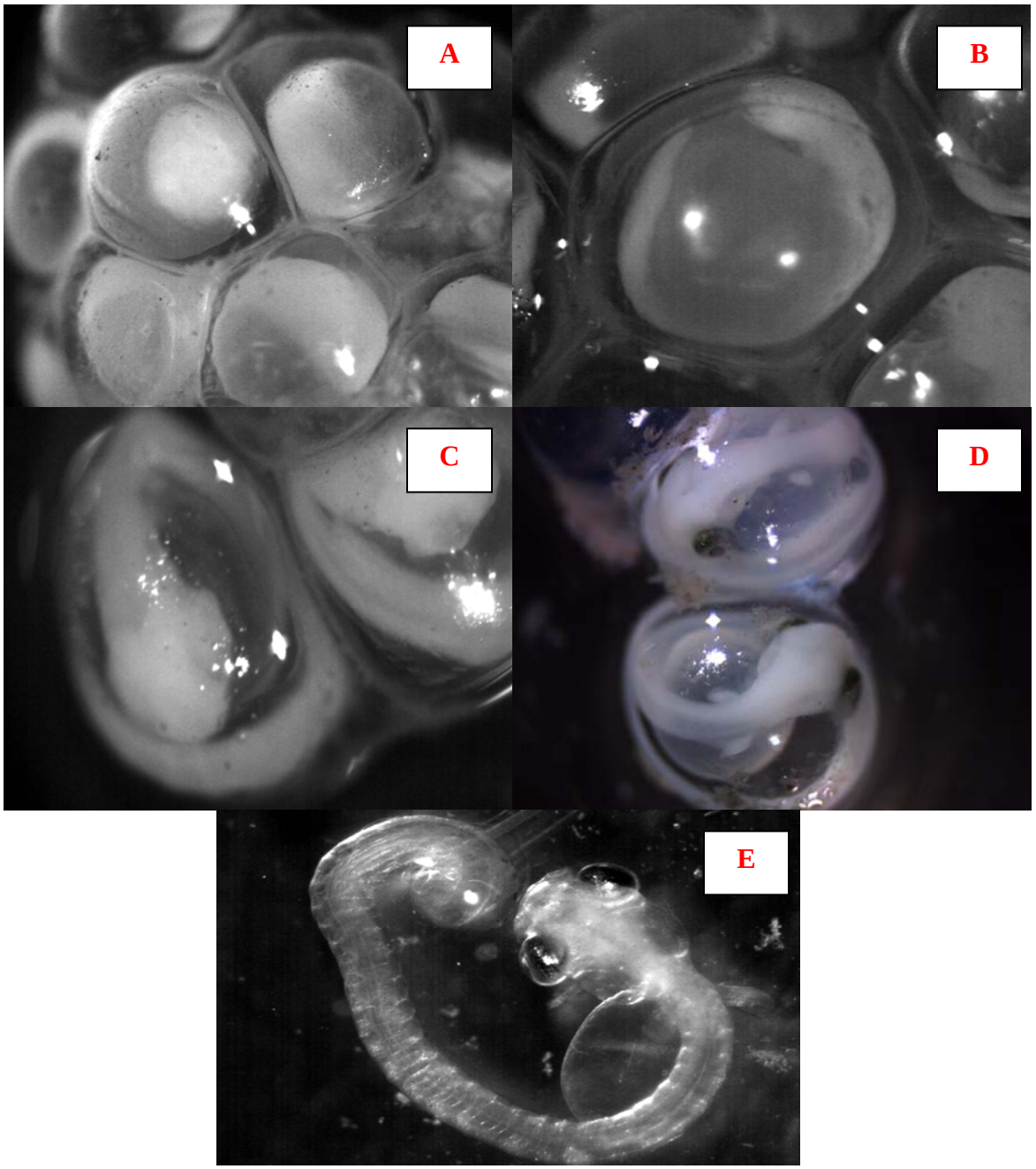

Figure 1. The developmental stages of herring from fertilization to hatch. A – fertilized eggs (3 DPF), B - developing embryo (11 DPF);, C – eyes are beginning to form (16 DPF), D – fully developed embryo approaching hatch (25 DPF), E – hatched herring larvae (30 DPF).

These results confirm previous studies showing that fertilization and hatching success in herring are generally highest at intermediate salinities and decline rapidly in full strength seawater and highly brackish (< 8 ‰) conditions (Dushkina 1973, Alderdice and Houston 1985, Griffin et al. 1998). Moreover, PWS herring also showed higher survival and hatching success at lower rather than higher salinity, similar to that reported for other stocks. This result may have implications for identifying potential stock supplementation release sites in PWS. Presumably, these would be located in areas where herring spawning has been documented, but no longer occurs.

Conclusions: Based on our preliminary findings, lower rather than higher salinity sites would be favored if adults are expected to have some degree of fidelity and return to their release site or area to spawn, although areas in which surface water salinity is intermediate (~16 ‰) would clearly be the most suitable. Identifying such sites will be an important part of integrating future stock supplementation studies with ongoing efforts to characterize nursery areas in PWS (see Objectives 2 and 7 in proposed 2008-2009 studies).

Objective 2: Determine the effects of delayed feeding on growth and survival of larval PWS herring. *Hypothesis: Delayed availability of live feed impairs subsequent feeding behavior and feed assimilation leading to elevated mortality in larval herring.*

Methods and Materials

Roe on kelp was collected from Prince William Sound and transferred to the Seward Shellfish Hatchery on April 2, 2007 where it was placed into a 300 L tank supplied with 5.0 L • min^{-1} seawater at approximately 32 ‰ and 4.2 °C (Figure 2a). After 5 days flow to the holding tank was changed to a well that provided seawater at 26 ‰ with a temperature ranging from 6.0 to 7.5 °C. Eggs/larvae were sub-sampled for histology, document development, measure length (larvae only) and to monitor feeding preferences every 2nd to third day until May 12, 2007. After this date, sampling coincided with any change in feed type or amount.

Hatching began on April 20 and was finished by April 22, 2007. Hatched larvae were collected in a 10 L container supplied with air to evenly distribute the fish in the water column. The larval fish were enumerated by counting the number in n=10 5ml samples drawn from the collecting container. The fish were placed in n=6 250 L rearing tanks supplied with flowing seawater (26 ‰) at a flow of 2.0 L•min^{-1}.

The 6 tanks were divided into 3 feed treatment groups. The first group (Fed) received live feed 3 days post-hatch (DPH). The second group (Delayed) was offered food starting at 10 DPH. The third group (Starve) did not receive any feed at all during the trial. The live feed production room is shown in Figure 2b. The Fed and Delayed groups were initially fed a live diet consisting of a combination of rotifers and two strains of artemia (SF and GSL strains) according to the schedule shown in Figure 3. Dry feed was introduced at 36 DPH (May 30) and fed in combination with a lower concentration of artemia until the termination of rearing at 65 DPH (June 28).

Figure 2. Larval herring culture tanks with degassing columns (a. top photo) and live feed culture tanks rotifers in rear and artemia in foreground (b. bottom photo).

