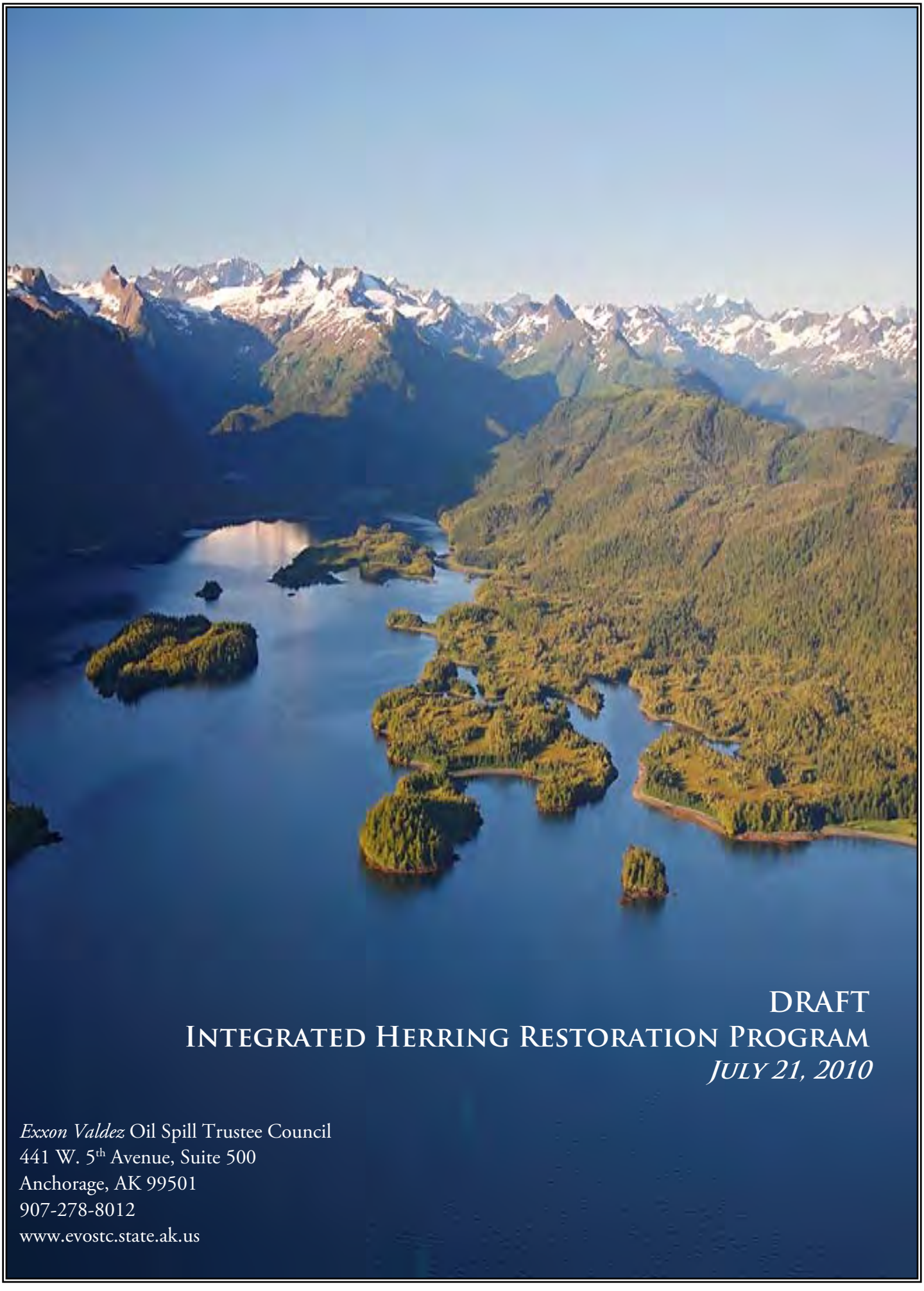

DRAFT
Integrated Herring Restoration Program
July 21, 2010

Exxon Valdez Oil Spill Trustee Council
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Preface

Herring are vital to many different species in North Pacific ecosystems, including humans. Herring transfer energy from zooplankton to upper level predators such as whales, sea birds and larger fish. The complex interactions among herring prey and predators make the examination of herring restoration challenging. Each step in the herring life cycle and the concomitant interaction with either food or predator could be a "bottleneck" point or limiting factor constraining recovery. Prince William Sound herring collapsed in 1993 and have not recovered naturally. It is time to consider potential restoration options that are based on the most likely limiting factors and rigorous science.

Since the 1989 oil spill, scientific research has been conducted on many of the injured species and services in Prince William Sound. Several recovering species have direct links to herring; and thus, herring are a keystone species necessary to support a full recovery of the ecosystem as a whole. Many recovering human services are also linked to the recovery of herring. It is likely that commercial fishing has the most far-reaching implications, with the economic effects of commercial fishing losses felt across entire communities. It is timely that herring restoration be examined now while there is still a viable, remnant stock from which to work. Additionally, the partnership which has developed between scientists and affected communities can carry this effort far.

More than twenty years have passed since the *Exxon Valdez* Oil Spill and herring numbers are still too low to sustain a commercial fishery. Herring are an integral part of every inshore ecosystem on the northwest coast of North America. We cannot consider the Prince William Sound ecosystem recovered from the effects of the oil spill until herring abundance has been restored—even if the collapse of herring cannot be linked directly to the spill.

I am pleased to acknowledge the hard work and dedication of the people who have contributed their time and expertise to the authors of this document: Catherine Boerner, Evelyn Brown, Rob Campbell, Doug Hay, Gary Fandrei, Paul Hershberger, Ross Mullins, Vince Patrick, Scott Pegau, Stanley "Jeep" Rice and Doug Woodby. I would also like to extend my thanks to the members of the Herring Steering Committee whose commitment to the restoration of Pacific herring in Prince William Sound has laid the foundation for the future of this important program.

Elise M. Hsieh, Executive Director

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PLEASE COMMENT

You can help the Trustee Council by reviewing this draft program and providing your comments. Comments are due by August 16, 2010 and you can comment by:

Mail:	*Exxon Valdez* Oil Spill Trustee Council 441 W. 5th Avenue, Suite 500 Anchorage, AK 99501 Attn: Integrated Herring Restoration Plan
Telephone:	1-800-478-7745 Collect calls will be accepted from fishers and boaters who call through the marine operator.
Fax:	907-276-7178
E-mail:	dfg.evos.projects@alaska.gov

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I. Executive Summary and Synopsis of the Restoration Plan

No one knows why herring in Prince William Sound (PWS) collapsed and no one definitively knows how to restore them. The PWS herring population, like all herring populations, fluctuates, but most herring populations rebound after periods of low abundance. This usually follows the suspension of fishing, but PWS herring have not recovered even after fishing has stopped for nearly a decade. It is clear that the present status of the population is severely depressed, but it is less clear if the present state is stable or if the abundance trajectory is improving or declining.

There are a number of approaches that might be successful at assisting with recovery of PWS herring, but none has been proven. Each approach invokes implicit biological assumptions that may be misconstrued or simply wrong. These assumptions often concern fundamental issues about factors affecting herring recruitment and interactions of herring with the ecosystem. Some of these uncertainties have been under investigation for more than a century, and probably will remain uncertain for some time. These limitations in knowledge and understanding impede efforts at herring restoration but do not necessarily stop it. A consequence, however, is that any effort at restoration will require careful efforts at validation to ensure that any changes in abundance are a consequence of a restoration activity and not a natural change.

Most approaches at restoration will be complex, expensive, and encounter both technical and procedural problems. Some approaches may actually be deleterious. These comments are not an excuse for inactivity, but they are a reason to proceed carefully and cautiously. Above all, the implicit guideline for an approach to herring restoration is do no harm. This report presents nine types of restoration activities that might be considered. Not all are necessarily feasible and the report includes and comments on the strengths and weaknesses of each. Further, the report outlines the essential scientific and procedural preparations that must be implemented before any restoration activity could be considered.

In distinct sections the report provides a brief background on the *Exxon Valdez* oil spill, basic herring biology, and potential factors limiting herring recovery. These are followed by a description of nine restoration options or activities. The report concludes with a restoration plan that consists of a list of recommended activities to be conducted in the next year prior to the initiation of any of the restoration options. Mainly these recommended activities will provide perspective about the cost and scale of efforts required for each of the options as well as essential information on the implications of the regulatory environment that could affect restoration work.

The restoration plan consists of three phases in time. A monitoring program to better understand recruitment, predator impacts, and demographic and biological changes within the herring population will proceed through all Phases.

Phase 1 (2012–2014) would consist of scoping activities related to the restoration options which would provide: (i) an external review of assessment methodology and sensitivity analysis of capability of current methods to detect change; (ii) a report defining the regulatory environment and implications for restoration work; (iii) a report on "scaling" restoration activities that would examine the effort and cost for different options; and (iv) a report defining decision points about when to initiate and suspend restoration activity. All scoping activities could then be synthesized into a single report that would systematically examine the restoration options relative to feasibility of cost.

Phase 2 (2014–2022) would initiate active implementation work on several restoration options, that are deemed feasible by the scoping activities. The option of possible "*future supplementation activity"* or a "hatchery" approach (Restoration Activity 8) is substantially more expensive that all other options. Its initiation would depend on preliminary contracts to investigate mass-marking technology and pilot-scale hatchery work. Such contracts would not necessarily imply that herring hatcheries are planned. Rather, in the event that they would be considered, this preliminary and relatively inexpensive preparatory work will have been completed.

Phase 3 would begin in approximately five to six years. If the schedule of activities outlined above is started, then likely the abundance trend of the PWS herring population will have been carefully monitored and the results of early restoration activity will be known. If PWS herring continue to remain at the current low level, and other restoration activities have not been effective, then decision makers should be prepared to consider the supplemental production activity (Restoration Activity 8) as a last resort to herring restoration. Based on work in Japan, this approach can successfully produce herring, but the cost of such work in PWS might be prohibitive.

Synopsis of the restoration plan: 2012–2022

This is a three-stage plan that will begin with immediate enhancement of monitoring and a set of scoping activities that are essential to define the regulatory environment, scale of potential activities and costs, and decision points relative to herring stock conditions that might initiate or suspend restoration activity. Stage 2 would begin selective restoration activities.

Stage 1: Monitoring and Scoping – 2012–2014

Preliminary Scoping. Through modest contract and/or workshops, conduct five different scoping activities related to the restoration options: (i) external review of assessment methodology and sensitivity analysis of capability of current methods to detect change; (ii) a report defining the regulatory environment and implications for restoration work; (iii) a report on scaling restoration activities relative to effort and cost; and (iv) a report defining decision points about when to initiate and suspend restoration activity. All scoping activities could then be synthesized in to a single report that would systematically examine the restoration options relative to feasibility of cost.

Stage 2: Selected restoration activity – 2014–2016

Restoration activity: Support research on the restoration activities that have the highest potential feasibility following the scoping in Stage 1.

Pilot-scale work

If Restoration Activity 9 is considered feasible, initiate contracts to investigate mass-marking and pilot-scale hatchery work. Such contracts do not necessarily imply that herring hatcheries are planned, but in the unlikely event that they would be considered, this preliminary and relatively inexpensive preparatory work will have been completed.

Stage 3: 2016–2022

In approximately five years, be prepared to initiate the supplemental production activity (Restoration Activity 9) if PWS herring continue to decline, and other restoration activities have not been successful.

II. Introduction

The Prince William Sound herring population collapsed in the early 1990's and has not recovered. Annual recruitment (year class strength) has been poor and the populations continues to be impacted by disease, predation, and oceanographic changes. Despite numerous studies directed at understanding the effects of oil on herring, the cause of the collapse and factors constraining population recovery are poorly understood. A combination of factors, including disease, predation and poor recruitment appear to contribute to the continued low population level of herring in the Sound.

The Integrated Herring Restoration Program (IHRP) examines the information and understanding about the complex factors affecting the PWS herring population, and provides a list of potential restoration activities, ranging from no activity to intensive activity. Although there are many scientific and technical complexities, as well as some political implications to overcome, this report tries to provide a decision tree that will aid decision makers in the future with difficult decisions on what can and should be done to restore herring in PWS.

Restoration plans for fish populations usually begin after stock collapse, not before. Awareness of a sustained collapse may not occur until long after it happens, sometimes years later. Pacific herring populations fluctuate naturally, so symptoms of a sustained collapse can be difficult to recognize. In PWS, symptoms of the collapse included reduced annual spawning and poor recruitment for several consecutive years. Sixteen years after the 1993–94 crash, the population has not rebounded as quickly as hoped.

Fish stock collapses are not rare events and recovery programs are becoming increasingly common. Over the last 20–30 years, rigorous scientific protocols have been established for restoration programs for many fish stocks. The concept is not new and the potential application to PWS herring is not necessarily unique. Usually initial restoration steps involve a curtailment of fishing and implementing of monitoring and assessment programs. Following the 1993–94 crash, the Alaska Department of Fish and Game (ADF&G) took the necessary steps to close the herring fishery and continue to monitor and assess the population on an annual basis. In effect, these were the first stages in an active restoration program.

A fundamental principle of fisheries management, like management of any renewable resource, is that harvested fish populations will increase reproductive output to compensate for removals by a fishery. The same principle applies as the first response for restoration activity: fishery closures. The basic assumption is that depressed fish populations will recover, reaching former levels of abundance when mortality from fishing is stopped.

In the context of the scientific approach to fish population restoration, the first approach of restricted fishing should be sufficient to promote recovery. If not, an "intervention" step may be considered. This involves some form of environmental manipulation, usually by promoting better survival of fish eggs or juvenile forms, as in a fish hatchery, but there may be other options and approaches. The main PWS herring restoration issue concerns the wisdom of implementing an "intervention" step. Specifically, is intervention warranted? If so, why and how could it be done? If not, why not?

There are many uncertainties to resolve before an informed decision about intervention can be made.. Not all of the uncertainties are biological or scientific – some legal and jurisdictional issues must be addressed before a second "intervention" step could begin. Before any intervention option can be attempted, there are unresolved issues of scale and policy that must be answered. For instance, how many "additional" herring would be needed to make a positive difference to PWS herring recruitment

and abundance? Also, uncertainty about costs must be resolved, especially for different types of containment facilities that would be required and would require financial support for staff, equipment, etc. There are serious, unresolved questions about legal and management jurisdictions. For instance, it is established that mass-marking of fish produced from restoration work is an absolute requirement for validation. Less certain, however, are the implications for working within the existing legal framework governing use of certain chemicals required for mass-marking. Similarly, legal concerns about disease, genetic issues and the movement of live fish would need to be addressed.

As years have passed without evidence of herring recovery, the *Exxon Valdez* Oil Spill Trustee Council (Council) has focused more closely on examining interventions to help restore herring to the PWS.. The main EVOSTC tasks directed specifically at potential intervention have been: (i) the 2006–2007 preparation of a draft (white paper) report on the feasibility of enhancement based on Norwegian and Japanese herring "hatchery" approaches – t he white paper also identified the requirement for developing "mass-marking" for PWS herring as an essential prerequisite for scientific validation; (ii) a series of EVOSTC-sponsored meetings in Cordova in 2008 that led to a draft report on potential intervention options and a list of important information gaps; (iii) the acknowledgement that mass-marking is a crucial component of any restoration-intervention approach, which led to the development of a "state-of-the-art" workshop on fish marking in Anchorage, October, 2009; and (iv) a new directive for the 2009 Invitation for Proposals that required herring researchers supported with EVOSTC funding to ensure that their project was integrated with other herring projects, plus a requirement that the research addresses fundamental issues concerned with potential PWS herring restoration.

Many of the issues in this report were identified and discussed during the series of meetings in Cordova in 2008 from which a list of potential recommendations was developed. A key recommendation concerned the adequacy of the present herring monitoring system in PWS. The concern was that the current system may not be adequate to establish a reliable baseline of the population, or even to monitor the present trends in abundance. Therefore, a period of "enhanced monitoring" is advisable as a prerequisite to any restoration activity.

A cautious approach is essential. If any restoration option is undertaken, it must follow rigorous scientific guidelines and criteria for evaluation and verification of intervention activities. It will require several more years before a decision to start intervention could, or should, be made. In the meantime, all current herring research activities funded by the EVOSTC have been designed to address basic questions related to intervention and further better understanding of the potential value of various intervention options.

There are four major sections that follow: Section III discusses the necessary herring biology required to understand the factors that limit herring at different life stages described in Section IV. Section V discusses the range of intervention options, beginning with none and ending with intensive. Lastly, Section VI suggests a sequential plan on which to base future program directions and decisions, thus, supplying future decision makers with the informational tools they will need.

III. Development of the IHRP (Integrated Herring Restoration Program)

The collapse and lingering decline of herring populations in Prince William Sound has stimulated discussions on restoration. The first management option, closing the fishery, has not resulted in an increase in the population sufficient to support a commercial fishery, but the fishery closure may have prevented even worse declines. Now, nearly 16 years later, restoration options are being considered, even though some may be controversial and risky. Salmon restoration efforts are common, but there has been more than 100 years of science and active hatchery operations in many countries to support this

group of species. In contrast, the information base on understanding the limitations facing herring and the related restoration science is rudimentary.

Biology and Science of Fish Restoration

The scientific concepts and principles of fish population restoration are well established but often not implemented systematically or successfully (e.g., Caddy and Agnew 2004; Walters and Martell 2004). In contrast to PWS herring, most fish stock collapses occur after periods of overfishing and habitat degradation. Therefore, the scientific basis for restoration of commercially important stock has been developed in the context of overfishing. The standard remedy to correct for overfishing is conceptually simple: reduce fishing pressure or suspend fishing completely. In practice, reducing fish catches can be difficult to implement and control, especially when there are multiple political jurisdictions (i.e., two or more states, provinces or countries) or geographically and technologically complex differences in fishing gear, monitoring and enforcement capabilities, etc. Restoration literature is rich on these topics, but these are moot points relative to the issue of the recovery of herring in PWS.