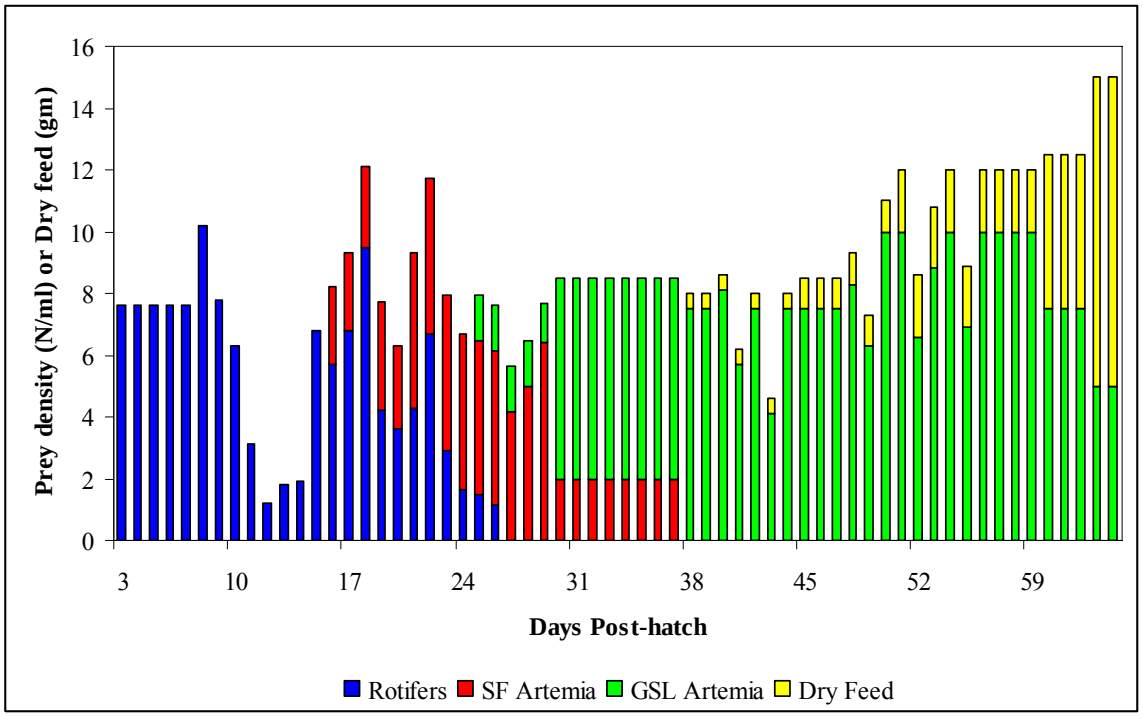

Figure 3. Schedule of rotifers, artemia and dry feed offered to larval herring from 3 days post-hatch until termination of rearing.

Results and Discussion

The development of embryonic and larval herring obtained from roe-on-kelp paralleled that of embryos and larvae obtained from gametes (Figures 1A to 1E). Eggs were first sampled on Day 3 post-transfer (DPT) when the embryo can be seen within the egg (corresponding to Figure 1B). Eyes were visible at 7 DPT and became pigmented by 11 DPT (corresponding to Figure 1D). The embryos continued to occupy a larger percentage of the egg until 17 DPT when hatching began, which was complete by 20 DPT.

Larvae measured 9.0 +/- 0.6 mm in length at hatching. Within 2 days, the yolk sac had diminished significantly and by 6 DPH was completely gone (see Figures 4A to 4C). This meant that by the time the Delayed feed group started feed the majority of the larvae had little to no egg yolk left. As noted above, feed was introduced to the Fed group 3 days post-hatch (DPH), whereas Delayed group first received food at 10 DPH.

Despite the rapid absorption of the egg yolk, the larval herring larvae were able to survive for some time without feed. For the Starve group, 100% mortality occurred at 23 DPD, whereas 100% mortality in Delayed group did not occur until 55 DPH. However, much of the mortality in these two groups also occurred within the second week following hatch, which indicates the importance that larvae be able to feed as soon as possible after hatching. Their ability to feed shortly after hatching was confirmed by gut squashes indicating the presence of rotifers in the GI tract as early as 4 DPH. The rotifers and artemia that made up the live diet and their presence in the GI tract of larval herring are illustrated in Figures 5A to 5G.

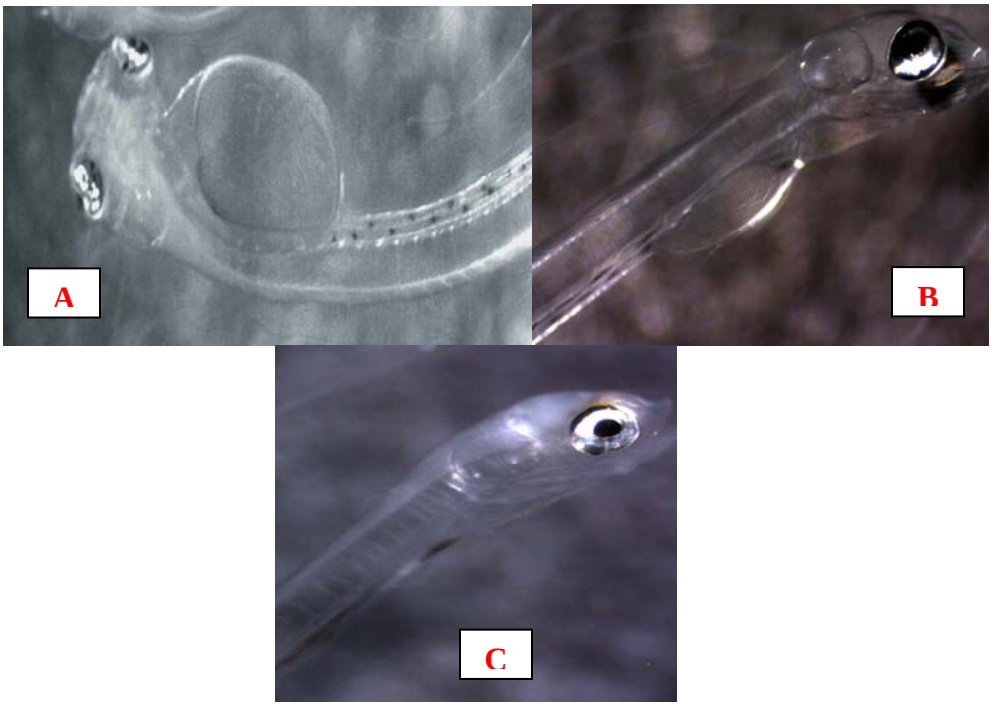

Figure 4. The progression of the yolk sac as the larvae develops. At Day 0 the larvae hatch with a large yolk sac (A). By Day 2 post hatch the yolk sac has diminished (B) and is completely gone within 6 days post hatch (C).