A basic assumption, applicable to nearly all approaches to restoration of fish populations, is that when fishing stops, populations will re-grow naturally, up to an approximate equilibrium level determined by the capacity of their environment – to a theoretical level known as the "carrying capacity". In general, this basic assumption seems to hold for herring: nearly all commercially harvested herring populations in the world have collapsed at some time during the last century and virtually all recovered (Hay et al. 2001).

Restoration through Intervention

Many commercial fisheries have collapsed in the last 50 years. At the same time rapid development in finfish aquaculture technology compelled some scientists to advocate artificial enhancement (i.e., "intervention") for restoring some fish populations. One possible "intervention" technique would be some form of herring hatchery, but there may be other, or additional approaches that might be considered as applicable in PWS, such as food supplementation or predator control.

The issue of restoration through intervention and particularly enhancement of marine fish populations is controversial. Part of the fisheries science community is steadfastly opposed to the concept of marine finfish enhancement. There is another component that is comfortable with the concept. However, even the detractors of the concept suggest that enhancement activity may be warranted when all other conventional management procedures fail. Even then, there are reservations about the efficacy of the approach if density-dependent factors regulating recruitment occur after the release of cultured fish.

Restoration options should be seen as a sequential process or "program" where natural recovery options are tried first, followed by intervention techniques – if possible or necessary. Caddy and Agnew (2004) provide a template of generic methodological steps that must be taken to restore depressed (usually overfished) populations. Most of their recommended steps, such as fishery closures and biological monitoring were already in place in PWS. From this perspective, the first response elements of a restoration plan for PWS had already been implemented, beginning at the time when catch quotas were reduced and also when the fishery was suspended in the mid-1990's. It was not considered as a "restoration plan" at that time, but the activities were the same. Therefore, the actions of the responsible management agency (ADF&G) were consistent with the essential "first response" elements of a formal restoration plan. The subsequent work of continued monitoring and assessment also could be viewed correctly as part of a restoration plan.

The main uncertainty of the PWS herring restoration plan concerns the problem of whether or not to take an additional step of intervention. Specifically what types of steps could be taken and how they could be implemented. The basis of that decision is the focus of this report. A chapter in a fisheries ecology textbook by Walters and Martell (2004) provides explicit protocols for the development, implementation and evaluation of a restoration program through supplemental production (Appendix B - Table 1a-c). Although the comments of Walters and Martell (2004) were directed mainly at artificial rearing and release, many of their recommendations apply to some of the other types of potential restoration options that have been considered for PWS.

Restoration Options

A series of community-based meetings in 2008 produced a list of potential intervention options. Participants included community members, scientists, and participants representing non-governmental organizations (NGO's), state and federal government agencies. These meetings were often difficult as participants struggled to find common ground as they considered a wide range of potential restoration options. Beyond a general agreement that herring are depressed, there was little consensus over the causes the herring decline, the extent or severity of the decline, the present abundance of herring, or what could, or should, be done to address the problem. Nevertheless, the meetings produced a preliminary list of options. However, decisions to proceed with any particular option require further information, in addition to the results of scientific work in progress. There are valid reasons for proceeding carefully and cautiously. A formidable reason concerns the issue of scientific validation. Any restoration program involving intervention will be expensive, and could even entail some adverse environmental effects. It is essential that the validity of the approach can be monitored and evaluated.

Beginning in 2009, all EVOSTC-funded research projects concerned with herring were designed to be mutually complementary – hence "integrated" – through the sharing of data and logistical support, etc. More projects were started in 2009 and 2010 and nearly every project will contribute some key information or understanding about either (i) the factors limiting herring recovery or (ii) the feasibility of one or more potential intervention approaches.

Criteria for Successful Restoration

Criteria for restoration have been defined provisionally as a time in the future when the PWS herring population meets the following criteria:

- spawning biomass has been above 43,000 metric tons for 6–8 years;
- two "strong" recruitments of age 3 fish in those 6–8 years (strong is $\geq$ 220 million fish);
- spawning occurring in 3 or more regions of PWS (e.g., North, East and West).

Meeting these goals means that the population is relatively healthy and stable, with a mix of age classes in the population, as opposed to one dominant age class. Because we do not fully understand the differences in survival of eggs, larvae or juveniles from the different spawning locations, there was consensus that three regional spawning areas within PWS was an important goal. The biomass target of 43,000 metric tons for 6–8 years was a mean of years during a good period, and it was thought these numbers would be more sustainable through tough years (swamping predators for example).

The duration of the program is roughly estimated at about 20 years. Probably it would take two or three years to initiate some of the pre-requisite work for some options, especially those that require mass-marking of herring.

Potential Problems for Restoration

If herring restoration were simple and inexpensive, almost certainly it already would have occurred. There are several fundamental problems related to the objective:

(i) ***Costs:*** Restoration activities can be very expensive, and EVOSTC already has expended significant funds to understand fundamental and practical issues of herring biology.

(ii) ***Scientific limits to understanding or knowledge:*** At the present time there is insufficient technical information required for certain restoration options, but this problem can be resolved with additional research. For example, it does not make sense to produce hundreds of millions of juveniles to be released in the late fall if the limiting factor is overwinter survival from starvation, climate mediated regime shift, or competition with hatchery released salmon, among various possible factors.. Or, could the production of additional herring result in an increase in predators? A better understanding of these factors will aid in the decisions of intervention strategies and locations.

(iii) ***Logistics and technology:*** PWS is remote and when coupled with the realities of harsh winters, all intervention strategies will need to be well-designed and safe for operation. These are solvable issues, but they are not trivial, and their solutions may be costly.

(iv) ***Limited accessible technical skill****:* For many activities, people with particular skill sets are required. Even if funds are available, it can be difficult to access specialized technical skill sets to work in remote parts of PWS.

(v) ***Institutional, procedural and legal issues****:* Surprisingly, this category represents one of the most difficult and formidable constraints to many potential herring restoration activities. Institutional, state and federal agencies have the legal mandate to protect fisheries and habitat through a series of procedures (e.g., environmental impact statement, permitting process with disease reviews); all of these processes will receive scientific and legal scrutiny, including from different interest groups. There are concerns for putting wild populations at risk, the use of chemicals in mass-marking, permitting, moving live fish, etc.

The Role of EVOSTC: Restoration by Intervention

A decision to investigate the feasibility of a particular restoration option does not necessarily mean that EVOSTC is committed to implementing a large-scale intervention program. Instead, the intention is to examine the implications of the concept, as it applies to herring in PWS. Full-scale intervention activities would require several years of preparation, mainly to develop and determine some technological issues, such as mass-marking of fish. Mass-marking and other technological activities are fundamental pre-requisites of any intervention activity. Therefore, because the development of these technological issues will take time, it is important that some investigations begin immediately. It also is important to understand that these investigations also could result in a definitive conclusion that the restoration activities are impractical or far too expensive.

IV. Herring Biology

Research of herring biology, supported by EVOSTC for more than 20 years, provides a foundation for understanding ecological factors affecting the PWS herring population and insight about which

restoration options are the most feasible. The following is a brief biological overview relevant to the restoration options.

Distribution

Pacific herring (*Clupea pallasi*) is one of about 180 species within the family Clupeidae (Order Clupeiformes). Pacific herring occur in waters of the continental shelf from northern Baja California to arctic Alaska, westward along shelf waters to Russia and south to Japan and the Yellow Sea. They also occur in some major estuarine areas of Arctic (Hay 1985) (Figure 2).

Life History

Herring have four distinct life stages: eggs, larvae, juveniles and adults. In PWS spawning occurs mainly in April, usually with durations of days or a few weeks. Annual mean spawning time is temperature-dependent and can vary, by a few days or even week. Eggs are adhesive and usually attached to vegetation. Hatching occurs in 2–4 weeks. After hatching, larval herring are small (~6–8 mm long) and are translucent. They move to the surface where they join the ichthyoplankton and thin. At this stage they may be advected over considerable distances, but probably are retained within the Sound. The larvae have yolks that will last a few days, followed by feeding on invertebrate eggs and small zooplankton, especially eggs and nauplii of copepods. As larvae grow, they begin to move and congregate in nearshore areas. By July, or about 10 weeks after hatching, they metamorphose into juveniles, gain silver pigmentation and begin to assume a typical herring shape. In the fall, the juveniles move into deeper water but nearshore habitat remains important for at least the first year, and they may spend up to two years in nearshore areas or bays before joining the adult population residing in deeper waters (Brown and Carls 1998). Copepods remain an important food for all life stages but adults also feed on larger crustaceans and small fish. During winter, as temperature and light decrease, food supply becomes limited and both young and adult year classes stop feeding functionally. Survival of young herring through the winter depends on the amount of food that was available in the preceding summer and their ability to store sufficient lipid reserves to sustain them over the winter. For the older age classes, winter is less limiting on direct survival but may affect their reproductive condition and spawning capacity in the spring (Carls et al. 2001).

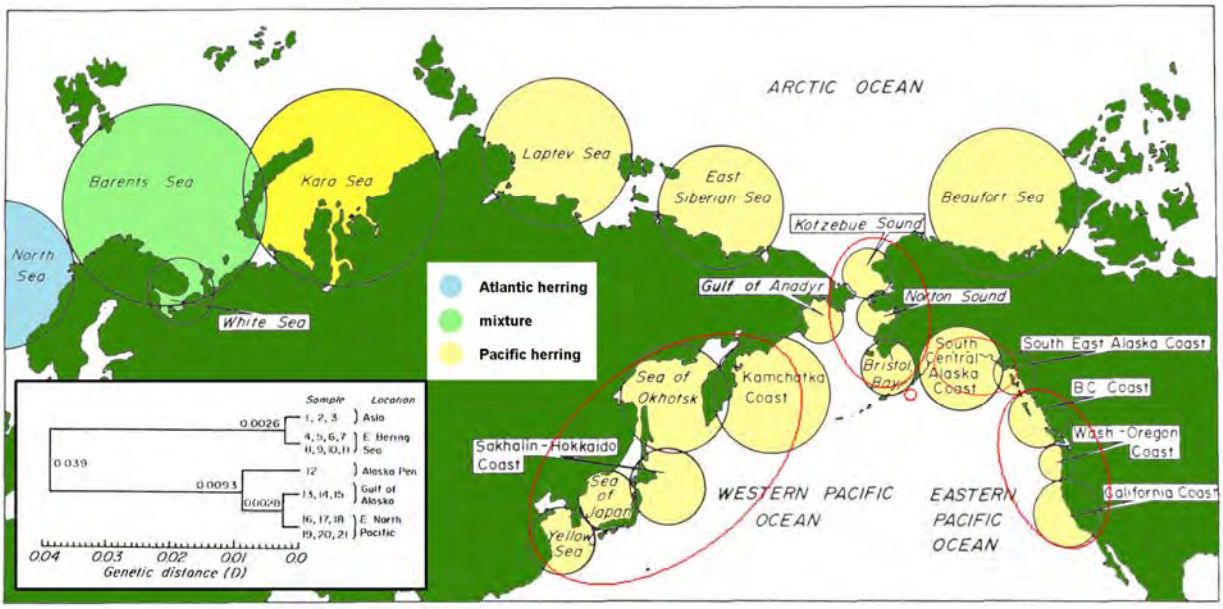

Fig.2. Global distribution of Pacific herring (adapted from Hay 1985)

Spawning Biology

Spawning in PWS typically takes place in April and the spawning season varies from five days to three weeks. Spawning locations may vary, but herring often spawn along the same beaches each year, although the volume of eggs and shoreline distances varies (Brown and Carls 1998; Carls et al. 2002). For example, from 1994 to 1997, the annual spawning beach length ranged from 23.3 to 68.5 km (Willette et al. 1998). Figure 3 shows Pacific herring spawning beds located throughout PWS based upon 1973–2006 data from the Alaska Department of Fish and Game (Moffitt, personal communication, 2006)

During spawning, the eggs attach to eelgrass, rockweed (*Fucus* sp) and kelp in shallow subtidal and intertidal areas. The eggs hatch in May, about 24 days after spawning depending on temperature (Hart 1973; Brown and Carls 1998).

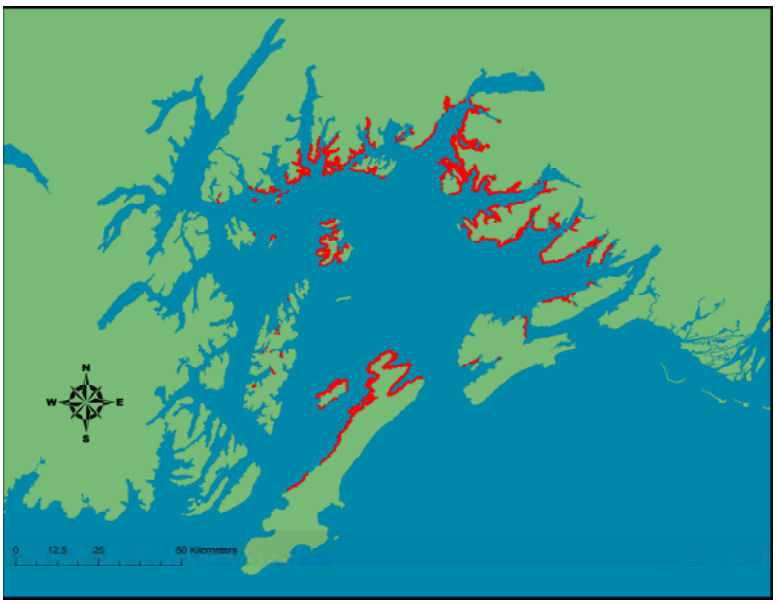

Fig.3 Pacific herring spawning beds located throughout PWS based upon 1973 - 2006 data from the Alaska Department of Fish and Game (Moffitt 2006, pers. comm.)

In PWS, adult Pacific herring rarely spawn before their third year and may live up to 15 years. The average life span of a PWS herring is nine years. After spawning in the spring, adult Pacific herring disperse from the spawning aggregations to multiple schools in deeper waters. The exact distribution of PWS herring in the summer months is uncertain, but in other regions herring typically migrate to open shelf waters to feed and return to sheltered in shore waters, in central and eastern PWS, in the fall to overwinter. The locations of the fall seine catches in the reduction fishery in the early half of the last century often was close to the entrance of PWS (Rounsfell and Dehlgren 1932; Brown and Carls 1998).

V. Potential Factors Limiting Recovery

Ideally, understanding the limiting factors would be a key to the deciding which intervention strategies have the best chance at success. A problem, however, is that this fundamental question has eluded scientific investigators throughout the world, studying herring, and other marine species. Very likely there are many different types of limiting factors (top down factors, bottom up factors), and they each will impact different life stages. One factor may be more effective in limiting recruitment of juveniles (e.g., winter availability of small prey), while another factor may be more limiting to adults (e.g., disease). The understanding is further complicated because the dominance of one factor not only may change with life stage or season, but also may change between years.

Lingering Oil

The PWS herring population was increasing prior to 1989, with record harvests reported just before the spill (Figure 3). After the spill, the 1989 year class of herring was one of the smallest cohorts of spawning adults recorded and by 1993 the fishery had collapsed with only 25 percent of the expected adults returning to spawn. To many it seemed obvious that the poor 1993 recruitment was a consequence of the spill that occurred four years earlier. The population collapse led to the closure of the commercial herring roe fishery, and ignited debate about the cause. Some remain convinced that the spill was the cause; others believe it was caused by natural systems (Rice and Carls 2007). We may never know the cause of the collapse with certainty or when it started because there is a conflict between data interpretations (Hulson et al. 2008; Thorne and Thomas 2008). While the cause of the original decline is clouded with unknowns that we cannot resolve, it is more important to understand why there is a lack of recovery.

Unhealthy fish were detected at the same time as the crash, and multiple stressors (including exposure to PAH's) can exacerbate some chronic infections to epizootic disease. Highly virulent pathogens continue to be present in the current population, and may continue to play a role as a limiting factor on the population. Disease surveillances did not occur in the previous years to the spill. Hydro-acoustic estimates of over-wintering populations were initiated in 1993, after the decline in population was detected. It is clear that the spill had some direct effects on eggs and larvae that were directly exposed to oil in 1989, but it is less certain that such exposure to oil led directly to the 1993 crash, although the 1989 cohort represented one of the poorest recruitments ever observed.

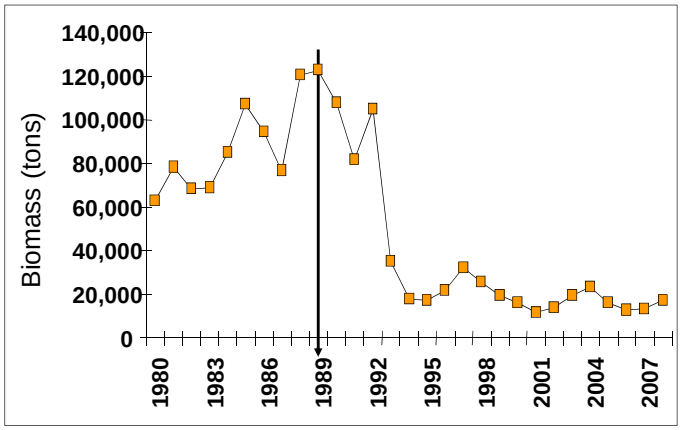

Fig 3. ADF&G ASA Model, 2008

For oil to be a cause of the current population depression, 1) lingering oil must have continued to exert new effects, or 2) the oil exposures of 1989 must have caused a persistent biological effects. There is no evidence of such persistent effects in herring. On the contrary, polynuclear aromatic hydrocarbon loads in the water are very low (Carls et al. 2006). Less than 0.2 percentof the shoreline has evidence of oil contamination (from lingering oil or from human historical habitation sites), and virtually none of that overlaps with the current spawning areas of herring (Boehm et al. 2004). Only trace concentrations of persistent organic pollutants (e.g., pesticides and polychlorinated biphenols) are detectable in intertidal areas.