Figure 6 shows the growth in length of larval herring for the 3 rearing groups from 3 to 64 DPH. All three groups showed little change in length up to 10 DPH. There was a slight increase in length for the Delayed and Starved groups by 6 DPH, but by 14 DPH their mean length was similar to that at hatching. The initial increase was probably due to energy stores in the egg sac that once depleted led to the decrease in length at 14 DPH. Despite receiving feed at 3 DPH, the Fed group showed little increase in length. This may have been due to the difficulties encountered with the rotifer culture in which actual prey density was lower than the target density (10-17 DPH in Figure 1) since this is reflected in the percent of larvae with food in their gut at this time (Figure 7). Moreover, length increased dramatically once artemia were added to the diet (Figure 1 – 16 DPH), which suggests rotifer density, nutrient value, or both were insufficient to support growth comparable to that provided by the addition of artemia. [Note: Decrease in length at 64 DPH likely due to size-selective sampling from low remaining population].

The percentage of larval herring with food in their gut also increased markedly once total prey density increased to 7.5 – 10.0 per ml. However, about 37 DPH the percentage of herring that had been feeding began to drop despite high levels of prey density (Figure 3). This corresponded with a change in artemia from the smaller SF to the larger GSL strain, as well as the introduction of dry feed, which likely contributed to the marked decline in the number of live larvae in both the Delayed (100% mortality at 55 DPH) and Fed treatment groups (> 90% mortality at completion of the study).

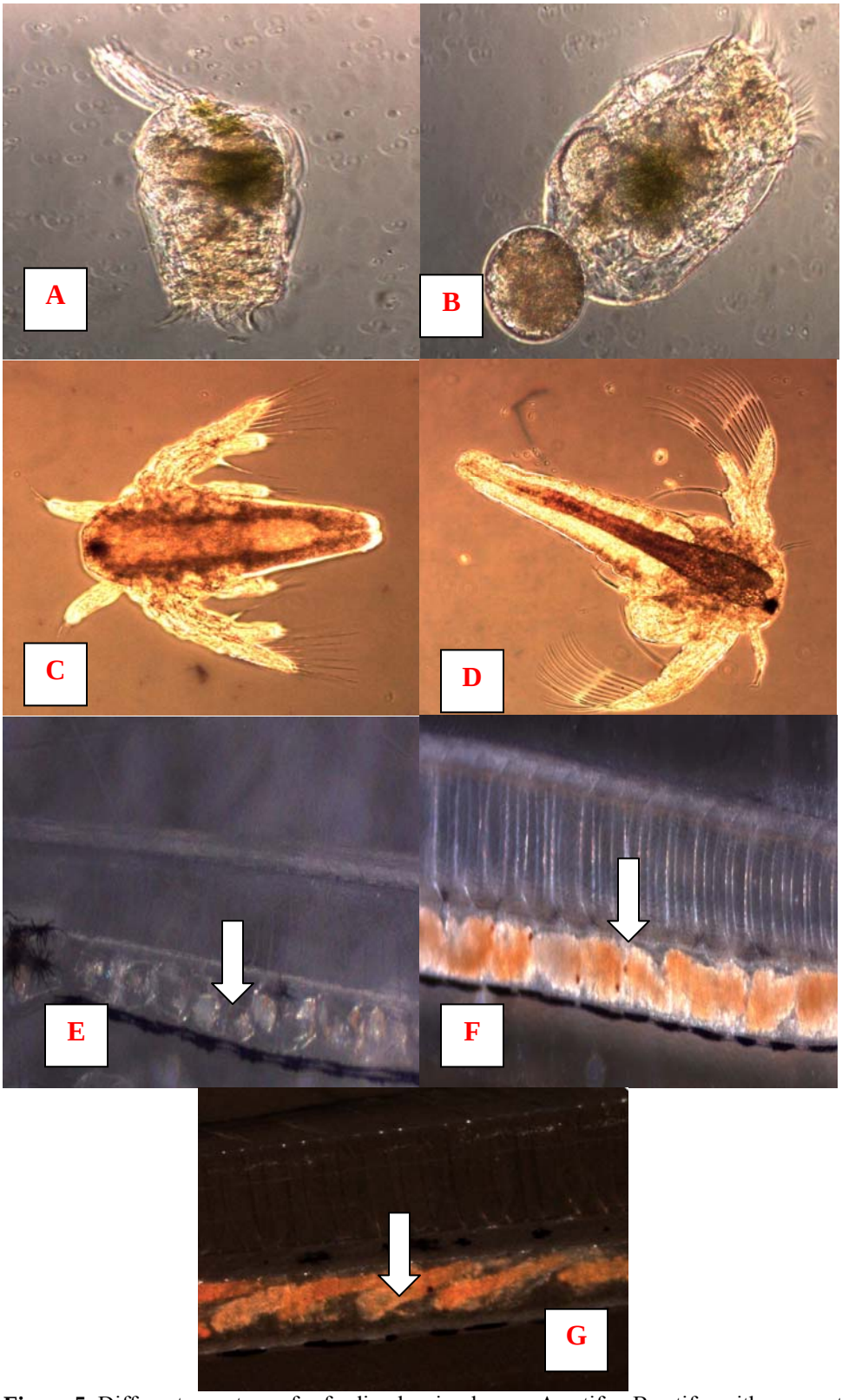

Figure 5. Different prey types for feeding herring larvae. A-rotifer. B-rotifer with egg cyst. C-San Fransisco strain of Artemia. D-Great Salt Lake strain of Artemia. E–rotifers in herring gut. F- SF strain of Artemia in herring gut. G–GSL strain of Artemia in herring gut. Note size difference between SF strain and GSL strain of Artemia. Arrows indicate presence of feed in GI tract.

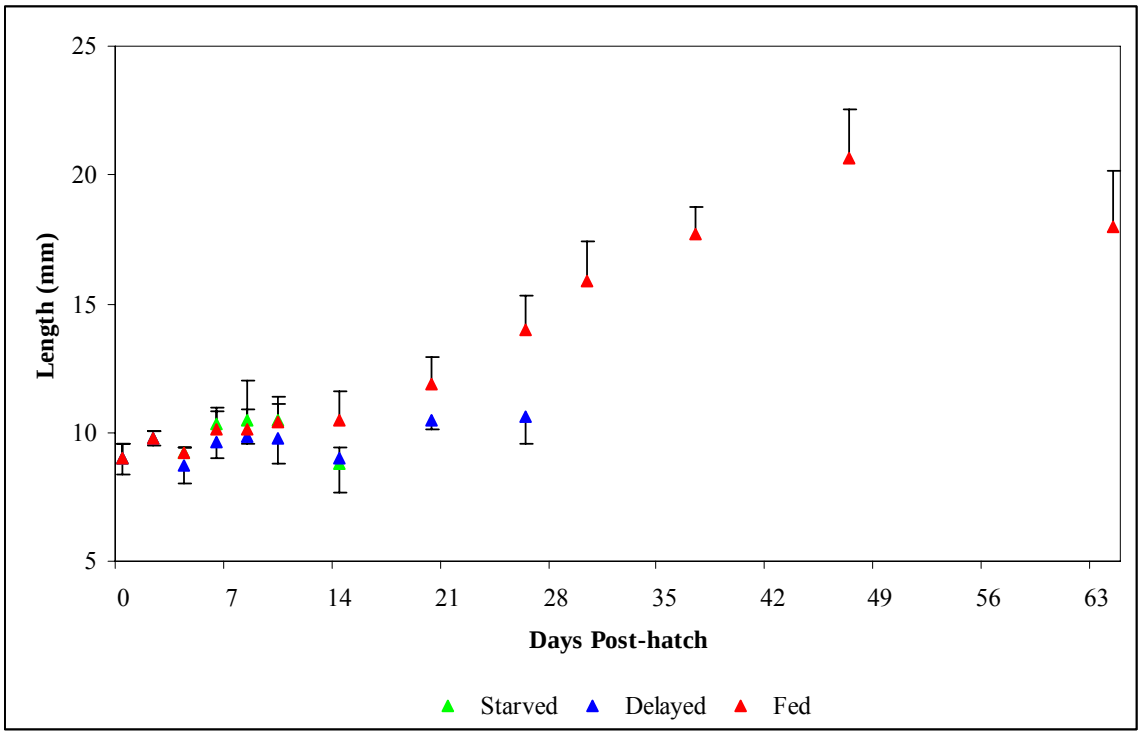

Figure 6. Change in herring larvae length from hatch to end of trial. Starved and Delayed feed groups were not sampled after 14 and 26 DPH, respectively, due to high mortality.