Lingering oil effects are not suspected as an explanation for the continued depression of herring. There is no evidence of significant herring exposure to oil in PWS after 1990. Unlike the habitat of certain other species (pink salmon, sea otters, and harlequin ducks), oil did not persist in herring habitat (open water and intertidal shorelines); thus, the herring population is not affected by a chronic source of lingering oil. Northeastern spawning areas were not affected by the *Exxon Valdez* oil spill, nor were

north-central spawning grounds (which are not currently utilized by the herring). There was little overlap between shoreline oiling and herring spawning on Montague Island and in the Naked Island group (another area not currently utilized by herring).

Spawning Habitat

Pacific herring spawn in shallow sub-tidal and intertidal water (Haegele et al. 1981). On rare occasions thick egg deposition can limit survival (Hay 1985; Taylor 1971), but that it rare in most spawning areas in PWS and elsewhere. Similarly, low oxygen or high temperature may kill or impair development of large numbers of eggs (Purcell and Grover 1990), but this is not an issue in PWS. On the contrary, herring spawning habitat in PWS is not considered impaired by human activity, pollutants, or natural factors. Therefore, there appears to be no credible limitation to herring recovery associated with spawning habitat.

Restricted Genetic Diversity

Genetic diversity in PWS herring, examined in 1995 and 1996 (shortly after the 1993 population collapse) was comparable to that of other healthy Northeast Pacific herring populations (Seeb et al. 1999; Beacham et al. 2008). This is not surprising since herring are a "metapopulation", meaning there is significant gene flow between adjacent herring populations throughout the Pacific west coast. Both gene diversity (heterozygosity) and allelic diversity (the number of alleles per locus) are high in PWS herring. The genetic diversity of PWS herring is similar to that of herring from Cherry Point but significantly higher than that of herring from San Francisco Bay. Both of the latter stocks are stressed. All measurements examined fail to demonstrate evidence of a genetic bottleneck among PWS herring capable of reducing recruitment success. According to observed genetic diversity, the 2.2 × 104 metric ton minimum spawning biomass threshold needed to conduct a commercial fishery is expected to protect the long-term genetic diversity of PWS herring. Even currently low population levels appear to be at least one thousand times higher than the upper bound on the evolutionarily effective population size of PWS herring. Gene flow is significant between southwest PWS and the Gulf of Alaska as well as within PWS, but subpopulations within PWS cannot be reliably differentiated. Because of large inter-annual genetic variation, further work with neutral DNA markers is unlikely to "resolve the question of whether demographically independent stocks occur within Prince William Sound or even in the northern Gulf of Alaska" (O'Connell et al. 1998). Any restoration option or intervention strategy needs to preserve genetic diversity.

Competition

With depressed population levels it is possible that another species has filled niches in the ecosystem that herring previously occupied. The competition for habitat or food at some life stage may limit the success of herring. Juvenile gadids, such as saffron cod or pollock, are often found in large numbers in the same habitats as juvenile herring. Although the Sound Ecosystem Assessment program found that there was no food competition between age 0 herring and pink salmon smolts (REF), there may be competition between these two species at different life stage or for different resources (Pearson et al. 1999) . At least one recent modeling project suggested that hatchery released salmon smolts are responsible for maintaining the depressed herring populations (Deriso et al. 2008), but the roles of competition as a factor preventing herring recovery remains uncertain.

Recruitment Issues

The net population increase or decrease is the result of factors that take the population down, such as disease, predation, senescence, and how that is balanced against the forces that increase the numbers, such as more food in the summer building up the energy levels to get through the winter. "Recruitment" refers to population increases as juveniles "recruit" into the adult population. After the 1993 crash,

recruitment was low in the 1995–1998 cohorts. Years with low recruitment also occur in other Pacific herring populations but consecutive low recruitment events are relatively rare (on the order of once every 50 years). However, 4-year to 6-year runs of low recruitment have occurred at other times in other herring populations, from Washington State to Togiak, Alaska. Strong recruitment from the lowest biomass levels has not been observed at PWS or Prince Rupert, but five of the ten examined herring populations (Togiak, Sitka, Craig, Queen Charlotte Islands, and West Coast of Vancouver Island) have generated extremely strong recruitment events from the lowest biomass levels. While the low recruitments from the 1995 to 1998 year classes are within the range of natural variability, recovery of PWS herring will require further good recruitment events, combined with increased adult survival from disease and other sources.

Oceanographic Conditions

Oceanographic conditions (mixing, temperatures) have a direct effect on primary product, and thus have a fundamental effect on the amount of energy transferred to the zooplankton that herring feed on. PWS oceanographic conditions vary annually (Gay 2007; Gay and Vaghan 2001), but do not explain the 15 years of poor recruitment in PWS herring. Pacific herring respond to climatic changes, with increases in some populations during warm conditions when plankton production is generally better than during cold years. The Gulf of Alaska populations have increased during the positive phase of the Pacific Decadal Oscillation, when the Gulf of Alaska is stormy, warm and the water is well-mixed (Brown 2006). The favorable conditions for these populations appear to be related to higher plankton production, as there are larger fish at equivalent ages when zooplankton are more abundant.

Disease

A potentially significant factor affecting PWS Pacific herring recovery is age-dependent mortality from three pathogens: mesomycetozoan *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus (VHSV), and filamentous bacteria (associated with cutaneous ulcers). A severe outbreak of VHSV began in 1993 and recurred again in 1998. Epidemics of *I. hofer*i peaked in 2001 and 2005. In general, newly recruiting 3-year-olds have the highest VHSV infection rates. VHSV infection rates decrease in older fish. In contrast, *I. hoferi* infection rates increase as herring age, thus affecting the largest and most reproductively capable adults.

The causes for sustained disease problems from 1993 through 2003 are not apparent. Immune suppression can be caused after acute exposure to oil, but no herring living today in PWS were alive and exposed in 1989, and no continuing exposure to lingering oil is suspected. At present, the relationship among disease and other factors, such as the lack of food, is not apparent. The PWS Pacific herring population remains too low to allow commercial fishing and there is no hypothesis to explain the continuing disease or adequate information to predict when disease problems will abate.

Predation

In the PWS ecosystem predation on herring transfers energy from zooplankton to predators, including humpback whales, harbor seals, birds, and other fish. In this role, herring may also significantly influence or control the grazing pressure exerted on lower trophic levels (Cole and McGlade 1998). Of these predator-prey interactions, the relationship between humpback whales and PWS herring has been identified as a factor potentially limiting recovery. Intensive foraging on aggregated winter herring may represent a significant source of mortality to herring, particularly if herring stocks are depressed and humpback whales numbers increase. A whale feeding on herring from October to mid-February (150 d), would consume about 4.5 × 105 herring. More than 100 whales were observed feeding on herring in winter 2008/2009 and have been estimated to consume the equivalent of a typical commercial fishery (Rice, personal communication, 2010).

Juvenile herring are heavily predated by multiple species of seabirds, including five species injured by the EVOS (Bishop and Kuletz 2007). Current research is focused on the spatial and temporal abundance of seabird predators in and around juvenile herring schools, as well as the physical and biological characteristics of the schools used for feeding. Juvenile herring are also heavily predated by multiple species of fish (Stokesbury et al. 2002; Brown 2003). ADF&G is currently collecting salmon stomachs to investigate salmon predation on herring and the Council has funded a three-year study to examine the effects of other fish predators. The estimates of juvenile herring consumption produced by these projects will aid in planning future restoration efforts as well as in assessing the role of predation on herring recruitment by providing data to both herring and ecosystem modeling.

Energy Consumption/Food Availability

Juvenile herring diets become more varied as they grow, though they continue to feed on copepods (Hart 1973; Norcross and Brown 2001). The energy content of available food also varies seasonally, lowest in late fall and highest in spring (Norcross 2001). Sufficient energy storage to maintain age 0 and age 1 juveniles over winter is critical to juvenile herring survival in PWS. Food availability declines in winter months (the highest percentage of empty stomachs is in December; Norcross et al. 2001) and fish in cold regions often fast or reduce feeding (Paul et al. 1998). Consequently, whole body energy content drops over winter; YOY juveniles either consumed relatively less energy than adults during this period or only those with the highest energy content in the fall survived (Paul et al. 1998). Based on research results on PWS herring juveniles, energy consumption appears higher than other populations (Sitka, Lynn Canal), and when coupled with food limitations, especially for overwintering age 0 juveniles, may be a limiting factor. Overwinter survival is probably one of the most important limiting factors in the recruitment of juveniles to the adult population for all stocks, and may be disproportionately important for PWS herring.

VI. Restoration Options

It may be possible to promote restoration of herring in Prince William Sound using intervention methods such as increasing over-winter survival of 0+ juveniles by artificial feeding during the late fall or the release of juveniles reared in hatcheries. However, every potential restoration option could be controversial and few have been tried or demonstrated to be technically feasible or cost effective. Further, the use of direct restoration activities may cause unintended adverse environmental outcomes such as the increase in incidence of disease to herring or other fishes. In some instances pilot projects can test the effectiveness and help to understand the factors limiting herring recovery. All potential interventions will benefit from improved knowledge on limiting factors that may affect the success of various intervention options.

Regardless of whether active restoration methods are used, monitoring will play an important role in the restoration process. Monitoring will be required as part of any active restoration program to evaluate the efficacy of various active restoration methods, the status of recovery, and the potential occurrence of unintended adverse impacts.

The following text presents a list and summary of restoration options (summarized in Table 1), starting with the least risky and lowest degree of intervention and progressing to the heaviest intervention.

Table 1. Summary and comparison of nine restoration options. The columns summarize the life stage, potential problems, benefits, start time and duration, cost, likelihood of validation and potential for harm. Costs estimates (in $thousands) are approximations.

Restoration activity	Life stage	Potential problems	Benefits	Start	Duration	Cost	Validation	Potential Harm
1. No action Continue existing ADF&G annual biomass estimates and allow for natural recovery	All stages	No direct restoration	Np harm, no cost	Ongoing	Long	No cost	Not applicable	Nil
2. Enhanced monitoring Enhanced monitoring to inform decision makers about choice of intervention options and to obtain supplemental information on recruitment, disease, post-winter YOY survival; comparative bay productivity	All stages	Modest increases in cost	Potentially improved management decisions, enhancement of all science projects	Immediate	Long >10 years	Moderate $250K-$1000K/y, but reduced after several years	Not applicable	Nil
3. Predator management Reduce mortality by controlling the level of predation on herring. Walleye pollock is a potentially major predator (and competitor) of herring during winter. A targeted fishery for pollock is a potential restoration option.	Age 0+ to age 1+ herring, increase survival in winter	Selective removal of predators without impacts on herring	Relatively simple approach utilizing local community support.	1-2 years	Short	Low $10K-$50K/y	Difficult	Moderate to High
4. Altering carrying capacity Concurrent research investigations would conduct field experiments comparing feed supplemented versus non-supplemented areas, etc.								
(1) Winter food supplementation. During winter, as temperature and light decrease, food supply diminishes and could become limiting for age 0+ juveniles. Food would be added to selected areas in PWS.	Age 0+, December to May	Potential technical challenges - getting food to herring and vice versa	Potentially a relatively simple and inexpensive	1-2 years	Moderate - > 5 years	Moderate Pilot-scale: $50-$100K/y, full scale: $100-$1000K/y	possible but requiring moderate research effort	Unknown
(2) Increase productivity in parts of PWS by adding additional nutrients: adding inorganic nutrients to increase fish production has been done successfully in lakes for many years.	All ages - increased nutrition from spring to fall.	Validation, and indirect effect on herring	Improved growth	1-2 years	Moderate - > 5 years	Moderate Pilot-scale: $50-$100K/y, full scale: $100-$1000K/y	difficult and perhaps expensive	Unknown
5. Disease mitigation A disease ecology approach involves a three tiered process								
(1) Monitor infection prevalence and intensity to anticipate future epizootics and evaluate efficacy of future disease management strategies.	Age 0+ juveniles and all older ages	Normal issues related to fish health research	Potentially improved management, scientific benefits	1-2 years	Moderate - > 5 years	Moderate $100-$200K/y	NA	Nil

Restoration activity	Life stage	Potential problems	Benefits	Start	Duration	Cost	Validation	Potential Harm
(2) Empirical studies to determine epidemiological relationships between environmental/biological factors and disease.	All ages	Normal issues related to fish health research	Potentially improved management, scientific benefits	1-2 years	Up to 5 y	Moderate $200-$400/y	"	"
(3) Develop predictive tools to forecast future disease epidemics.	Age 0+ juveniles and all older ages	Normal issues related to fish health research	Potentially improved management, scientific benefits	1-2 years	3-5 years	Moderate $~$200/y	"	"
6. Managing competition Herring may be out-competed by pollock at the overwintering age-0 stage. If pollock is a significant competitor of herring, removal of that competition has the potential to reduce overwintering mortality.								
Selectively remove pollock by a fishery targeting that species. Targeting juvenile pollock may be difficult because it often co-occurs with herring but a selective fishery for adult pollock is feasible.	Age 0+ juveniles and all older ages	Potential controversy; capture/mortality of non-target species, disposal of pollock catch	Relatively simple approach utilizing local community support.	1-2 years	Short	Low ~$50K/y but additional cost possible for EIS review	Difficult	Moderate to High
7. Relocation of stranded eggs Two strategies were identified: relocating stranded eggs, and relocating spawn to seed underutilized bays.								
(1) Relocation of stranded egg involves moving eggs stranded on the shore back into the water to improve their viability or moving them to another location believed to be more favorable for survival.	Eggs and larvae	Basic assumptions may be valid; potential damage to healthy spawn.	Probably none	1-2 years	Short	Low ~$100K/y	Difficult,	Low to moderate
(2) Relocation of spawn, by picking kelp laden with spawn has the advantage of a higher probability of having more viable embryos survive till hatching.	Eggs and larvae	Basic assumptions probably are invalid. There is potential for damage to healthy spawn.	Development of expertise useful for other restoration options (supp. production).	Soon - 2010-2011	Short	Low ~$100K/y	Simple to prove ineffective	Low to moderate
8. Improved management strategies Harvest strategies change may be needed to rebuild the stock. This effort would include a public process involving the Alaska Board of Fisheries, stakeholders, and ADF&G personnel, possibly including a workshop. Changes may include protecting spawning areas from staging and anchoring boats, revising fishery thresholds, and restricting practices that induce disease.	Spawning adults	No implementation until the fishery is reopened and no effective validation.	Low costs to implement and potentially improved sustainability of the fishery.	Uncertain	Indefinite	Low ~$50K/y (max) - but potential loss of future fishery revenue	Not certain, probably impossible	

Restoration activity	Life stage	Potential problems	Benefits	Start	Duration	Cost	Validation	Potential Harm
9. Supplemental production Supplemental production would release cultured herring to supplement natural recruitment to assist recovery of the population to historical levels. This would be the most intrusive alternative, would require the most infrastructure, probably has the most risk from disease, and most costly of all alternatives.								
(1) Pilot-scale tests	eggs to 0+ juvenile (age 6 months)	High cost, long development and implementation period	Could follow established prototypes from Japan	1-2 years	1-5 years	Moderate-high $300-1000K/y	Necessary	low
(2) Herring hatchery or hatcheries - shore based or transportable within PWS	eggs to 0+ juvenile (age 6 months)	High cost, long development and implementation period	Direct addition of fish to the population	> 2years, requiring development of mass marking technology	minimum of 10 years	High-very high $5,000K/y (or higher)	possible, necessary requiring mass marking	unknown

1. No Action (allow natural recovery)
A serious restoration option is to take no direct action and wait for natural recovery. This would require monitoring of the population to determine abundance trends in the herring population. Keeping tabs on population trends will inform and aid decision makers about choices of intervention options. This option would require the continuation of the current ADF&G surveys and annual biomass estimates.

2. Enhanced Monitoring
This option provides supplemental information, such as evaluations of recruitment, trends in disease, post-winter survival by young of the year, and relative productivity of various nursery bays. Enhanced monitoring also might lead to a better understanding of the role of disease, predictability of disease outbreaks, and potential disease management practices that reduce disease impacts. Monitoring of herring populations and quantification and measurement of critical life-history attributes might also allow for the development of better predictive models of herring stocks, more protective fisheries management practices, and longer-term sustainability of the stock.

The tools and understanding developed by monitoring and research would be expected to provide fisheries managers with better predictions of herring populations allowing for more adaptive management practices that will be needed even if active intervention is implemented. The greatest advantage is that no ecological manipulation is required. The disadvantage is that it does nothing to restore herring populations.

3. Predator Management
The goal of predator management is to reduce mortality by controlling the level of predation on herring. Herring are a common prey item of fish, birds, and mammals, and predation is, therefore, a likely factor limiting recovery of herring in PWS. Predator management can be accomplished by altering the behavior of a predator (known as "hazing"), or by outright removal of the predator. Clearly, there are a number of herring predators whose abundance and behavior cannot be manipulated, on legal and moral grounds: Two major mammal predators in PWS (humpback whales and Steller sea lions) currently are listed as endangered species. Moreover, an important consideration for the recovery of herring populations is that they are prey to avian predators still listed as "not recovered" from EVOS. However, there are a number of significant fish predators on herring, including groundfish (walleye pollock, cod and halibut) and salmon. Behavioral modification of fish predators is not possible, but they may be removed by targeted fisheries. Walleye Pollock in particular has been identified as a potentially major predator (and competitor) of herring during the winter period, particularly the juveniles that are struggling for survival in their two years, and an expanded, targeted fishery for that species is the most feasible restoration option.