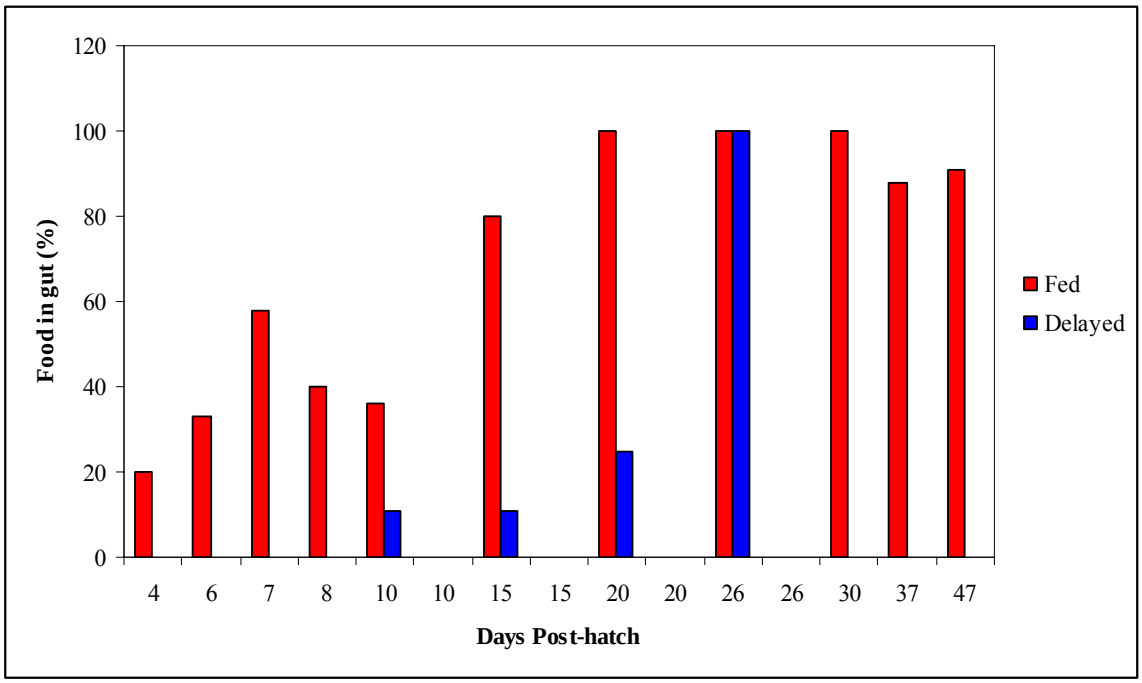

Figure 7. The percent of larval herring with food in their gut from the time of first feeding until 47 DPH.

This increase in mortality in the larvae may have been due to an early transition in prey size (SF to GSL) and also prey type (i.e. introduction of dry feed). As part of the longstanding protocols established for herring culture at Akkeshi, artemia are fed through 45 DPH, and dry feed is introduced at 20 DPH. This probably facilitates the transition to dry feed since it allows the larval herring to feed on small live prey for a longer period of time and thereby reach a size (> 30 mm) where dry feed can be more easily eaten and assimilated (Yamamoto 2001). Future rearing efforts will follow the feed schedules used at Akkeshi, which were not available until completion of our studies in 2007.

Conclusions: Despite problems associated with feed transition and high mortality in the Fed group near the end of the study, the growth rate that we produce in our first trial compares favorably to that for larval herring at the Marrowstone Field Station (Paul Hershberger, USGS, personal communication) particularly when differences in water temperature are taken into account (Figure 8). On a temperature specific basis (i.e. degree days), PWS herring appear to grow more rapidly than those in Puget Sound. [The decrease in length on the last sample date was likely due to size selective sampling because of the small number of fish remaining. Note also the increased variance]. Interestingly, our observed growth rates were also comparable to those reported for Akkeshi Bay herring on a temperature adjusted basis (data not shown, Yamamoto 2001). Such differences may also simply reflect rearing protocols, but our results are encouraging for future culture efforts for stock supplementation. Importantly, however, rearing temperature has a direct effect on the number of days it takes larval herring to reach a specific length. For example, at rearing temperatures of 12 to 13 °C at Akkeshi Bay, larval herring reach 20 mm in length in less than 30 days of rearing, whereas at the 6 to 7 °C that we obtained from the seawater well at the SSH, it took nearly 50 days to reach this length. These cooler rearing temperatures will markedly reduce the size that larval herring will reach by late summer to early fall and potentially affect recruitment. Moreover, we conclude that tank hydraulics (size, shape, water flow) had a major effect on our ability to present live and dry food to maximize feeding opportunity in the larval herring. The rearing space and culture tanks that were available given the timing and budget of the project were marginally adequate to accomplish the pilot studies. As shown below, our culture system differed considerably from that developed for stock supplementation of herring in Japan. Accordingly, our proposed future rearing efforts incorporate important changes in culture technology, as well as heated rearing water to achieve absolute rates of growth comparable to those obtained for larval and juvenile herring at Akkeshi (see Objective 1 in proposed 2008 – 2009 studies).

It is worth noting that even minor delays in the onset of feeding have a major effect on larval survival. We eventually observed a 100% loss in the Delayed group (55 DPH), suggesting that highly extensive supplementation projects, such as moving roe-on-kelp to potential nursery bays, must take into consideration the timing of the accretion in the forage base. Failure to do so may lead to newly hatched larvae being placed in areas where food is not yet available, which would clearly limit the likelihood that these fish would grow and survive. This would be particular concern for transfers of roe-on-kelp from spawning areas in the southern part of PWS to nursery areas further north, where rising surface water temperature and increasing plankton abundance occur much later.

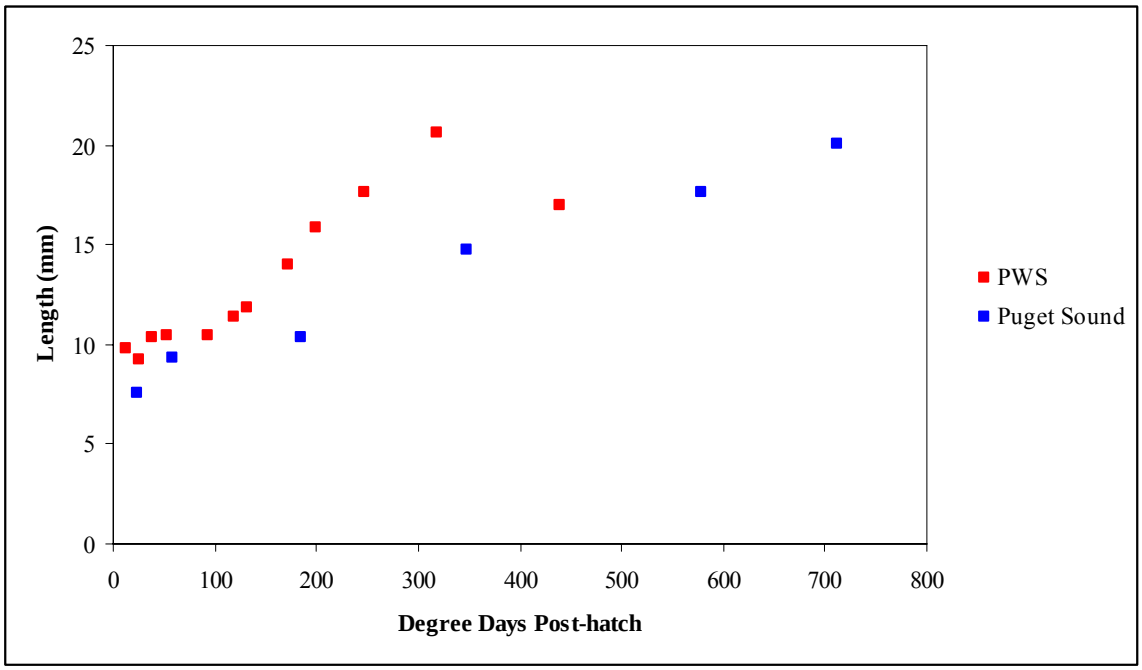

Figure 8. The growth in length of PWS and Puget Sound larval herring adjusted for rearing temperature differences using degree days.

Finally, as we note above, our initial attempts to culture rotifers as live feed was not without difficulty. Due to the logistics of getting the rotifer system online and the cultures to Seward, as well as the timing of hatching of the larval herring, it was necessary to begin harvesting rotifers at a high level before the culture reached a high reproduction rate. This resulted in harvest rate that exceeded the rate of reproduction. Hence, it was necessary to reduce the harvest level below the optimal prey density to avoid a complete loss of the rotifer culture. It also required that we make supplemental purchases of rotifers to maintain a basal level of rotifer additions to the culture tank. We plan to address this issue in future work by initiating rotifer production at least 10 to 14 days before the time of first feeding for the larval herring to sustain the high demand for this important source of live feed (see Objectives 1 and 3 in proposed 2008 – 2009 studies).

Objective 3: Determine if calcium sensing receptor proteins (CaSR's) are expressed in the tissues of larval herring. *Hypothesis: CaSR proteins are expressed early in development, particularly in tissues associated with salinity sensing and regulation.*

Materials and Methods

Post-hatch larval herring collected on multiple dates were preserved in fixative for later analyses of CaSR expression. Tissue localization of CaSR proteins using immunocytochemistry was performed using methods described previously by our group (Nearing 2002; Hentschel et al. 2003). Briefly, paraffin-sections containing specific herring tissues were deparaffinized, exposed to antibody blocking solution, and then incubated with one of several available anti-CaSR antisera. Bound anti-CaSR antibody was localized using affinity purified peroxidase conjugated secondary antiserum. Appropriate controls to evaluate nonspecific binding of antibody were also included.

Results and Discussion

Immunocytochemical staining showed that CaSR proteins were present in multiple tissues during early development (red staining in Figures 9A to 9D). In view of the euryhaline life history of herring, this finding is not surprising, even though our ICC method utilized a CaSR antibody developed from a salmon CaSR. CaSR proteins are highly conserved (Yamaguchi et al. 2000), which reflects their fundamental role in calcium homeostasis and salinity sensing. Moreover, CaSR proteins are clearly expressed in the skin and GI tract (Figure 9C and 9D), which are responsible for osmoregulation in this species prior to the appearance of gills (Jones et al. 1966). There is also CaSR positive staining evident in the olfactory epithelia, suggesting a possible role for sensing of prey (Figure 9B), and integration with nutrient adsorption in the GI tract. Similar CaSR expression has been found in the olfactory bulb of salmon (Nearing et al. *in press*).

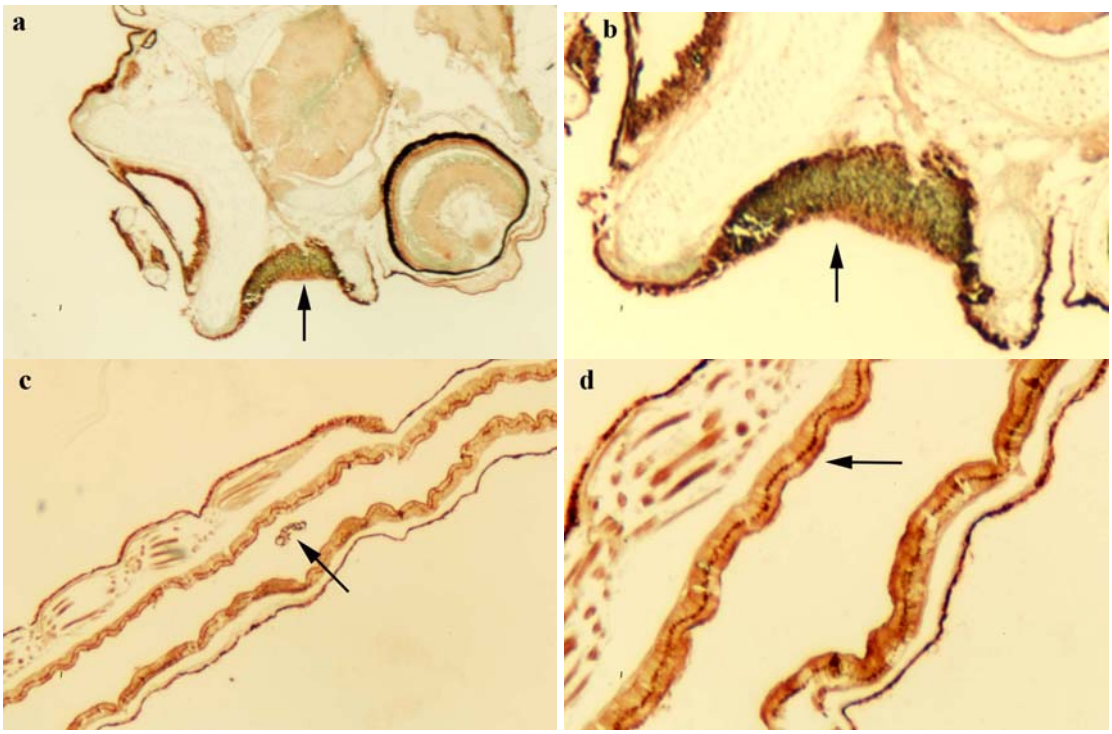

Figure 9. Calcium sensing receptor staining in larval herring. Photos a and b – head showing eye and olfactory epilthelia (arrow). Photos c and d – GI tract. Note artemia in GI tract in photo c (arrow).