Predator management is a controversial approach. The simplest form of predator control would be fishery for some of the dominant fish predators. More controversial would be the hazing of marine mammals or birds (possible during the spawning events). It has the disadvantages of having no manner to directly test the efficacy, some of the predators are endangered species, and relying on reduction fisheries practices. This option would require a preceding Environmental Impact Statement (EIS) review. It would also require a public process involving the Alaska

Board of Fisheries and reconsideration of potential effects on Steller sea lions, now protected under provisions of the Endangered Species Act.

4. Altering Carrying Capacity

Herring feed in the winter when food is available, and that winter feeding improves their condition (Rice 2007). Overwintering starvation (or predation on nutritionally stressed individuals) is a potentially large source of mortality for herring, particularly for juveniles, so supplying supplemental food to young herring during the winter may lead to improved year-class strength.

Food may be a limiting factor for at least part of the herring life cycle. During winter, as temperature and light decrease, food supply diminishes and could become limiting, especially for age 0 juveniles. Survival of young herring through the winter depends on food availability in the preceding summer and the lipid reserves that sustain herring over the winter (Blaxter and Holliday 1963; Hay et al. 1988; Paul et al.1998; Vollenweider and Heintz 2007). For older age classes, winter survival is less precarious, but food availability may affect their reproductive condition and spawning capacity in the spring (Carls et al. 2001). Therefore, the food environment experienced by herring prior to, and during, winter may influence year class strength and reproductive capacity. These observations indicate that if food supplementation were feasible, especially to juveniles that are concentrated in shallow, nearshore habitats, then it might lead to improved survival.

There is a wide variety of marine feeds that have been developed for aquaculture that could be used towards this end, some manufactured (pellet food and the like), some more natural than others (e.g., *Artemia* eggs and nauplii); each have some advantages and drawbacks in terms of price, simplicity, and nutritional value.

A slightly different approach may promote increased productivity in parts of PWS by adding inorganic nutrients to increase fish production, as has been done successfully in lakes for many years (Hyatt et al. 2004). Fertilization has not been attempted in the coastal ocean, mainly due to problems of residence time (i.e., dilution by tidal flushing) and scale (the vast amount of nutrients required). Even in well-constrained lakes, nutrient additions have usually been of a single, limiting nutrient, and unbalanced nutrient ratios have often lead to unintended consequences (blooms of algae types that are grazer resistant, for instance). Rather than adding allochthonous nutrients (i.e., nutrients that are brought in from an external source), it is also possible to enhance the movement of autochthonous (i.e., local) nutrients by moving deep water to the surface. Deep water is generally nutrient enriched (by the degradation of sinking organic matter); nutrient levels in the deep waters of the North Pacific are among the highest in the world ocean (Reid 1961, 1965).

Nutrients usually are prevented from being transported upwards and mixed to the surface by temperature or salinity gradients. Such gradients are especially pronounced in PWS, where the large amount of fresh water input every spring and summer create a relatively fresh surface layer overlying deeper, nutrient rich water. However, it is possible to move deep water to the surface, which will increase nutrient concentrations and enhance production; the technology has been used for many years for shellfish aquaculture. A series of simple calculations suggest that

artificial upwelling may enhance growth in fish stocks (Kirke 2003), though those calculations were done for a low latitude reef ecosystem.

The surface waters of PWS usually are stratified in summer (Vaghan et al. 2001), which tends to reduce nutrient fluxes to the surface. Most primary production occurs in April and May (Eslinger et al. 2001).) Mechanical "upwellers" could be used to enhance late-summer production: the technique has been recently demonstrated in the open ocean (Grabowski et al. 2008). Age-0 and age-1 schools inhabit nearshore areas and by late-July locally enhanced production and increased food availability could then be expected to result in increased energetic reserves in young herring which could lead to a concomitant reduction in overwintering mortality (Norcross et al. 2001).

There are many questions that need to be addressed prior to initiating an overwintering feeding or nutrient enrichment program. Within overwintering bays, it is important to have some understanding of the current winter carrying capacity. Measurements of how much food is available to overwintering herring can be assessed by plankton surveys. It is also important to understand the bioenergetic requirements of herring during winter, in order to determine how much food is required. However, the bioenergetics of herring are fairly well known (Megrey et al. 2007). Finally, surveys to enumerate herring and their competitors, as well as the location where supplemental feeding should occur, are needed in order to determine how much food would be required.

To assess the effectiveness of an overwintering feeding program, it would be important to monitor winter survival as well as the energetic condition of the fish. A comparative approach, where one or more bays are manipulated and others are not, would permit testing whether or not food additions improved overwintering survival and by how much. A potential test of the effectiveness of feeding supplementation could be based on fatty acid (FA) profiles. If the FA composition of manipulated bays were different than the profiles of non-affected bays, then this would be reflected in the FA of herring that consume the food. Therefore, FA testing, combined with other tests, could determine if manipulation led to increased feeding of herring, and if the effects of the manipulation were limited to local areas, or whether the possible movements of herring among different bays, obscured any local effects. Similarly, to assess the effectiveness of a late summer nutrient enrichment, it would be important to also monitor the effectiveness of the upwelling system (with measurements of nutrients and productivity), as well as to follow survival and energetic condition of the fish. Again, a bay-to-bay comparison would be required to determine if nutrient additions were effective.

The technological requirements for a feeding program are fairly modest. There is a requirement for technological development of the method used to deliver the food, and evaluation of the nutritive composition of the food. Aquaculture nutrition is a mature science, and there are many aquaculture feeds currently available that might be used for herring. Similarly, a late summer nutrient enrichment program could use existing upweller technology. Some upwellers are powered by waves, others by mechanical pumps; it is likely that an enclosed bay (which receives less wave action) would require the use of the latter. Both of these restoration options would need to be informed by synoptic, broad scale surveys of overwintering bays in PWS; high-speed,

cost-effective survey methodologies (optical and acoustic) are required to collect the necessary data at the appropriate scale and at a reasonable cost.

The approach depends on being able to identify the location of overwintering juveniles and providing an appropriate feed for them. It is important that any such program not attract predators or competition for the food resources. A full scale program may require repeated feeding at several locations within Prince William Sound. Advantages of this approach are that cultured herring are known to eat commercial feed, so the cost is likely to be moderate. Also, it may be possible to mark the fish using the feed. Disadvantages include the need to identify appropriate feeding locations, feed the target species without creating more predation or competition, and ensure the fish can metabolize the food.

5. Disease Mitigation

A potentially significant factor limiting PWS herring population is age-dependent mortality from three pathogens: the mesomycetozoan *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus (VHSV), and filamentous bacteria (associated with cutaneous ulcers). A severe outbreak of VHSV and ulcers began in 1993. Epidemics have cycled through the Pacific herring population in PWS about every four years with decreasing severity since 1993. However, epidemics of *I. hoferi* have been observed in more recent years.

The causes of the persistent disease are not apparent. Unfortunately, there are no long-term disease data sets for other herring populations or other species with which to make comparisons. Immune suppression can be caused after acute exposure to oil, but no herring living today in PWS were alive and exposed in 1989, and no continuing exposure to lingering oil is suspected. An original hypothesis was that disease was a sporadic event associated with exceeding carrying capacity (Marty et al. 1998), but the 1998, 2001, 2002, and 2005 disease events occurred when the population was relatively low. How the current levels of disease and their interaction with other factors, such as predation or poor nutrition, affect mortality rates at the different life stages is unknown.

Traditional disease management strategies involve an integration of infection prevalence and intensity monitoring with mitigation strategies, including prevention with prophylactics, treatment with appropriate therapeutics, and adaptive disease management practices that are evaluated by continued disease monitoring. Although this proven process typically works extremely well in hatchery situations, where fish are monitored and manipulated under semi-controlled conditions, the traditional disease management process is not appropriate in situations involving populations of wild marine fish, including Pacific herring in Prince William Sound. For example, administration of prophylactics and therapeutics to populations of wild marine fish are complicated by issues involving ecosystem scale and fish community dynamics, and are typically not considered appropriate for populations of wild fishes. These complications have historically prevented the advancement of disease management in populations of wild fish; however, the field of disease ecology has recently emerged and is offering creative ways to mitigate and manage diseases in wild populations.

A disease ecology approach is similar to that employed by the World Health Organization (WHO) and Centers for Disease Control (CDC), and involves a three tiered process involving:

(1) Establishment and continuation of infection prevalence and intensity monitoring and surveillances. This component is required to monitor changes that signal the emergence of future epizootics and to evaluate the efficacy of future disease management strategies.

(2) Incorporation of empirical studies intended to determine the basic epidemiological relationships between environmental and biological factors influencing infection / disease prevalence.

(3) Development of predictive tools, based on known epidemiological relationships, which will be useful in forecasting the potential for future disease epidemics.

Combined, this three-tiered approach will provide the basic epidemiological information necessary to develop and validate adaptive disease management strategies intended to mitigate the effects of future herring disease outbreaks in PWS; these adaptive management strategies can then be evaluated and adjusted through continued monitoring for infection prevalence and intensity. A very clear advantage of this approach over that employed by the WHO and CDC involves utilization of the natural host (Pacific herring), rather than mammalian surrogates for humans, in empirical manipulation studies.

Interaction between the disease mitigation and supplemental production options

Disease principles, relationships, and adaptive management strategies addressed in the Disease Mitigation option are also critical and intimately tied to the success of restoration option 8: Supplemental Production. Disease is a natural phenomenon inherent to populations of both wild and hatchery fishes, with both groups of fish sharing similar causes, exacerbating factors, and principles of disease. For example, viral hemorrhagic septicemia causes large epizootics among populations of wild Pacific herring (Traxler and Kieser 1994, Meyers and Winton 1995, Meyers et al. 1999, Hedrick et al. 2003), and often causes epizootics in impounded herring used for the closed pound spawn-on-kelp (SOK) fishery that has occurred in PWS (Hershberger et al 1999). As a result of extremely large quantities of infective virus shed into the water during active epizootics (Kocan et al. 1997; Hershberger et al. 1999; and Hershberger et al. In Preparation), some have questioned the impacts of the closed pound SOK fishery on initiating epizootics and deleterious population-level effects to wild, un-impounded herring.

6. Managing Competition

Several species of fish occasionally compete with herring for food resources, so competition may be a partial limitation to recovery of herring stocks, particularly at early life stages such as overwintering age-0. Recent work (Deriso et al. 2008) suggests that competition (and predation) from juvenile salmon released from hatcheries in PWS may be limiting the recovery of herring.

Juvenile walleye pollock (*Theragra chalcogramma*) is also a significant competitor to herring in PWS (Sturdevant 1999; Purcell and Sturdevant 2001). Juvenile pollock inhabit the same nursery bays as juvenile herring; the energetic content of pollock tends to increase over the winter, while that of herring declines (Paul et al. 1998; Kline 2008). This suggests that herring may be out-competed by pollock during the winter, which would add to overwintering mortality (pollock is also a predator of herring, and predator control is dealt with in another section). If pollock is a

significant competitor of herring, removal of that competition has the potential to reduce overwintering mortality.

The removal of pollock may be accomplished by a selective fishery specifically targeting that species. In practice it may not be possible to specifically target juvenile pollock, because it often co-occurs with herring. A selective fishery for adult pollock could be accomplished more easily and would result in a concomitant reduction in the number of juvenile pollock the following year provided that there is a strong stock-recruitment relationship. To be successful, some basic knowledge of the biology of pollock in PWS would be required, including estimates of stock size, age structure and distributions. Also, it will be important to estimate the number of pollock needed to be removed to have the desired impact. As well, there would be no need to develop specific fishing gear technologies for this option; pre-existing gear and methods could be employed.

7. Relocation of Stranded Eggs

Two strategies were discussed in the 2008 Cordova meetings: relocating stranded eggs, and relocating spawn to seed underutilized bays. Neither strategy involves impoundment, handling of adults, the lengthy propagation or feeding of larvae and juveniles; hence, the logistics and costs are minimal.

Relocation of stranded egg involves moving eggs stranded on the shore back into the water to improve their viability or moving them to another location believed to be more favorable for survival. Some participants in the 2008 Cordova meetings considered stranded eggs to be a waste. They advocated a strategy to salvage the "wasted spawn" to reduce mortality at the egg and through the larval drift stages of life. Some of the assumptions for moving stranded eggs may be challenged, however. In a study that examined the collection and transfer of such eggs to a new location, most of the eggs were found to be viable, even after extended periods on the shore (Hay and Marliave 1988). Further, many of the stranded eggs were naturally re-immersed in water on subsequent tides.

Relocation of spawn, by picking kelp laden with spawn, would be more intrusive, but has the advantage of a higher probability of having more viable embryos survive till hatching. Because the picked kelp could be held in a predator-exclusion structure, such as a herring impoundment, high hatch rates could be expected. This mechanism would permit the possible seeding of bays removed from the current spawn areas. Advocates of this approach, however, should realize that prior work in BC was unsuccessful. Although billions of eggs were collected and transported to a new location, there was no subsequent spawning in the location in the years following the transfer (Hay and Marliave 1988).

Advantages of the approach are that the manipulation of eggs may allow them to be marked, handling is relative low, infrastructure is low, and, hence, the cost is relatively low, giving this alternative some attraction. Disadvantages include potential harm to existing eggs during the collection process, the low likelihood of being able to manipulate enough eggs to detect an effect in the population, and it bypasses very few potential bottlenecks (e.g., predations, overwinter survival of age 0) in herring recovery, so it has a lower likelihood of success.

8. Improved Management Strategies

The recovery goal outlined in this plan requires a biomass above that currently used to open the fisheries. Therefore, changes to harvest strategies may be needed to allow full rebuilding of the stock. Such changes may include protecting spawning areas from staging and anchoring boats to reduce disturbance to the eggs, changing the fishery threshold, and restricting practices that tend to induce disease. Advantages of the approach include low costs to implement and potentially improved sustainability of the fishery. The disadvantages include not being able to implement until the fishery is reopened and no direct measure of how the changes affect the population.

9. Supplemental Production

Supplemental production would release cultured herring to supplement natural recruitment to assist recovery of the population to historical levels. This would be the most intrusive alternative, would require the most infrastructure, probably has the most risk from disease, and would be the most costly of all alternatives.

Rationale and overview

Raising early life stages of herring in captivity avoids high rates of mortality occurring at larval and juvenile stages. This approach appears to be successful in Japan where herring are cultured successfully, released into the natural environment as juveniles, and recovered years later as adults. If this approach were tried in PWS, all fish released must be marked to provide a basis for evaluation of the program. The success of a supplemental program may depend on the duration of the rearing period: longer is better, up to a maximum of a year. Therefore, the duration of the captivity period is uncertain at this time, but a spring release would avoid potential starvation in the winter, and would release juveniles at the time of the spring bloom when wild food abounds.

Mass-marking technology would need to be developed and authenticated before enhancement activities could be considered. Also, a "core" monitoring program to measure natural impacts on the PWS herring population must be in place. Supplemental production approaches could be costly and it is essential to determine valid cost estimates required to ensure success. Uncertainties involve unresolved questions of scale. Specifically, how many juvenile herring would be required to effectively supplement natural recruitment, and what would the program cost? <u>These questions could be addressed in a "white paper" that considers the scale and costs of a supplemental program.</u>

As an approximate guide to the probable scale of a supplemental operation, a 10 percent increase in the present annual recruitment of about 200 hundred million age 3 recruits, would require the addition of 20 million age 3 herring. Probably the mortality between the time when supplemented herring are released (as 0+ herring in their first winter) and the time when they join the spawning population (as age-3 or age 4 recruits) is substantial (>90%). Therefore, it may require the rearing and release of at least 200 million herring juveniles, and perhaps more, to achieve even a modest (10 %) increase in recruitment of 10 percent. Production of this magnitude is in the same ballpark as the hatchery releases of salmon in PWS.

An advantage of supplementation is that it adds fish directly to the ecosystem and technology exists for rearing large numbers of juveniles. Another advantage is that it involves very low

impact on the wild population in that relatively small numbers of herring are required as sources of eggs. For instance, a relatively good cohort of herring in PWS at the present time might consist of about two hundred million fish (or 20 thousand tons), about ten times greater than the sizes of most recent cohorts). Even full-scale supplementation would not attempt to rear twohundred million herring, but even if it did, it would require the eggs from only about one ton of herring (or 0.005% of the present population). Probably a more realistic number for potential supplemental production would be the addition of 20 million fish (approximately the same number as the number of released pink salmon released annually). Even allowing for considerable mortality (90%), etc., such supplemental production (20 million additional recruits) would require the gametes (eggs and sperm) from about one ton of herring.

Disadvantages of a supplemental production option include the potentially high costs associated with the duration of the herring rearing period and the potential for the release of diseased or inferior stock among numerous unintended and undesirable consequences. Probably it would require 2–3 years to establish the efficacy of a mass-marking technology, although it is likely that such an approach can be met successfully, provided that permitting issues can be addressed. The time required to conduct pilot-scale experiments is at least several years. Another three years may be needed to implement full-scale supplemental production. Once released it would require 3–4 years before some of this hatchery-produced cohort recruited to the adult population. Therefore, it would take at least six years and probably several more before the success of the project could be evaluated.

Supplementation facilities

The types of containment systems that might be used for mass-rearing of PWS herring require further discussion and innovation. Traditional shore-based facilities, which require massive volumes of pumped sea water, provided to fish housed in large tanks, are probably not the prototype for work in PWS. A drawback from such an approach is that the release site would be confined to the immediate vicinity of the shore-based hatchery. This may be a problem because the optimal locations for release may be elsewhere. It would likely be best to release hatchery-reared herring in multiple sites, especially in habitats that are known to be natural habitats for juvenile herring. Such widely distributed release would be simplified if SP-herring were reared in floating facilities that could be towed to one of more release sites. Experience with herring bait pond operations in British Columbia and Washington State shows that the capture, confinement and movement of live herring can be difficult. Herring do not react favorably to being moved with dip-nets or confined to small net-cages, even for short periods. Often such handling results in abrasion and scale loss followed by disease outbreaks. It follows that such practices must be avoided during the conceptual design phases of any potential herring project.

Validation approaches—the essential requirement for mass-marking

Regardless of the place, duration or larval containment method, all fish released must be marked to allow the efficacy of the program to be determined. This fundamental requirement must be established early in the enhancement schedule of activities. There are positive spin-offs that accompany a well designed mark-recapture programs as they would also provide means to address fundamental questions about factors limiting recovery. There is also the potential for controlling the release site environment in a manner that can inform the efficacy of other restoration alternatives.

Although artificially reared herring can be successfully released to the wild, there is still uncertainty about whether such releases actually increase the population, displace naturally produced fish, or merely become supplemental food to enhance predator populations. Similar debates continue about supplemental production of other hatchery-produced species, such as salmon, and the answers are not necessarily clear. A resolution to such a debate involves a marking or tagging program of naturally produced species, done in conjunction with releases from supplemental production. However, the implementation of such a tagging program for wild herring would not need to be initiated until the technology for mass-rearing and mass-marking of hatchery-reared herring is well established. This would require a few years.

Pilot-scale experiments
Any full-scale supplemental production program (i.e., release of herring juveniles reared in captivity) must be preceded by pilot-scale experimental projects that establish a protocol for effective mass-marking. This is not trivial and it took several years of preliminary work before Japanese researchers were able to meet this requirement. Work in PWS can build on Japanese experience, but pilot-scale experimental work is essential because conditions differ. The best pilot-scaled program would provide the information needed for developing a full-scale in situ herring marking and rearing program.

VII. Monitoring and Core Data Collection

Any restoration activity will require basic information about the PWS herring population. Annual assessments of spawning stock biomass (SSB) are essential – both for any intervention activity as well as for the continuity of responsible management. Regardless of which, if any, restoration option is undertaken, monitoring will play an important role in the restoration process. Monitoring will be required as part of any active restoration program to evaluate the efficacy of various active restoration methods, the status of recovery, and the potential occurrence of unintended adverse impacts.

Enhancement of monitoring for stock assessment
Currently, an annual stock assessment is completed by ADF&G. Data requirements for a minimal management plan require samples of the spawning population to determine the age and size structure. These data are supplemented by assessments of the relative abundance of herring spawn (measures as the cumulative distance of spawn along shorelines). Further, these data are often supplemented by acoustic surveys in selected parts of PWS. Due to funding and staffing constraints, the current surveys are not as comprehensive as needed to gain a working understanding of the current state of herring in PWS.

***Top-down process monitoring*:** Predator and disease monitoring need continued monitoring. We understand both processes exist, but we have less understanding of the dynamics of both processes across years and life stages. Both of these processes will continue to occur with wild fish but also come into play with enhanced fish.

Disease monitoring: Regular collection of specimens would be used to test for the presence of pathogens. Further, there must be a capability to evaluate the extent of epizootics as they occur.

Predators/competitors: The abundance and distribution of important predators/competitors to herring is required, particularly as they affect the early life stages and recruitment. In general, this will require a combination of field surveys and subsequent laboratory analysis to evaluation trophic relationships of herring and other species that consume the same prey as herring.

***Oceanographic monitoring*:** Oceanographic monitoring of the physical and biotic environment in PWS must continue. Environmental conditions affect the growth environment for herring, which in turn may affect survival, especially the over-wintering survival of age 0+ juveniles. Further, the amount of planktonic food transfer between PWS and the Gulf of Alaska can impact the ecosystem within the sound (Cooney et al. 2001).

VIII. Implementation Plan

Restoring herring is a complex problem, from scientific, technical, legal, and political perspectives. The path to successful restoration is not obvious or simple. Every path is likely to be controversial, including the speed along the path. Every potential restoration activity will require a sequence of difficult decisions and probably the information available may not be fully satisfactory basis for most decisions. Given this uncertainty, the plan outlined below is designed to make progressive advancements in better understanding of the technical efficacy and limitations, financial costs and legal implications of potential restoration activity.

Moving forward toward the goal of a restored herring population will require time and careful evaluation of the present status of herring and the possible impact of potential activities. A "phased approach" is best, with each of several phases focusing on different stages of the development of the program.

A defensible, scientific approach to herring restoration in Prince William Sound would be to approach the issue in incremental steps, or stages. At the beginning of each step there would be an objective and a set of activities that would be evaluated at the end of the step. We suggest that the earliest steps of a "conceptual phase" that began approximately in 2007, are already completed. We are now at the beginning of a second "scoping stage", but the components of the first stage are described below to provide a context for the subsequent components of a restoration plan.

The initial phase of herring restoration has likely already occurred in the form of four distinct activities: (1) a series of meetings in Cordova in 2008 that developed a list of potential restoration activities; (2) beginning in 2010, a restructuring of EVOSTC-funded research proposals concerned with herring to ensure that all were inter-connected and addressed issues or questions related to one or more of the potential restoration options; (3) in 2007 a report (white paper) that reviewed the efforts of herring restoration, and related activities in other countries; and (4) in 2008–2009 a workshop and report on issues related to mass-marking and tagging of herring, which could be essential components for validation of any herring restoration program.

Although there is a list of nine herring restoration options (summarized in Table 1), none is fully developed to the point of implementation. Mainly, the uncertainties concern factors that can be examined in the first year or two (2012–2014).

Stage 1 – Monitoring and scoping stage: strategies and feasibilities (2012–2016)
The preliminary steps taken in stage 1 helped to define and understand the potential options, but it also led to the understanding that there are other aspects that must be addressed prior to initiating any active restoration intervention or activity. This stage should begin in 2012 and be completed in 2013 or 2014 and consists of ensuring that all of the technical, scientific, legal and administrative components are in place prior to any active restoration work. It also includes specific requirements, to begin as soon as possible, to enhance monitoring.

Monitoring is essential, so that recruitment factors are better understood. Three types of monitoring can be distinguished:

(1) Recruitment monitoring, mainly associated with contract research directed at the pre-recruitment life stages of herring biology and ecology. This includes oceanographic monitoring, sampling for juvenile herring in bays, and other work, some of which is currently in progress as part of contract work funded by EVOSTC for the years 2010-2013. This specific monitoring should be re-evaluated after three field seasons (in 2012) with the intention of reducing the effort (perhaps by half) and selecting and retaining the most productive and informative monitoring measures on recruitment, to be continued for the next 4–20 years but at a reduced level.

(2) Top-down monitoring, mainly associated with predation on all life stages, but with particular emphasis on bird, mammal and fish predation of older juveniles and adult herring. Enhanced "top-down monitoring" should begin in the next 1–2 years, and continue for about five more years, followed by a longer period when it is re-examined, reconfigured and conducted at a reduced level.

(3) Herring population monitoring, with special emphasis on age composition, geographic distribution, and spatial and temporal variation in size and age within PWS.

Six scoping tasks are defined, each of which should result in a stand-alone report. The completion of this scoping stage would be an assessment and evaluation of all of the information that would provide essential details about cost and scale of effort related to each potential restoration option.

> ***Scoping Tash One: Summary of Past and Ongoing Herring Projects***
> This first scoping task will provide a comprehensive review of the past and ongoing herring projects related to herring. This review will help determine where data gaps exist, what long-term data sets are available, and indicate any causative factors and the prospects for restoration activities.
>
> ***Scoping Task Two: Monitoring evaluation***.
> The second scoping task is to ensure that the present monitoring systems for herring can adequately detect change in abundance, either for increases or declines. This can be done

best in a small workshop of scientific and technical experts, mainly from ADF&G and NOAA. The workshop would culminate in a written report, that will review present and past monitoring and assessments. The workshop report would compare procedures used for herring assessments in PWS and (i) comment on the relative strengths or weaknesses relative to assessments done elsewhere, especially in the northeast Pacific, (ii) estimate the sensitivity of the present approach, in terms of the ability to detect changes in recruitment or abundance, or other demographic or ecological changes in herring, such as spawning location; and (iii) recommend specific changes, as required that might be essential for the restoration options. This work could be done within the next 12 months.

Scoping Task Three: Defining the regulatory environment.
A different but concurrent preparation task is to develop a stand-alone report that would describe the implications of restoration activity relative to the regulatory environment in PWS. For instance, most restoration activities involve movement of live fish, and this aspect falls under jurisdiction of the State of Alaska. There also are regulatory implications of fish disease, etc. Prior to initiating any restoration work there must be a well-defined method for determining the regulatory implications for specific restoration activities. This report could be done by contract, or perhaps by a short-term secondment (1–2 weeks) of state or federal personnel who understand the broad range of regulations that might affect any of the potential restoration activities. This work could be done within the first 12 months.

Scoping Task Four: Scaling restoration activities.
A fourth concurrent task, relevant to some, but not all, potential restoration activities, is to determine the "scale" of changes that must be made to make a significant impact. For instance, some restoration activities attempt to improve survival of young herring entering the adult population – or the technical term is "recruitment". It is necessary to estimate the level of increase in recruitment that would be required to make (1) a detectable difference and (2) a significant increase, so that the PWS herring population could be restored. Similarly, if the objective of a restoration activity were to decrease predation rates (i.e., increase survival) of adult herring, then there is a requirement to know how much activity is required to make a detectable difference. Completing this preparation activity could be done in a small workshop or some short-term contracts that would prepare a definitive report on the scale required for each of the potential restoration activities. This work could be done within the first 12 months.

Scoping Task Five: Defining key biological decision points.
A fifth preparation activity is to examine and define criteria and "decision points" for guiding restoration work. It is essential to have defined criteria that would provide a quantitative basis for (1) deciding when to initiate any restoration activity and (2) when to suspend restoration activity, if the population is recovering or if the population fails to recover. Changes in abundance, although probably the primary factor affecting restoration work, are not the only criteria. For instance, restoration activity might also be based on changes in spatial distribution (especially if all herring were confined to specific areas) or pronounced changes (voids) in demographics (i.e., missing several cohorts in the age

composition). This "pre-restoration" activity could be initiated in a workshop, held within a year that would produce a set of workable criteria and restoration points.

Scoping Task Six: Costs and directions.
This activity concerns issues of scale and cost and the temporal duration of potential restoration activities. Many restoration activities would be very expensive, especially if conducted at a scale that would impact the entire PWS. Some would require nearly a decade or more to be fully implemented and evaluated. The costs of such work could be prohibitive, requiring more than the EVOSTC budget. Prior to initiating any restoration activity, it is essential that the approximate costs of each activity be examined, both for the implementation of pilot-scale work and the potential start of full-scale restoration. This preparation task follows as a logical outcome of the four other tasks (monitoring, regulation, scaling and decision points). It will require about one year to complete all of the other preparation tasks. At that time, during the spring or summer of 2013, the information from each of the four preparation tasks would be assembled and examined by a small team of specialists representing skill sets that could establish a credible evaluation of the costs and time associated with each of the potential restoration activities. The composition of this team could be decided during the coming year and could include some of the same people who contributed to the four other preparation tasks reports.

Stages 2 and 3 – Implementation and monitoring (2014–2022)
Stage 3 is not fully defined and must wait for the full development of stage 2 (enhanced monitoring and scoping tasks). The recommendation is to pursue the restoration options that (i) do no harm; (ii) are the least expensive, and (iii) appear to have some chance of success that can be validated.

The list of options (Table 1) begins with "no action" as Restoration Activity 1. The choice of this option would depend on recent trend in PWS herring abundance between 2009 and 2014. If herring abundance appears to be increasing, this may be an acceptable option. If the trend were for continuing deterioration of herring, then the no-action option should be dropped in favor of one or more active intervention options. A key decision point concerns the level of herring abundance that is deemed to warrant sufficient concern to lead to the implementation of restoration work. This decision point should be defined in a scoping workshop in (see Scoping Task Four above).

Of all of the restoration options, the simpler and lower cost option would be to re-evaluate the fishery threshold as an aspect of "Improved Management Strategies." The near-term cost would be foregone harvest revenue.

Predator management, in the form of a target fishery for pollock (Restoration Activity 2), also addresses the "managing competition" option (Restoration Activity 5). The predation hypothesis is that pollock predation on herring has led to some reduction of the PWS herring population, so that reduction of the predator biomass would lead to enhance herring survival. The competition hypothesis is that juvenile pollock may compete for similar zooplankton food as juvenile herring ; therefore, reducing adult pollock would lead to a reduction of juvenile pollock, hence, reduced competition. The main problem with this option is that it would be difficult to verify its success

because herring populations might have increased even if a pollock fishery did not occur. In contrast, it would be relatively simple to confirm that restoration by pollock removal failed, and this would occur if herring has not increased after fisheries targeting on pollock. The advantage of this approach is that the work is relatively inexpensive, but the public reaction could be mixed, especially if there were no market for the captured pollock (i.e., wasted) or if there were significant bycatch, especially of herring. The conceptual basis for the competition-reduction hypothesis may require more attention. The premise is that the reduction of adult pollock would eventually lead to a reduction of juvenile pollock that compete with herring for limited food. This premise requires more scrutiny.

Two options to alter carrying capacity through altering carrying capacity or food supplementation (Restoration Activity 3) also could be conducted for a moderate cost, although there are some technical details that remain uncertain. The key hypothesis is that survival of age 0+ herring may be restricted by food limitation. The addition of relatively small amounts of food could stem the over-wintering mortality that may limit herring recruitment in PWS. Although technical uncertainties exist, this option addresses interesting hypotheses based on early work by Norcross, Brown, Paul and others (see references). It would be difficult to confirm the hypothesis that herring recruitment improved following feeding (the evidence would only be circumstantial), but if there were no marked improvement in recruitment following feeding supplementation, corroborated by sampling of herring juvenile nutritional condition, then this option could be discarded as ineffectual. There may be opportunities to develop tests that monitor unique natural chemical signal in supplemented food (such as fatty acid profiles) that would provide a basis for tracking the fate of supplemented food, and whether it is utilized effectively by herring.

The disease mitigation activity (Restoration Activity 4) does not involve active restoration activity, but it seeks to determine if there are any underlying relationships between environmental factors and the incidence of herring disease. In general, this approach involves leading edge, technically sophisticated and rigorous scientific research. It is relatively modest in cost and has negligible impact on herring with potential to provide significant benefits.

The premise for the relocation of stranded eggs (Restoration Activity 6) may be flawed, because stranded eggs are not necessarily doomed, and tidal actions may re-immerse some stranded eggs. Also, herring eggs can develop normally for extended periods in air provided that temperatures are not extreme and the eggs do not become desiccated. Although the cost of relocating eggs is relatively low, there could be deleterious impacts on normally developing eggs. Perhaps a redeeming aspect of this activity is development of egg acquisition protocols for possible herring hatchery work (Restoration Activity 9), if that were to develop. There is virtually no way to evaluate the efficacy of egg relocation, but it is possible, and likely, that the approach could be ruled unsuccessful, or the implicit assumptions about herring stock structure were incorrect, if herring did not establish or re-establish in the transplanted locations (Hay and Marliave 1988).

The improved management strategies activity (Restoration Activity 7) is based on the assumption that flaws in herring fishery management may has contributed to the 1993 collapse,, but hard evidence for this is lacking. Perhaps the major concern is that some impoundment operations may have led to the spread of disease.

The final restoration activity of supplemental production (Restoration Activity 9) or herring hatchery(s) is the most difficult and expensive and has the longest duration. Ironically, it is the one activity that has a successful prototype, based on Japanese work. This concept and details of this approach have been examined elsewhere, but the major reservations about proceeding with this approach include the high cost, the unknown potential for disease transmission from such a hatchery, as well as unintended genetic effects on the wild stock./. If this approach were attempted, it must be preceded with pilot-scale work on mass-marking (recommended) and pilot-scale rearing facilities (perhaps recommended but not immediately). Even with the successful completion of preliminary mass-marking and pilot-scale work, this supplemental production option should only be undertaken as a "last resort", if and when there has been demonstrable failure of the preceding approaches to increase herring abundance and a deterioration of natural production.

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Appendix A - Terminology

Recovery – Recovery is the return of the PWS herring population to some defined level. This can occur naturally or through restoration activities.

Restoration – Restoration is the recovery of the PWS herring population through human actions.

Intervention – Intervention describes the activity that attempts to either increase PWS herring birth rates or reduce PWS herring mortality.

Enhancement – The goal of restoring the herring population in a habitat that is capable of sustaining it.

Integrated program – An ecosystem based program organized around common goals/hypotheses determined and implemented through involvement by impacted communities and scientists to develop a teamwork that creates efficiencies, open communication, and inter-related activities that inform each other to achieve the program goals.

Supplemental production – the release of cultured herring to increase the existing herring population.

Intensive aquaculture – Rearing of herring using traditional hatcheries and artificial environments.

Extensive aquaculture – using natural habitats (bays) to rear herring

Recruitment - the process of older juveniles becoming sexually mature and joining the adult population. This definition is specific to Northeast Pacific herring.

Gamete - sperm or unfertilized ova, prior to release from adult fish

Egg – fertilized ovum, adhesive and sessile, within the inter-tidal and shallow sub-tidal zone, with developing embryo, and hatching in ~ 3 weeks

Larva – recently hatched embryo, living off yolk sac (~5 days) and feeding on small (~100 μm) zooplankton, living in surface waters (mainly top 20 m) and part of the zooplankton community, although most abundance in nearshore habitats. In general, larvae are long and thin, with little resemblance to adult forms.