Conclusion: These findings confirm our hypothesis that CaSR's are present early in life in herring, and also support our suggestion that naturally occurring compounds that activate the CaSR may aid in early growth and development. Concentrations of an important class of these compounds in live feed, polyamines, can be enhanced through simple alterations in the salinity of the water in which the rotifers and artemia are cultured. An important part of our scope of work for larval culture in 2008 and 2009 will be to examine the effects of these dietary additions on larval growth and survival (see Objective 3 in proposed 2008 – 2009 studies).

Objective 3: Collaborate with Japanese researchers to transfer herring culture techniques developed in Japan to Alaska. *Hypothesis: Herring culture techniques in Japan are suited for stock supplementation in Prince William Sound but may require refinement for a large-scale program to raise recruitment to sustainable levels.*

Materials and Methods

Contact, travel and collaboration with Japanese herring culture researchers were facilitated by Dr. Shinji Harakawa of Hakuju Institute for Health Science Co., Ltd. (scientific collaborator of MariCal, Inc.), and Mr. Naoki Tojo of Hokkaido University. Dr. Harakawa and Mr. Tojo provided initial contact information with the principal investigators involved in herring culture research and also coordinated obtaining approval to meet the researchers and view their operations. New regulations adopted by the Fishery Research Agency of Japan, which recently took control of the previously independent Fishery Research Institutes, required that all information exchange on the part of institute scientists be cleared through FRA. After nearly two months of effort, written approval was provided by Dr. Koji Nakamura, International Coordinator for the Incorporated Administrative Agency at the FRA. Dr. Linley was then able to travel to Japan and meet personally with herring researchers and observe stock supplementation efforts as part of this official visit. Despite the complications associated with obtaining approval for the visit, and the timing of the visit coinciding with several national holidays, a total of 4 days of meetings, facility tours and presentations occurred in late April 2007. This included time at both the Hokkaido National Fishery Research Institute (HNFRI) in Kushiro, as well as the Akkeshi Field Station. The herring investigators with whom I interacted included Dr. Takahiro Matusbara, Section Chief Resources Enhancement Section, Coastal Fisheries and Aquaculture Division, HNFRI, Dr. Masaaki Fukuda, Director of the Coastal Fisheries and Aquaculture Division, HNFRI, Dr. Nobuyuki Ohkubo of the HNFRI, Mr. Murakami Naoto and Mr. Fukunaga Kyohei of the Akkeshi Research Station, and Dr. Ryuzo Yanagimachi of the Univ. of Hawaii.

History of Herring Fisheries and Stock Supplementation in Japan

Artificial propagation of herring in Japan has its origins in the development and eventual collapse of what had once been one of the largest herring fisheries in the world. Although a herring fishery had been conducted in Japan for nearly 600 years, catch records for the modern fishery show its rapid growth from the late 1800's and subsequent decline over the next 70 to 80 years (Morita 1985). The modern fishery targeted two major stocks in the Northwest Pacific; 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 5000 tons annually. Interestingly, the Hokkaido spring herring stock sustained a harvest over a much longer period of time 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. The collapse of these fisheries, coupled with development of methods for the culture of other species of marine fish, prompted investigations for artificial propagation of herring by the Japan Sea Farming Association beginning 1981 that resulted in the initial attempts at stock supplementation in 1987. Now nearly 20 years later, these efforts continue today and are now directed by the Hokkaido National Fisheries Research Institute of the Fishery Research Agency of Japan. The main research facility is at Akkeshi Bay in eastern Hokkaido near Kushiro.

Herring Culture Technology

The propagation techniques for herring were developed largely from prior studies of the culture requirements for sea bream, flounder and porgy. These techniques and there application to stock supplementation are detailed in the Yamamoto (2001), which provides the basis for most of what is reported below. The document provided to Dr. Linley is being translated to English and will be used to guide our pilot-scale studies over the next two years together with personal contacts between Japan and the ASLC.

Akkeshi Bay Field Station Facilities: Early experimental efforts showed that herring adapted well to existing methods for marine fish and many of these have been retained to the present with minor modification. In general, the incubation and rearing facilities at the Akkeshi Field Station are generic with respect to their utility for multiple marine fish species. The field station consists of three building modules that are nearly identical in layout and operation. Each module houses between 8 and 10 50m^3 rearing tanks supplied with flowing seawater. The typical layout is shown in Figures 10a and 10b, which is the module used for herring production. The combined incubation and rearing tank design is shown schematically in Figure 11. Water enters at the top of the tank and exits through the screened outlet at the bottom (light arrows). The flow to each tank ranges from ~ 35 $L \bullet min^{-1}$ during incubation and early larval rearing to 140 $L \bullet min^{-1}$ shortly before transfer to net pens for final rearing (i.e. 1-4 total water exchanges per day). Circulation in the tank is maintained by air stones located in each corner of the tank (dark arrows). Stainless steel heating coils connected to a boiler are located along both sides of the long axis of the rearing tank and are used to heat the water to a range of 8-13 °C. The bottom of each tank has a painted grid that provides a track for an electric-eyed controlled vacuum to remove excess feed, waste and dead fish (Figure 12).

Figure 10. Photos inside the herring culture module at the Akkeshi Research Station showing typical tank arrangement (a. top) and tank outlet (b. bottom).

Brood Stock Collection, Spawning and Incubation

Brood stock are collected by purchasing adult herring returning to spawn in Akkeshi Bay that are landed in the local fishery and sold in the fish market(s). The fish are purchased within hours after landing to ensure the gametes remain viable. [Herring egg viability, for example, remains above 80% up to 6 hours post-harvest, but declines to ~ 10% after 24 hours]. Individual fish are checked for ripeness and any fish that have not reached final maturation are discarded. Gametes are obtained from both males and females by evisceration. If eggs remain in the skein (ovary), these are separated using a soft spatula.

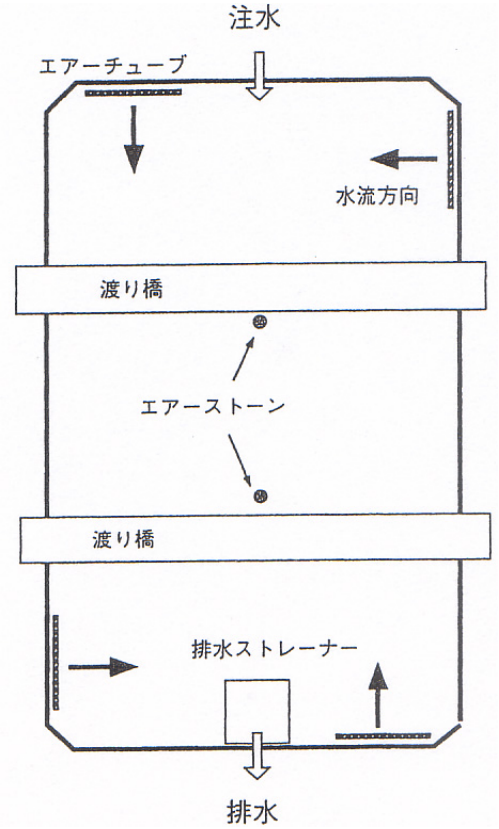

Figure 11. Schematic diagram of the typical incubation and rearing tank used for marine fish culture at the Akkeshi Field Station.