Metamorphic – process of change between larval and juvenile forms (pigmentation beginning, physical change)

Juvenile – the stages between the larvae and sexually mature adult. Young juveniles begin to assume the adult form and develop silvery-colored scales. In general separate cohorts begin to aggregate together and form schools. In general the young juvenile stages are retained in nearshore habitats, but may venture into offshore (continental shelf areas) during their second or

third years. The duration of the juvenile stages usually ends at age 3 or 4 when the fish are sexually maturing and joining adult schools.

Adult – the sexually mature stage, beginning at age 3 or 4 (36–48 months of age). Adults may form sub-populations that may, or may not migrate to shelf waters for summer feeding. In general adult herring form dense aggregations during winter months and remain relatively immobile and feed opportunistically.

Mass-marking – the ability to place a physical or chemical mark on large numbers of fish in order to determine their place of origin

In-situ – taking place in the original environment; not moved

Carrying capacity - The maximum population of a particular organism that a given environment can support without detrimental effects

Otolith - Calcareous particles found in the inner ear

Appendix B – Critical Steps In Program Design

Table 1a - Critical steps in program design – (adapted from Chapter 13, Walters and Martell, 2004). The discussion of development steps in a generic marine fish enhancement program was adapted to and Excel sheet. The comments in the right column indicate the present state of PWS herring relative to an enhancement program.

Critical steps in program design		**Comment**
Step 1	**Make management priorities and trade-offs clear and acceptable**	Tradeoffs could be difficult if the cause of low herring abundance was related to the pink salmon hatchery programs. This critical step asks "what if the Prince William Sound herring stock cannot co-exist at high levels of abundance with other stocks?"
Step 2	**Demonstrate recruitment overfishing or unsuccessfully rearing in the wild**	This step is fully met. Annual stock assessments are done annually. There is no fishery, so there is no concern with recruitment-overfishing, unless herring are taken in significant quantities and bycatch (or killed by collateral damage) in other fisheries. This seems unlikely.
Step 3	**Show that enhanced fish can successfully recruit in the wild**	This has been shown by Japanese work.
Step 4	**Show that total abundance is increased by the enhancement contribution**	This step has NOT yet been shown by Japanese work. Although potential restoration methods used in Prince William Sound may resemble those used in Japan, the objectives are not necessarily the same. The best way to meet this objective is to extend the culture time as long as necessary to reduce, or eliminate, density-dependent competition with wild juveniles.
Step 5	**Prevent continued overfishing**	This step is not applicable at the present time. The fishery is closed. This step is only relevant if and when the stock "recovered" to a level that supported a fishery. If that happened restoration efforts should cease. If they continued, then management rationale for restoration would have changed – from a "conservation and restoration" program to a "production" program.
Step 6	**Ensure that fishery regulations are adequate to prevent continued overfishing of the wild population (unless there has been a policy decision to 'write-off' the wild population**	This step is not applicable at the present time. The fishery is closed. This step is only relevant if and when the stock 'recovered' to a level that supported a fishery.
Step 7	**Show that the hatchery production system is sustainable over time, if it is to be permanent.**	This step is not applicable at the present time. The fishery is closed so enhancement is being considered for purposes of restoration, not production.

Table 1b - Critical steps in monitoring– (adapted from Chapter 13, Walters and Martell, 2004). The discussion of development steps in a generic marine fish enhancement program was adapted to and Excel sheet. The comments in the right column indicate the present state of PWS herring relative to an enhancement program.

Monitoring and experimental requirements		**Comment**
Step 1	**Mark a high proportion of all released fish**	First, marking methods need to be established. Then broad marking programs should assess the survival of enhanced and wild herring. Probably the Japanese ALC marking procedure may be a guide.
Step 2	**Mark some wild fish in addition to hatchery fish**	See comment above: Marking methods need to be established.
Step 3	**Vary the releases among years, including the number released, time of release and release areas.**	This step applies more to species such as salmonids. For herring it may be advisable to monitor success of releases among different areas.
Step 4	**Monitor changes in recruitment**	This should be possible with routine bio-sampling of the PWS herring population.
Step 5	**Monitor changes in fishing mortality**	This would depend on the re-establishment of a fishery. If stocks recovered to the level that would support a fishery, then enhancement would be unnecessary.
Step 6	**Monitor changes in reproductive success of released fish**	One way this could be done is sampling of maturing adults in the fall and winter, prior to spawning. Monitoring also could include fecundity analyses, quantification of ovarian atresia (counting atretic oocytes) and egg size of spawning fish.

Table 1c - Things that can go wrong– (adapted from Chapter 13, Walters and Martell, 2004). The discussion of development steps in a generic marine fish enhancement program was adapted to and Excel sheet. The comments in the right column indicate the present state of PWS herring relative to an enhancement program.

Things that can go wrong		**Comment**
Step 1	**Failure to produce fish that successfully recruit to the spawning population**	Japanese work indicates that cultured herring can survive and spawn but it is essential to develop a mass-marking system for any released fish in PWS.
Step 2	**Direct exploitation of wild fish to provide hatchery seed stock**	This is a real, but relatively small concern with the assumption that, following Japanese practices, there can be relatively good survival from hatching to the juvenile stage.
Step 3	**Post-release competition between hatchery and remaining juvenile fish**	This may be the most pressing concern. Monitoring and research should attempt to determine the optimal release time. Based on the information in this report, later releases of larger juveniles may reduce possible competition for scarce food resources in the late fall and early winter.
Step 4	**Increase in predation and disease risk for remaining wild fish**	This is a major concern, given the present high incidence of disease in Prince William Sound herring. It is especially troubling that the viral disease (VHS) tends to break out in crowed conditions.
Step 5	**Selection under enhancement conditions for traits that are inappropriate**	This is only a concern if enhancement activities had a long duration.
Step 6	**Attraction of fishing effort by unregulated fisheries**	Probably this is not an issue.

Appendix C – Herring Enhancement White Paper

Herring enhancement in Prince William Sound: feasibility, methodology, biological and ecological implications

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1 Overview and synopsis

The subject matter within this report is very broad. Some topics included in this report have received a lot of attention from researchers. Other topics have received very little. Not all topics are strictly scientific. The potential background information for examination of herring enhancement is substantial and includes hundreds of scientific papers and books on herring biology and enhancement of marine fish. There is almost no literature specifically on the topic of herring enhancement. Many of the issues involved with the subject of marine fish enhancement are not resolved. Also there are many unresolved issued concerned with the Prince William Sound herring population. Any attempt to summarize or distill all the available information into a single report demands severe condensation. That is the case in this report.

The issue of enhancement of marine fish populations is controversial. There is an influential part of the fisheries science community, mainly from the ecological side, that is steadfastly opposed to the concept of marine finfish enhancement. There is another component, mainly the practitioners, who are comfortable with the concept and worry little about biological implications. However, even the detractors of the concept suggest that the activity may be warranted when all other conventional management procedures fail. Even then there are reservations about the efficacy of the approach if density-dependent factors regulating recruitment occur after the release of cultured fish. This is a focal point for this issue in Prince William Sound.

The available information about herring in Prince William Sound indicates that some limitation to abundance occurs at early life stages, prior to recruitment which occurs mainly at age 3. Recent work on juvenile herring ecology within Prince William Sound indicates that some herring, especially those residing in specific bays or inlets, have inadequate nutritional resources to survive their first winter. If such mortality is a factor limiting recruitment to the adult spawning stock, then perhaps enhancement could promote better survival of this stage. There are several distinct life history stages of herring however, and they interact spatially and temporally. Also, there are interactions (predation and competition for food) with other species. Therefore a conceptual matrix is developed to show the intra-cohort and inter-cohort interactions, and also interactions between herring and other species. Based on a review of available data on survival of specific life history stages, this report suggests that enhancement activity, if it proceeds, probably should retain cultured herring until the end of the 'fall juvenile' stage. At this time, well-nourished herring juveniles may withstand a relatively good chance of surviving the winter period when feeding opportunities are limited.

This report avoids advocacy but the concluding sections present a review of the current fisheries management and ecological factors that should be addressed prior to the initiation of enhancement activity. The decision about whether enhancement should proceed, or should not proceed, is not addressed explicitly in this report. However, this report is designed to assist those charged with making such a decision. The report points out the failures of previous attempts at marine fish enhancement, especially for Atlantic cod in Atlantic waters. The report also describes the results of recent Japanese research. Their results of mass rearing of herring are impressive – even startling – but it is not clear whether their obvious success at herring culture is actually having any positive effects on the wild herring populations. This statement is based on

reviews of such activity by some Japanese scientists who question the validity of the approach. Nevertheless, if enhancement activity is undertaken in Prince William Sound, the Japanese experience would be a source of invaluable technical protocols. Acquisition of such technical details, however, would require direct contact with the Japanese agencies engaged in this work because only brief technical summaries are available at the present time.

Several major scientific reviews about marine fish enhancement activities are unanimous on one key point: it is futile to release young cultured animals into the wild prior to the time when intense density-dependent processes may result in intense mortality. There is no doubt that this point is valid and it is emphasized, and perhaps over-emphasized, in the following text. Although this is a valid and useful comment it may be difficult to identify the point in the life history of herring in Prince William Sound where such density-dependence might occur. Based on the experience with Atlantic cod rearing, it seems clear that release of herring larvae, after a short culture period, would precede the impact of density-dependent processes. Instead, based on the considerable work on herring juveniles in Prince William Sound, it seems that a release time approximately at the end of the first summer feeding period may be the best time to avoid possible density-dependent mortality associated with food limitation and winter survival.

Readers not familiar with biological and fisheries literature may have difficulty understanding why concepts like density-dependence can invoke so much discussion and so little consensus. Probably that is the case with herring in Prince William Sound and perhaps other issues related to herring in Prince William Sound. Density dependence interactions in herring can be complex because there are several distinct life history stages that may, or may not, occupy different parts of the regions (i.e., different depths) and eat different food. It is possible however, that all life history stages within Prince William Sound may overlap, spatially and temporally, at some times of the year. A major uncertainty about Prince William Sound herring biology concerns the role of the shelf waters as summer feeding areas for adults. In other areas of the eastern Pacific the adult component of large herring populations are migratory, and feed intensely during the summer on shelf waters. Juvenile herring tend to reside close to sheltered, nearshore waters. Presumably Prince William Sound herring have the same migratory habits but this aspect has not been explicitly documented or described. It is important because the shelf feeding waters would provide the major source of food for adult herring (age 3 and older). Such feeding migrations would lessen the potential for density-dependent interactions between adults and juveniles within Prince William Sound. This report recommends clarification of this issue.

Another factor affecting density-dependence is the potential for competition from other species. In Prince William Sound there are several large populations of other major species. The role of inter-specific competition for food with herring is not clear. To assist with any decision about the efficacy of herring enhancement, it would be useful to clarify the potential for competition for food between herring and other species. It would be especially useful to understand spatial and temporal variation of such potential interactions. If enhancement activity were undertaken, decisions must be made about the time and location of releases. Such decisions would benefit from knowledge of the location-specific risks for food competition and possible predation.

A review of factors leading to the low biomass of herring in Prince William Sound, or related issues such as biomass surveys or assessments, are not included in this report. Instead, after a brief review of the present state of herring in Prince William Sound, the report reviews relevant literature and information (unpublished reports and some personal communication) that are

mainly from Norway, Canada, United States and Japan. Relevant Norwegian work is concerned mainly with the history of mass larviculture and implementation of experimental and mass production mesocosms for larval rearing. Canadian and American work is related to early life history and larval rearing, reproductive and spawning biology and ecology and impoundment of spawning herring. Japanese work on herring enhancement has been conducted for over 20 years but, until recently, little has appeared in the mainstream literature. Some of the most relevant information in this report has been provided through personal communication with Japanese researchers. The report makes a number of recommendations. Mainly the recommendations are suggestions about the merits and limitations of certain technical approaches, such as how to move eggs, incubation, feeding, marking, etc.

There are some sections of the following report which are, admittedly, tedious and banal. Some readers may balk at wading through text that seems to be long on speculation and short on conclusions. This may be especially so for non-biological readers. For this reason I have added a distinct section called 'Prologue'. The prologue consists of questions and answers. The questions raise points that I think many readers may ask. The answers try to avoid technical terms and jargon but retain accuracy. Still, it is clear from some helpful preliminary reviews that some answers provided here may generate debate and discussion. If so, that could be a useful outcome.

The header of this report states ***'Final Report – September 2007'.*** This version made a number of small editorial and formatting corrections to the previous version dated June, 2007. Helpful comments by reviewers and others were incorporated into earlier versions but the author takes full responsibility for any errors or omissions. This final version has corrected a number of typographical and syntax errors. A few points have been clarified but no substantial deletions were made to the text of earlier versions.

2 Prologue: to enhance or not to enhance - questions and answers

Herring enhancement, through the culture of eggs, larvae and juveniles may seem straightforward, but the concept has profound and complex biological implications. There are technical challenges related to mass production of marine fish but, based on Japanese experience, probably these can be mastered. Such massive production, however, does not necessarily imply successful enhancement. Attempts at enhancement of marine fish started over 100 years ago, but all early attempts were unsuccessful. These early attempts did not recognize that the concept of enhancement makes certain implicit assumptions about ecosystems and factors that limit marine fish abundance – specifically the relationship between the abundance of adult fish (the 'stock') and the numbers of younger fish (the 'recruits') that join their ranks each year – usually known as a 'density-dependent' relationship called 'stock-recruitment'. Some interested readers may not be familiar with these concepts, yet still be interested in the feasibility and problems related to the enhancement of herring. For such readers the following questions and answers may provide some better understanding of the issues. These questions and answers may also reveal something about the present state of knowledge, limitations of knowledge, and technical capacity to do enhancement.

QUESTION: Is it possible to raise large numbers of herring larvae and juveniles in captivity and release them into Prince William Sound?
ANSWER: Yes, it is relatively simple.

QUESTION: How long would you have to raise them before they are released?
ANSWER: Probably a minimum of 6 months, and perhaps longer.

QUESTION: Will the released fish survive and join the spawning population in Prince William Sound?
ANSWER: Yes, it is almost certain that ***some*** cultured herring will join the wild spawning population. This has been done successfully in Japan.

QUESTION: Will these released fish help to increase the herring population in Prince William Sound?
ANSWER: It is not clear whether cultured herring will add to the existing population or merely displace wild herring that are competing for limited resources.

QUESTION: Are there ways that enhancement can be evaluated?
ANSWER: Yes. The released fish can be marked. Survival of released fish can be compared to the survival of wild (natural) fish – but this requires a lot of work. A potential concern with marking programs, however, is that there usually must be some form of fishery to capture the marked individuals.

QUESTION: What are the most important things to learn before enhancement is considered?
ANSWER: One is the time or age when 'density-dependent' factors limit survival. Another is clarification of the herring stock structure in Prince William Sound – how many populations exist there? Yet another is the geographical range of Prince William

Sound herring in the summer. Specifically, do some or most adult herring migrate out of the Sound to feed on shelf waters?

QUESTION: What is 'density-dependence'?
ANSWER: The growth of every animal population is limited by something. When population growth is restricted by the population size or density, this is called 'density-dependence'. Fishery ecologists still argue about the nuances of the definition.

QUESTION: When does density-dependence occur in Prince William Sound?
ANSWER: Nobody knows for certain. In Prince William Sound there is evidence that it happens during the first year of life – mainly in the winter.

QUESTION: How does density-dependence limit herring survival?
ANSWER: In many areas within Prince William Sound there is not enough food for herring to survive over the first winter of life.

QUESTION: Why would enhancement be required now and not earlier, say 20 years ago?
ANSWER: That is not clear. Recent research indicates that food may limit the survival of age 0+ herring. Presumably this was a not a severe limiting factor 20 years ago.

QUESTION: Can there be limiting factors that occur at other times, say for the egg stage?
ANSWER: Usually even the most severely depressed herring stocks produce sufficient eggs and larvae to allow recovery. For example, during the 1960's and 1970's the spawning biomass of the Norwegian spring spawning herring declined to about one percent of its biomass, but the population recovered rapidly when fishing stopped. The decline in Prince William Sound is not yet that severe.

QUESTION: Can there be limiting factors that occur at adult stage?
ANSWER: Yes, and perhaps these are important at the present time. However, the critical adult habitats are usually on shelf waters, where many herring populations feed. If there were some general decline in ocean feeding conditions, or a decline related to increased predation on the adults, we might expect to see similar impacts on all Gulf of Alaskan stocks, but stocks adjacent to Prince William Sound appear to be doing well.

QUESTION: Are there any key biological issues that need to be examined?
ANSWER: It would be useful to know if adults feed on shelf waters. There appears to be uncertainty about the distribution of adult herring relative to the shelf waters adjacent to Prince William Sound. It would be unusual if Prince William Sound herring did not migrate to these shelf waters to feed – but if they really do not utilize this habitat for summer feeding, then their distribution may be confined mainly to the waters within Prince William Sound. If so, adults, pre-recruits and juveniles may be competing for the same zooplankton food (especially copepods) within Prince William Sound.

QUESTION: What is the implication of the time of 'density-dependence' for enhancement?
ANSWER: Releases of cultured herring prior to the period of density-dependence will not help to increase the total abundance of herring.

QUESTION: Would enhancement be expensive?
ANSWER: Yes, but enhancement should not be considered only as a short-term activity.

QUESTION: Is enhancement a remedy for recovery of the Prince William Sound herring population?
ANSWER: It may be, but it may still be too early to consider as an option.

QUESTION: When should enhancement be considered?
ANSWER: Only as a last resort, when all other conventional approaches have failed and after a review of the rationale for enhancement indicates that it is warranted and feasible. Probably, in Prince William Sound, the 'conventional' approaches, that consist mainly of catch controls and fishing gear controls, already have been fully implemented.

QUESTION: Can enhancement create new problems?
ANSWER: There is a possibility of negative ecological impacts on wild fish (i.e., competition for food, alterations of genetic diversity, and risk of increased disease).

QUESTION: Can the uncertain aspects of enhancement be identified based on existing information?
ANSWER: Probably the main points can be identified – and a key one is the time of density-dependence or life history stage of density-dependence. The second aspect concerns required scale of operations. Probably this is among the largest type of marine fish enhancement project ever contemplated.

QUESTION: Is herring enhancement in Prince William Sound a concept worth consideration?
ANSWER: This depends on the motivation and willingness to undertake an expensive project with no promise of success - but it might work.

QUESTION: What will determine success?
ANSWER: Success will depend on the willingness to follow some well-established principles concerning biological and technical procedures. Many aspects about enhancement are not clear, and the most important is whether it should be attempted at all.

QUESTION: Is more review required?
ANSWER: Some greater clarification about spatial variation within Prince William Sound is advisable, especially about stock structure issues. For instance, if there are spatially discrete populations (that might not be genetically distinct) then it would be essential to know how enhancement efforts would apply to each population (or sub-population).

QUESTION: Do we understand the biology well enough to proceed?

ANSWER: There are many unknowns, and all seem important. It would be comforting to better understand the roles of food, predation, intra- and inter-specific competition for food in the survival of age zero herring, during the first year of life. These are relevant to the density-dependence issue.

QUESTION: Disease is an issue for Prince William Sound herring. How could that affect enhancement activity?

ANSWER: The impact of disease on an enhancement program is not clear. At worst, the confinement of herring in high density situations could exacerbate the problem. At best, it may be possible that cultured herring, after exposure to disease at young life history stages (and probably suffering increased mortality following such exposure), may develop some resistance to disease.

QUESTION: Do we understand enough about enhancement technology?

ANSWER: Yes. Most of the necessary detailed information is available, although not necessarily in the scientific literature. Especially important is the technology for marking cultured fish prior to release. This is essential. This process needs more investigation and probably could follow Japanese experience.

QUESTION: How might an enhancement project begin?

ANSWER: Because of the many uncertainties, if it were to start it probably should begin in relatively small, incremental steps. Such steps could be used to provide feedback about the direction and efficacy of the concept.

QUESTION: What are the next procedural steps?

ANSWER: First, the basic question of whether herring should, or should not be subjected to enhancement efforts should be formally addressed prior to initiation of any major enhancement activity. For instance the present paper does not present a rationale for enhancement. The rationale needs be done elsewhere and should address socioeconomic and conservation biology concerns. Second, pilot scale projects should be initiated to address technical problems, such as the number of eggs required, survival rates of cultured fish, food requirements and successful application of chemical marks to young released fish.

3 Introduction

This report reviews scientific literature on marine fish enhancement in general, and herring enhancement in particular, relative to the possible enhancement of herring in Prince William Sound, Alaska. Preparation of this report has been a struggle to reconcile two opposing perspectives about marine fish enhancement. It is clear from the literature that there are strong differences of opinion about the scientific merits and biological rationale for the concept. Skeptics focus on the problems and pitfalls of enhancement programs and are adamant that conventional approaches to stock recovery, such as those described by Caddy and Agnew (2004), must be tried first. Advocates point to the failures of conventional management and the apparent successes of the rapidly expanding mariculture industry.

The interest in stock enhancement and related forms of activity such as marine fish aquaculture and sea ranching has rapidly expanded in recent years. Some researchers do not endorse enhancement activity and dismiss the concept while others advocate careful, precautionary approaches to this subject. Advocates of Prince William Sound herring enhancement should understand the biological and management problems related to this task and should not underestimate the severity of many of the basic concerns. On the other hand, if herring enhancement must be done, it should benefit from the results of relevant research and experience elsewhere, especially in the work conducted on the west coast of Canada, United States, Japan and Norway during the last 30-40 years. The report attempts to explain the factors which require a cautionary approach and discuss the technical approaches used elsewhere.

3.1 Brief background to Prince William Sound herring

A major oil spill occurred in Prince William Sound in 1989, and this is known as EVOS (Exxon Valdez Oil Spill). This spill was followed by an enormous volume of biological work that examined impacts of the spill (see for example the AFS volume edited by Rice et al. (1996) or the series of papers in the Canadian Journal of Fisheries and Aquatic Sciences, Volume 59 (2002). There also has been considerable debate about the severity and duration of the impact on herring. Post-spill estimates of spawning biomass seem to have been contentious, but there is general agreement that there was a major decline of herring in 1993, four years after EVOS. There is general agreement that (1) spawning biomass declined since 1993 and has remained low and (2) recruitment since 1993 has been unusually low (Fig. 1). The causes of the decline and the subsequent low abundance levels have been examined in many studies since 1993 but the explanation for both the decline and lack of herring recovery remains uncertain.

3.2 What is enhancement and what is herring enhancement?

There is potential for ambiguity in the term ‘enhancement’ as it has been used and defined in recent fisheries literature. For example Bell et al. (2006) define ‘stock enhancement’ as the ‘process of releasing cultured animals to increase yields beyond levels supported by natural recruitment’. The generality of this definition is widely accepted but it is possible to distinguish between releases of cultured animals as ‘mitigation’ or ‘restoration’ activity versus releases to

'augment' natural production. For instance Radke and Davis (cited in Table 1 by Molony et al. 2003) use the term 'enhance' as the 'production and release of fish to increase stocks above original levels'. In this context, the implication is that the results of enhancement will provide an increase in numbers or biomass to levels exceeding natural carrying capacity. Hay and McCarter (2006) use the term 'enhancement' in a very different way: in a spatial or geographical context as the 're-establishment' of herring to discontinued spawning areas or 'introduction to new areas'. They state "there are also many potential spawning locations which have never been documented as spawning areas but still appear to have all the appropriate vegetative substrates and local oceanographic conditions that are found in heavily utilized areas". Based on spawn data analyses and herring spawn transplant experiments, Hay and Marliave (1988) state that herring "enhancement" or "re-establishment" does not appear to be possible at the present time. Further, they suggest that if herring spawning habitat is lost, we cannot necessarily expect the impacted stocks to spawn in other locations nor can we realistically expect that new spawning habitat can be created by habitat manipulation. Therefore when used in the context of spatial analyses of herring spawning, the term 'enhancement' has a different meaning than that proposed by either Bell et al (2006) or Molony (2003).

In the present report the term 'enhancement' is used to mean 'the release of cultured herring to supplement natural recruitment so as to assist recovery or restoration of the population to historical levels'. In this sense, the use of the term enhancement refers explicitly to the biomass (or numbers) of the herring spawning stock biomass (SSB) and there are no implicit assumptions about the geographic distribution of spawning areas as noted by Hay and McCarter (2006).

This definition of enhancement is not complex, although some could argue that the present biomass levels are within the range of normal variation, and if so, such attempted enhancement would be a form of 'augmentation'. On the other hand, if present levels of spawning biomass are too low to allow for normal recruitment, and especially if the present low levels are associated with anthropogenic activity, then enhancement of recruitment would clearly be a 'mitigation' process. For the purposes of this report, no further reference to this distinction will be made, except for brief mention in the concluding sections. Readers with an appetite for more definitions, however, should consult Molony et al. (2003, Table 1) that have listed definitions from published literature.

3.3 The biological issues: if enhancement is a solution, what is the problem?

The suggestion of enhancement of Prince William Sound herring could be seen as a specific solution to an undefined problem. There is no dispute that present abundance of Prince William Sound herring is low (Fig. 1). The 'problem' is the uncertainty for the cause(s) of the herring decline: there is not unanimity about the reason(s) for the decline and failure to recover (see, for example the review by Carls et al. (2002) or Pearson et al. (1999). More recent but brief commentary by local experts such as Moffitt (2005), confirm the uncertainty of the explanations for the decline, but point out that there are several interacting factors including environmental factors and disease. This uncertainty has a direct bearing on the rationale for any potential enhancement. In the views of many skeptics, this uncertainty may be sufficient reason to preclude further consideration of this approach. Such skepticism is well founded. Overly-eager

proponents of marine enhancement projects should be aware of the spectacular failures in earlier approaches. The most noteable is the multinational, century-long project attempting to enhance Atlantic cod (*Gadus morhua*) (Solemdal et al. 1984) and this is described below. These earlier flawed efforts have led some to question the validity of such approaches (i.e., Grimes 1998) or categorically reject them (MacCall 1989).

This paper does not attempt to identify the biological problem related to the causes(s) of the herring decline but it does try to focus on aspects of biology that proponents of enhancement should be aware of – specifically the issue of the life-history stage in which density-dependent mechanisms limit survival. The ecological factors that limit herring abundance can be elusive (Lasker 1985). Walters and Martell (2004) and Blaxter (2000) point out that, relative to enhancement programs, if the carrying capacity is limiting at a stage or age that occurs after the time of release, then probably enhancement efforts will be worthless because they will not produce 'additional fish'. At best they will result in the replacement of 'naturally produced' fish with 'cultured' fish. This is a vital issue. Therefore a close examination of the different life history stages of herring follows in the next section.

4 Relevant herring biology

4.1 Life history stages and density-dependence

Herring have several different life history stages that differ in duration, size and temporal location (Hay and McCarter 1997). There are over-lapping generations so there is potential for both 'intra-cohort' and 'inter-cohort' interactions – mainly predation and competition – as well as interactions of all herring life history stages with other species. These life stages are depicted in Table 1 that shows the 'within-cohort' interactions as a cohort develops from egg to adult. There are four stages shown in six rows: (1) the egg stage; (2) the larval stage; (3) age 0+ and age 1+ juvenile stages; (4) the pre-recruit and adult stages.

4.2 The egg stage (Row 1, Table 1)

During the egg stage there may be intra-cohort density-dependent 'competition' for oxygen. Maximal survival from fertilization to hatching occurs at moderate egg densities (Galkina 1971, Hourston et al. 1984, Stevenson 1962). On the other hand, the *rate* of egg mortality by scavenging predators may be higher in very low densities (i.e., << 100,000 eggs/m2), such as those that occur in parts of Puget Sound (Palsson 1984) and elsewhere. Therefore in most spawning areas, there will be a scavenging community of benthic grazers that may eliminate some but not all eggs. The optimal egg density is probably a trade-off between the highest density that will minimize the loss to scavenging predators and the minimal density that will provide optimal gas exchange.

4.3 The larval stage (Row 2, Table 1)

The next life history stage is the yolk-sac larval stage that, after about 5-10 days (duration is temperature-dependent) becomes a feeding yolk-sac larva. For most fish species this is a period of extraordinarily high mortality (by predation) and rapid growth among the survivors (Houde 1989). Sometimes there is potential for 'within-cohort' predation, by the largest individuals eating the smallest, as documented in Norwegian enclosures (Wespestad and Moksness 1989) but the frequency of this in natural settings is uncertain. There also may be a risk of predation by juveniles of the older generation, and, in some circumstances, adults – but mainly cannibalism would be limited because (1) larval distribution soon becomes dilute and (2) each life history stage tends to have different spatial niches – older juveniles are deeper and slightly farther offshore.

An unresolved issue is the role of competition for food among larvae – or whether larval food availability limits population growth. In a seminal paper Cushing (1983) advised that, in most larval fish populations, the larvae were 'too dilute' to graze down their food supply. On the other hand, food limitation – or ability to feed - is generally thought to be a key factor regulating larval survival in some clupeid species such as anchovy. For instance, the role of turbulence and wind in regulating access of larvae to patches of food has been embraced as a key hypothesis known as 'Lasker's Windows' (named after the prominent scientist Reuben Lasker). The topic of food availability for herring could fill volumes but there appears to be a consensus that starvation in Pacific herring larvae is not common. For instance, Robinson and Ware (1988) found no evidence of this. Rather, predation appears to be a factor controlling Pacific herring larval survival. Predation by jellyfish (Aurelia) can reduce larval populations by up to 10 % a day (Purcell 1989, 1990; Purcell et al. 2000, Arai and Hay 1983). Jellyfish are only one of many species that prey on larval herring.

The biological literature is awash with papers on larval fish feeding and survival in laboratory settings. Many of these papers are on herring but very few are useful for understanding the issue of Prince William Sound herring enhancement. The exceptions are the papers that comment on feeding rates and methods of mass culture (sometimes called 'larviculture'). The most useful practical literature is mainly from Norway and concerns the rearing of herring in 'mesocosms' or very large containers that allow for mass rearing in plastic cages or bags, as well as concrete and semi-natural outdoor enclosures.

4.4 The juvenile stages (Rows 3-4, Table 1)

This stage develops after 2-3 months of life when herring larvae 'metamorphose' from anguilliform (or 'eel-like') larvae into creatures that resemble small versions of adult herring. There are two stages. The age 0+ (sometimes called age 'zeros') begin at weights that are only a small fraction of a gram and finish their first summer, at an age of about 6-7 months, usually with a size of about 80-100 mm and weight of about 5-10 grams. The age 1+ juveniles are considerably larger and usually there are distinct, non-overlapping size modes of each age group (Stokesbury et al. 1999a). There also may be variation in the weight of juveniles that varies 'within locations and among years' and 'within years and among locations'.

The age 1+ juvenile stage seems to reside in different depths and location than age 0+ herring. Routine juvenile surveys in the Strait of Georgia (Haegele 1997) shows that most – but not all - have moved out of the area by mid-summer. In British Columbia (BC), they move to offshore shelf areas where they have access to rich feeding opportunities in upwelling areas. Probably the migration patterns of herring in Prince William Sound are similar.

The age 0+ juvenile stage warrants careful examination relative to enhancement of Prince William Sound herring. Studies of juvenile herring in the Strait if Georgia (Haegele 1997), like those of Norcross et al. (2001) and Stokesbury et al. (1999 a, b) show that there is spatial variation in the growth of age 0+ (or age 'zero') juveniles in Prince William Sound. This is attributed to spatial differences in food availability. Further, Prince William Sound studies that have examined the energy content of this stage have concluded that food is limited (Paul and Paul 1998, 1999, Paul et al. 1998). It is interesting that Swedish work makes a similar conclusion about juveniles in the Baltic – specifically that sometimes food is limiting at the age 0+ stage (Arrhenius and Hansson, 1999).

Other studies also implicate the age 0+ stage as potentially interesting because juvenile surveys show that indices of the abundance of age 0+ juveniles are significantly correlated to the size of the recruiting cohorts. This has been described in the Baltic (Axenrot and Hansson 2003) and by Hay et al. (2002) for the Strait of Georgia. As discussed later, if estimates of the abundance of the age 0+ stage provide adequate information for the prediction of the recruiting cohort, then it appears that this would be a stage representing the minimum size for release from enhancement. In the case of the Strait of Georgia (Hay et al. 2002) the positive and significant correlation is between the juvenile abundance and the estimated number of individuals about 2.5 years later when they recruit at age 3 (36 months and estimated by age-structured analyses). This was a log:log comparison and the correlation, while significant, was not striking. Rather, it seems that the best prediction came from years when the juvenile abundance was very low – in such years the corresponding abundance of recruiting cohorts also was low. ***The implication is that the size (and age) of juveniles by the time of mid-summer of their first year of life, may be a minimum target for the required period of enhancement.***

4.5 The pre-recruit and adult stages (Row 5-6, Table 1)

Recruitment to the adult (spawning) stock occurs at about age 3 (36 months) although this may be a year earlier for a few individuals (especially males) and later, at age 4 or 5 for others, especially females. The present literature on Prince William Sound herring does not describe seasonal migrations in and out of the Sound to coastal shelf waters. In most areas of the Pacific coast, including San Francisco Bay (Spratt 1976) and other locations, herring move to shelf waters to feed in the summer and fall. Many return to inside waters, (such as the Strait of Georgia) to over-winter. Similar migrations occur in northern BC waters. Also, it seems that in the Bering Sea, adult herring stocks move away to deeper shelf waters in the south-west, and away from the shallower spawning areas in Bristol Bay. *What happens in Prince William Sound*?

Presumably there must be some utilization of the shelf because, based on simple comparisons or available 'habitat', Prince William Sound would not be large enough to support the abundance of

herring that were seen there in the 1970's and 1980's (Woodby et al. 2005). This assertion is based on the simple analysis made by Hay and McCarter (1997) that adult herring 'habitat' may be simply defined as the available space between 0-200 m. For example, the Strait of Georgia has only about 30 % of its area that would be suitable for continuous adult feeding habitat (Table 1 in Hay and McCarter 1997). Based on a maximum high density of about 10g/m^2 (or 10 mt/km^2) the Strait of Georgia should be able to maintain an adult herring population of about 30,000 mt (metric tons). This estimate is much lower than recent biomass estimates which frequently exceed 100,000 mt (Schweigert 2004). The simplest explanation for the difference is that most herring migrate from the Straight of Georgia to shelf waters off the west coast of Vancouver Island to feed during the summer.

The distributions of depth strata of the Strait of Georgia and Prince William Sound are quite similar. Therefore it seems reasonable to assume that the biomass of herring in Prince William Sound might be approximately similar to that of the Strait of Georgia, and generally this is what the stock assessments of the 1980's and 1990's indicate (Woodby et al. 2005). Therefore it also seems reasonable to assume that Prince William Sound herring also move to shelf waters to feed in the summer.

5 Herring habitat and density-dependence

Herring habitat is determined by the composite of abiotic and biotic factors affecting herring. Often, however, the term habitat is used in the context of a single factor, such as food or temperature. In this report the term habitat is sometimes used in the context of food or space. To be suitable habitat for herring there must be a suite of suitable conditions including water temperature, oxygen concentration, depth range and so on.