Whole testes are placed in nylon bags, which are then compressed to separate the tissue and sperm. The ripe eggs from 5-10 females (n=50,000-100,000) and sperm from 5-10 males are pooled and mixed in 15 L containers for several minutes after which ~ 5 L of seawater is added. Immediately after the addition of seawater, a wire wand imbedded with coconut hair is immersed and swirled in the mixing container as adhesive substrate for the fertilized eggs (Figure 13). The wands are then transferred directly to the rearing tanks where they are suspended in the water column using an over head line and bottom weight (Figure 14). The resulting incubation density is ~ $n=10^6$ eggs per $50m^3$ tank.

Figure 12. Photo of electric-eye sensing vacuum system used to clean tank waste.

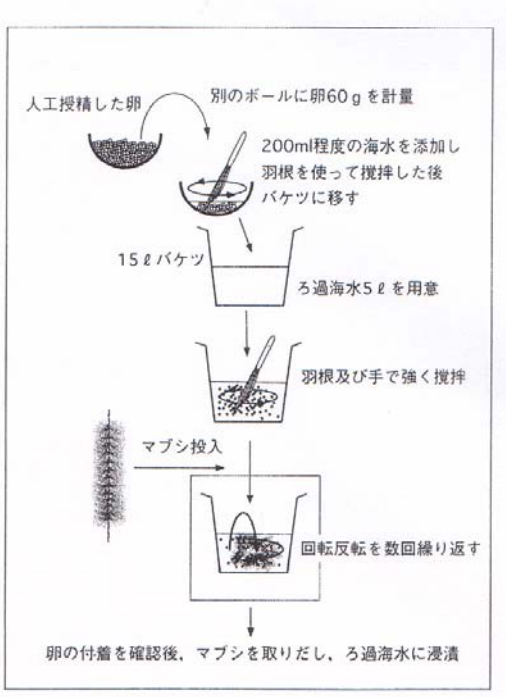

Figure 13. Schematic representation of dry fertilization method and egg adhesion to artificial substrate.

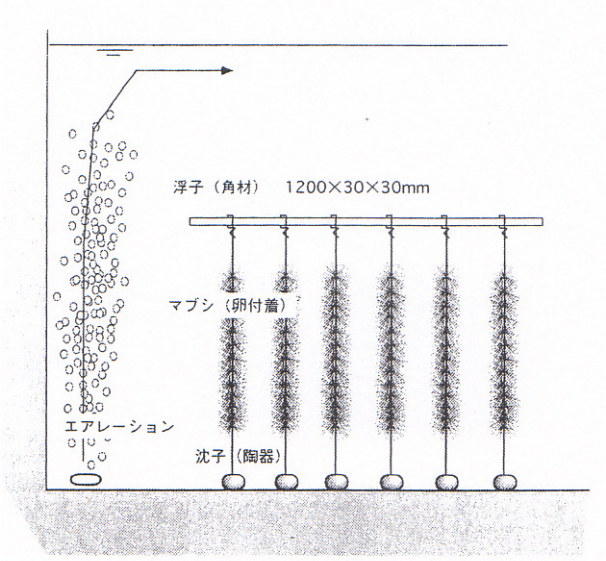

Figure 14. Typical arrangement in rearing tanks of coconut hair wands used as artificial substrate.

Larval rearing

Hatching at 10 °C occurs in 11-12 days with a rate of survival ranging from 40-60%. Rotifer feeding begins immediately after hatch and extends for a period of approximately 15 days. Artemia are introduced 8 days post-hatch and fed for 5 weeks, whereas dry feeding begins at day 20 post-hatch and extends for 7 weeks. The actual feed protocol used at Akkeshi is shown in Figure 15. Although live feed protocol used at the SSH in 2007 is close to that at Akkeshi, there are notable differences. First, rotifer density ($N \bullet ml^{-1}$) peaks at higher concentration at Akkeshi, but terminates earlier. Secondly, artemia as well as dry feed are introduced much earlier in the protocol at Akkeshi. The Akkeshi live feed protocol also includes a green water phase for the first 20 days of rearing that consists of live feed of a standard and DHA+EPA enriched algae (Chlorella Industries). Early enrichment of live feed with Ω-3 series fatty acids may contribute to the high post-hatch larval to juvenile survival reported at Akkeshi Bay by promoting rapid GI tract development. For many marine species, Ω-3 enrichment begins in the artemia phase, which is the method that we employed in our 2007 studies at the SSH. Our future rearing efforts at the ASLC will be adjusted to incorporate these differences in timing, prey concentration and feed enrichment.

Growth and survival at Akkeshi Bay during larval and juvenile rearing are shown in Figures 16a and 16b. At rearing temperatures of 12-13 °C growth is rapid and fish reach metamorphose length of ~ 30 mm in about 40 days for rearing. In contrast, in our rearing studies at 6-7 °C at the SSH, the larval herring reached only 20 mm after 40 days of rearing. This will be an important consideration for future stock enhancement work and supports our emphasis on developing larval rearing protocols based on use of increased rearing water temperature to accelerate growth through the larval phase. A protracted larval rearing period under colder water temperatures also increases the risk that the fish will feed less aggressively which can delay the onset of foraging with the attendant consequence of high mortality. At Akkeshi, post-hatch to juvenile survival is typically 40-50% (Figure 15b), which is near the upper end for reported values (Alderdice and

Hourston 1985). This may also relate in part to temperature and salinity interactions, which show that larval mortality is maximal when low (high) salinity is coupled with low (high) temperature. Salinity at Akkeshi Bay is typically 32-33 ppt (T. Matsubara, Hokkaido National Fisheries Research Institute, personal communication), hence elevated rearing temperatures may help potentiate the higher post-hatch survival.

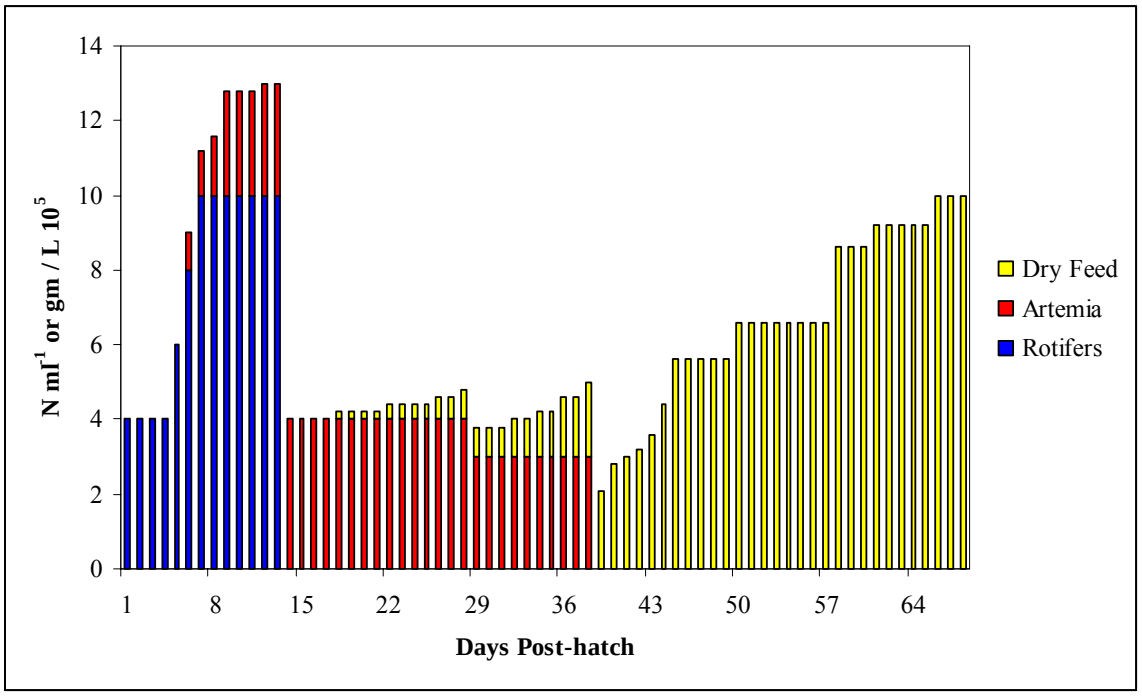

Figure 15. Live (number • ml^{-1}) and dry feed (gm • 10,000 L^{-1}) protocol for rearing of larval herring.

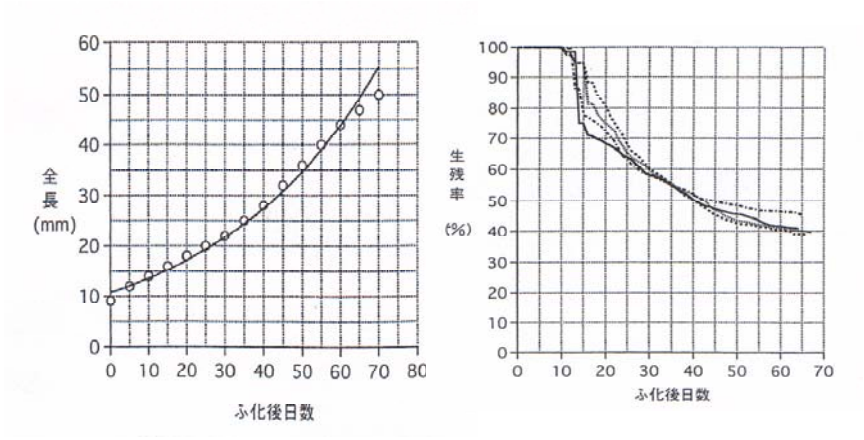

Figure 16. The rate of growth (a. left panel) and survival (b. right panel) for 70 days of tank-based rearing at Akkeshi.

Cage culture

Juvenile herring ranging in size from ~ 50 mm to 70 mm in length are transferred to net pens in Akkeshi Bay in early July where they are reared for an additional 1-2 weeks. Water temperature in the bay at this time approaches 15 °C so growth is rapid and fish reach ~ 80 mm in length by the time of release. This growth is reflected in the feeding rates and feed frequency, which range between 5 to 10% per day and 4-5 times per day, respectively. This is an important feature of the stock supplementation program at Akkeshi and a major reason for including this as a component in our pilot studies (see Objective 1). Under optimal temperature and salinity conditions, net pen culture can result in release of larger and healthier juvenile herring in to PWS. It also provides the flexibility to better refine release timing since rearing density constraints are typically not an issue. Mortality during cage culture at Akkeshi is reportedly very low and may be due, in part, to the apparent lack of potential pathogens such as VHSV and ICTH that are highly problematic for herring in PWS. Whether the pathogens are actually absent or the fish simply do not express the disease is not known, but there have been no reported disease outbreaks during any period of larval to juvenile rearing at Akkeshi.

Recruitment

Juvenile to adult recruitment at Akkeshi has ranged from approximately 1% to more than 12% over the 20 year period that supplemented fish have returned to the bay. Increased recruitment has coincided with improved culture techniques that have led to increases in the size of juvenile releases in recent years. Coincidently, the lowest rates of recruitment occurred during the mid-1990's at the time that the numbers of fish released each year were at a historic peak (~ 600,000 – 700,000). It is unclear, however, if the low rates of recruitment at this time were due to density dependent factors operating within Akkeshi Bay. Evidence suggests that high rearing densities or elevated water temperature during incubation and larval rearing may have contributed to a high incidence of spinal deformities which were observed in these fish before release (T. Matsubara, Hokkaido National Fisheries Research Institute, personal communication). Nevertheless, the program has clearly succeeded in contributing adults to the spawning population. In fact, based on post-release monitoring and otolith sampling of adult fish in the commercial harvest, the contribution of supplemented fish over the last 10 years has averaged approximately 20%.

Conclusion: Stock supplementation techniques developed at Akkeshi have been successfully employed for nearly 20 years to provide a material contribution to the local stocks of herring as well as stocks elsewhere in Japan. These techniques appear to be suitable for adoption to construct a similar effort in Prince William Sound.

Overall Summary, Conclusions and Recommendations of our 2007 Project

The history of herring stock supplementation in Japan clearly demonstrates the technical feasibility of intensive culture of juvenile fish for release into the natural environment

and their recruitment to the adult population, particularly on a local scale. Stock supplementation for herring has occurred in Japan for nearly 20 years and the techniques for the culture of juvenile herring have been carefully refined. Much of this development stemmed from research on the culture requirements of other marine species, which supports the hypothesis that these cultures methods can be adopted for the culture of juvenile herring for release into PWS. Our preliminary studies in 2007 confirm this. Under optimal conditions, PWS herring grow and survive at rates similar to those reported for Japan and Puget Sound. Our findings also demonstrate that CaSR proteins are abundantly expressed during early life history. This suggests that rearing techniques that influence CaSR function, such as those involving CaSR activating dietary supplements, may be highly beneficial for increasing growth and survival under intensive culture and post-release conditions.

However, our results also demonstrated that the laboratory-scale rearing system we developed in 2007, as well as some of our rearing techniques, will require substantial modification if we are to achieve a consistently high degree of productivity for culturing live feed and growing juvenile herring to reach a size by late summer to early fall that will maximize their recruitment after release. To do so, we have made several important revisions to our proposed scope of work for 2008 and 2009. These include:

1. Increased focus on salinity, temperature and feeding protocol effects on larval growth and survival (Objectives 1, 2 and 3).
2. Expanded rearing space with tanks having hydraulic characteristics that optimize feed availability and presentation.
3. Improved utility infrastructure, particularly access to heated water to accelerate early development and larval growth.
4. Investigation of naturally occurring dietary supplements to promote early GI tract development, growth and immune system function
5. Testing to determine if cultured herring possess a biochemical otolith mark that will allow them to be distinguished from wild herring.

These and other related objectives are described in greater detail in our revised proposal for 2008 and 2009.

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