5.1 Habitat areas and density-dependence – is any stage-specific habitat limiting?

A common position among most commentators on marine fish enhancement is that it is not a worthy activity if density-dependence mechanisms are prominent after the time of fish release (i.e., Blaxter 2000, Walters and Martel 2004). In general, it seems that most commentators believe that this is the situation for species such as herring that inhabit large ocean areas. This common reservation is simple and sensible: if there is a point in the life history where the carrying capacity of the environment or habitat restricts survival, then efforts to expand a population beyond the carrying capacity are pointless at best – and harmful and wasteful at worst (MacCall 1989).

Some scientific reviews on marine fish enhancement (i.e., Blaxter 2000) preclude consideration of species like herring - probably because it seems unreasonable to consider manipulating populations like the Norwegian spring spawning herring or the Bering Sea herring that inhabit such vast ocean areas. In these populations the relative numbers of recruiting fish in many years is phenomenally large compared to the real (or imagined) capacity of culture operations that would be required to produce them. Herring populations in the eastern Pacific, however, are different than those of the Atlantic or western Pacific because the maximal population sizes are

relatively small (< 100,000 t) and they have a tight ecological connection to nearshore habitats. For Pacific herring it is usual to think of stock:recruit relationships as population-specific interactions between the mature, adult stock and the new recruits joining them each year (Williams and Quinn 2000a, 2000b).

Hay and McCarter (1997a) describe the apparent relationship between available habitats for different life history stages of Pacific herring. They point out that in most populations a limiting factor to maximal population size (SSB) is shelf area. Although their analysis is simple, they point out that the available shelf area (defined as the surface area between 0 and 200m) for Prince William Sound is about 17,000 km^2 (Table 1, p 562, Hay and McCarter 1997). Surveys of larval distributions show large inter-annual variation in the Strait of Georgia. Larval distributions may change substantially among years, with some years having most concentrations on the east side or north (Hay and McCarter 1997b). The key point is that the larval distribution was substantially greater that the spawn distribution, and extended to many areas where spawning did not occur. ***A relevant conclusion from these st udies is th at spawning habitat or larval rear ing habitat was NOT a limiting fa ctor in the Strait of Ge orgia.*** This also might apply to Prince William Sound.

5.2 Lessons from the Strait of Georgia

Juvenile surveys (Haegele 1997) showed that juveniles were more abundant around the perimeter of the Strait of Georgia and there were substantial size differences among juveniles from different areas, similar to results described for Prince William Sound. The conclusion is that Prince William Sound, like the Strait of Georgia, has adequate spatial habitat to support the nearshore-resident juvenile stages of the spawning stock.

The ***within-cohort juve nile*** density-dependence factors may not be the same as the density-dependence factors that operate ***between the adult SSB and the si ze of the recruiting cohort*** – unless the adult cohort can graze down the available food used by juveniles during their first summer. Therefore, as suggested by Lorenzen and Enberg (2001), much of the density-dependent factors that operate at the adult stage in herring may be on growth – occurring after recruitment. This assertion is consistent with the observation of relatively consistent sizes of juveniles as they recruit (estimated by the size of scales) followed by increased cohort-specific variation in size in older ages (Hay et al. 2001).

5.3 Lessons from Prince William Sound

Foy (2001), Norcross et al. (2001), Norcross and Brown (2001), Foy and Norcross (1999a, 1999b) and Paul and Paul (1998a, 1998b, 1999) provide evidence that (1) herring juveniles may not feed sufficiently during the summer to accumulate sufficient energy to see them through their first winter. *This observation is very relevant to the issue of enhancement in Prince William Sound.* (2) There is spatial variation in the nutritional state of herring juveniles in different parts of their range (within Prince William Sound). Specifically, the energy content of herring juveniles, at the end of their first summer of life, varies geographically within Prince William

Sound. The geographical differences in energy content appear to be relatively consistent over time (Norcross et al. 2001).

The implication from the results of Foy (2001), Norcross et al (2001) and others is that the juvenile carrying capacity may be limiting in some locations of Prince William Sound and that it also may vary over time, within locations. It may be useful, however, to distinguish between the nearshore carrying capacity of juveniles versus the offshore shelf-feeding carrying capacity of the adult stock. In some years, the carrying capacity of the juvenile stage may be restrictive, so that the recruiting cohorts may be small. In a year, or a succession of years, when large numbers of juvenile cohorts are produced, one would expect classical density-dependence between SSB and recruiting year class, similar to that described by Myers (2004) and others. On the other hand, if there were years, or succession of years with bad recruitments, then perhaps other forms of population limitation are operating, such as a limitation of the carrying capacity of some (or most or all) of the juvenile rearing areas – and especially the areas that support large numbers of age 0+ herring in their first year. *An implication of this is that any enhancement activity should stress the age 0+ juvenile period and that enhancement should only be considered when the spawning stock is low relative to historical levels.*

5.4 Depth strata, herring habitat and density

The question of available spatial habitat for herring may be important and it may be instructive to compare the spatial distribution of habitats between Prince William Sound and the Strait of Georgia (Table 2). The rationale for this comparison is that adult herring (those that are recruited to the adult spawning stock and usually are age 3 or older) spend the summer months feeding on waters off the continental shelf. This is the post-recruitment stage of sexually mature (or maturing) individuals otherwise known as the spawning stock biomass (SSB).

The significance of the potential utilization of shelf waters for feeding is simply that Prince William Sound probably comprises only part of the habitat used by adult herring. Summer feeding migration of adults from the Sound would reduce potential competition for limited food resources – because adult herring can feed on the same zooplankton (copepods) that are consumed by juvenile herring. Conversely, if Prince William Sound herring did not migrate from the Sound, then the density of herring (prior to the 1993 crash) would have been extraordinarily high. For instance Hay and McCarter (1997a, Table 1) list the maximum density of Prince William Sound herring as 8.8 g/m^2 (or 8.8 mt/km^2). This estimate is based on a maximum spawning biomass (plus catch of 150,000 mt in 1991) and a presumption of a spatial habitat of about 17,000 km^2 which includes the adjacent shelf waters. Although the maximum estimate of 150,000 mt (from Funk and Harris, 1992, cited in Hay and McCarter 1997a) may now seem unreasonably high, it is reasonable to assume that Prince William Sound may sometimes have had a spawning biomass of at least 100,000 mt. If so, and if the available spawning habitat were about 9000 km^2 (from Table 2), then the maximum density of Prince William Sound herring would be over 11 g/m^2 (11 mt/km^2) making it the most dense of any herring population in the world. However adjacent herring populations in Southeastern Alaska and BC also have a high density at about 10 g/m^2. The point is, however, that in the absence of contrary information, it is probable that Prince William Sound herring also use the adjacent shelf

waters for feeding. Such shelf feeding would reduce the potential for intra-specific and inter-cohort competition for food, as shown in Table 1. In particular, the seasonal departure of some or most of the adult component of the stock would reduce competition between adults and age 0+ juveniles in the late summer and fall (when they are large enough to take the same copepod prey as the older age 1+ juveniles and adults) and age 1+ juveniles. If the adult component of the Prince William Sound herring stock followed the migratory patterns seen in BC herring, then they probably would leave the Sound immediately after spawning and not return until the fall, around October or November.

5.5 Habitat limits on herring abundance

Hay and McCarter (1997a) also suggest that the distribution of herring populations is determined by the availability of the habitat for key life history stages: the egg stage (spawning habitat), larval stage (retention areas), juvenile habitat (nearshore, shallow protected habitats), adult feeding habitat (usually shelf waters with high zooplankton density related to oceanographic factors, especially upwelling) and over-wintering habitat (usually nearshore quiet areas). For example, along the Pacific coast of North America herring spawn only in relatively sheltered areas – and almost never in open waters. This is a major difference between Pacific and Atlantic herring that spawn in shallow open shelf waters. If spawning in such protected areas is a requirement for Pacific herring, this would explain the distribution of herring populations between California and Northern Washington State that are usually associated with small coastal indentations, usually river mouths and estuaries. San Francisco Bay is an example of a large estuary – and in most years has maintained a substantial herring population (> 20,000 mt).

5.6 Continental shelf as the ultimate limiting factor

An important ecological limit to herring abundance on the coasts of BC, SE Alaska and the Gulf of Alaska may be the geographical area of the shelf waters where adult herring feed – and not necessarily the spawning habitat or the geographic area (km^2) of juvenile habitat. In BC, there appears to be ample herring spawning habitat: herring have, at one time or another, utilized almost 25% of the BC coast for spawning (Hay and McCarter 2006). Similarly, there may be more larval herring habitat than is required, although this is harder to define. Replicate field surveys showed herring larvae were broadly distributed relative to their spawning areas, although they appeared to stay in the vicinity of the shore (Hay and McCarter 1997b). Perhaps even more important is that larger larvae seemed to move inshore, close to shallow waters. The evidence for this is based on the unexpectedly high incidence of large larvae captured with small nets fished in shallow nearshore areas that are ordinarily not sampled with the larger, open water plankton nets used in systematic surveys (Hay and McCarter 1997b). Similar observations have been made about the distribution of large herring larvae in the Baltic and in the southern North Sea. This implication is that in some areas, such as the eastern Pacific, the absence of large herring larvae in field samples may not represent only larval avoidance of sampling gear by larger, faster larvae, but rather the movement of these larvae towards shallow, macrophyte-rich areas where herring begin the juvenile phase of their life. Therefore the relative abundance of

nearshore shallow areas may provide critical habitat for herring larvae as they metamorphose into herring juveniles.

5.7 Questions about shelf waters and Prince William Sound herring

The role of the continental shelf as a possible feeding area for Prince William Sound herring is not clear. There is very little reference in the literature to the occurrence of herring on the adjacent shelf waters. To some this may seem like an arcane point but it may be important. In most areas of the North Pacific, the adult component of large herring populations move to shelf waters to feed in the summer. Presumably this also occurs in the water adjacent to Prince William Sound. Access to the adjacent shelf waters probably would expand feeding opportunities by a factor several times greater compared to the potential to feed only within the Sound. For instance, the total area of the "Prince William" district (Fig. 2) is 7885 km^2 for depths from 1-100m , and 8990 km^2 for depths from 101-200 m (Table IV-1 in Ronholt et al. 1978). In contrast, Table 2 (this report) shows that the total area within the Sound is 9059 km^2 with about 3400 km^2 for depths of 0-100 m and about 5300 km^2 for depths from 101-200 m. Therefore, if habitat is simply defined as the preferred depth range of herring, access to the shelf waters adjacent to Prince William Sound would nearly double the available habitat for adult herring, - and it would triple the available habitat between 1-100m. It seems very likely that herring do use this habitat and this author (Hay) has observed adult herring captured as incidental bycatch during research surveys conducted in the Gulf of Alaska in the early 1960's. The research survey conducted at that time, a survey of demersal resources in the Gulf of Alaska and Bering Sea, has been described by Rohholt et al. (1978). They do not report explicitly on the numbers or locations of herring catches but they do note (in their Table V-3) that herring were captured in all six surveys conducted throughout the area. Rounsefell (1930) describes locations of herring catches in the 1920's in the extreme south-west of Prince William Sound, (Manning Bay, Macleod Harbor, and Elrington and Prince of Wales Passages and Puget Bay). These seem to have been areas supporting summer fisheries. Some locations (i.e., Puget Bay) were well outside of Prince William Sound. These observations suggest that some herring do move from the Sound into adjacent coastal waters.

6 6 Review of enhancement – related work

A considerable amount of research has been conducted relative to laboratory culture and rearing of larval marine fish, including herring. Usually the work on herring was directed at some purpose other than enhancement. Regardless, some of the results are applicable to this review. Much, but not all of the work was conducted in Norway, Japan, Canada, the United States of America and the United Kingdom.

6.1 Global activity – general considerations

The global activity related to marine finfish enhancement and sea ranching has increased rapidly in the last ten years. Born et al (2004) provide an impressive list of the numbers of countries undertaking enhancement projects and they also show lists of species and other information related to the duration of projects, numbers of individual fish released, etc. They also make a plea for better reporting so that the efficacy of 'stock enhancement' can be better evaluated. They provide a review of some of the biological and methodological requirements related to this rapidly developing field – specifically the need for standardized nomenclature and reporting. Further they briefly discuss the issue of whether enhancement is an appropriate approach relative to more conventional approaches (input and output controls) to fisheries management. In the case of Prince William Sound, where nearly all herring fishing has been suspended, some of these issues are not applicable. Born et al (2004) point out that the FAO Code of Conduct for Responsible Fisheries, to which the United States is a signatory, provides technical guidelines (through Article 9, FAO, 1995) that apply to the development of aquaculture and culture-based fisheries. Probably this FAO report would not be an obstacle to future enhancement efforts in Prince William Sound but proponents should be aware that there are general internationally-accepted protocols for such activity. General protocols that provide basic guidelines to all enhancement projects are provided by Bartley and Leber (2004), but these do not comment specifically on herring.

At the conclusion of this report there is a checklist, adapted from Walters and Martell (2004). This checklist covers all of the aspects of enhancement considered in the FAO reports, and more,

6.2 Review of applied technology and applications

Many countries, or research agencies within various countries, have embarked on marine fish enhancement programs. Norway has been involved with fish culture and related work for over a century. The Norwegian work with the culture of marine larvae (larviculture) provides some useful information relative to Prince William Sound herring enhancement. The long Norwegian experience with larval rearing of Atlantic cod, and the participation of other countries in the same exercise, may represent one of the most revealing failures in fisheries science. It would be regrettable if herring enhancement proponents were to naively advocate and resurrect such a failed approach – so in the text below, there is a section that briefly explains the Norwegian failure (and the same failure as repeated in Canadian, American and British agencies).

Summarizing research activities that have relevance to herring enhancement could be done according to country (mainly but not exclusively Norway, Canada, United States and Japan). Alternately it might be done according to life history stage, beginning with egg stages followed by larval stages, juvenile stage and so on. This life-history-stage is the approach taken in the following pages except for the Japanese work which is unique and difficult to dissect into stages. Also, most of the Japanese work is not accessible through the conventional scientific literature. Only the Japanese agencies have attempted to raise large quantities of juvenile herring for mass release, as an attempt to enhance local herring stocks. These attempts, while scientifically interesting, still are not a demonstrable success – or failure. Nevertheless, the results to date are very useful for the purposes of understanding issues related to enhancement in Prince William Sound.

6.3 Sources of eggs

Compared to many other marine fish species, access to fertile, viable herring eggs is simple. For nearly every conceivable type of enhancement activity that might develop in Prince William Sound, all would involve securing eggs (from a 'donor site') and moving them to a new location (a 'recipient' site) for incubation. There are four general ways this can done. Each is listed below with some comments.

6.3.1 Method 1. Stripping and artificial fertilization

Eggs can be extracted from live, ripe females and artificially fertilized. All parental stock is killed in this process so this method is suitable only for small scale experimental work. The major disadvantage is that it is difficult to be certain of the spawning readiness of females. If they are slightly immature, they may still have eggs that can fertilize, but the overall fertilization rate may be relatively low. Also, there is some indication that during the preparation of sperm solutions, the presence of blood from the surgical removal of testes could contaminate eggs with blood thereby limiting viability of the egg (D. Alderdice, pers. comm.).

If artificial fertilization methods are used it is important to avoid exposing eggs to seawater prior to fertilization. Maximal fertilization rates can be achieved by introducing eggs into a previously prepared sperm solution. Herring have extremely adhesive eggs so one method of mass culture is to extrude eggs from the female onto an artificial substrate such as a plastic screen or Nitex ™ (a fabric used for plankton nets). This was a successful artificial substrate used in experimental work (Hay 1986). Before eggs were placed onto this screen material, the screens were soaked in fresh sperm-containing seawater. Fertilization occurred instantly as the eggs were extruded from the female.

6.3.2 Method 2. Naturally spawned eggs

Moving eggs from natural spawns will provide an excellent source of eggs for experiments, and possible pilot-scale enhancement experiments. The main problem with this approach is that some naturally spawned eggs will be lost to the environment. Also, there may be habitat damage as eggs are removed. Such removals, if small, are not a conservation concern although there could be some negative impacts on spawning areas.

There is a risk of a serious impact on natural spawning areas if naturally spawned eggs were the main source of eggs used for enhancement. First, there would be direct loss of eggs (even a small enhancement project would involve billions of eggs). Second, there would be damage to the spawning habitat – and remaining eggs. Probably there would be considerable direct mortality to eggs that would be squashed, or dislodged or uprooted during this phase.

6.3.3 Method 3. Impounded herring

Eggs could be used from operations like 'spawn-on-kelp' fisheries in which impounded or semi-impounded captive, spawning fish, are forced to spawn on suspended or removable substrate. Perhaps material such as web netting from purse seine webbing, or vegetation natural substrate – such as *Laminaria* used for 'spawn-on-kelp' operations could be used for the purposes of

acquiring eggs for enhancement. In these situations, captive herring spawn on natural or artificial substrate that is prepared and suspended in ponds or cages.

Probably this approach may be the most reliable and the least controversial for potential enhancement projects. There is a roe-on-kelp fishery in Prince William Sound and the technical expertise at establishing impoundments exists in the area. There is a substantial 'grey' literature on experimental impoundments in BC during the 1980's when these were under consideration as possible alternatives to the roe fishery. Two of the most useful reports are by Kreiberg et al. (1986) that present designs and methodology for 'towable netpens' and Kreiberg and Solmie (1987) that provide a basic biological guideline for impounding herring.

It is unclear if disease may be a factor to consider when planning a potential enhancement project. If so, it seems probabe that disease issues might arise more frequently from impounded herring. This issue of disease is discussed briefly as a separate topic.

6.3.4 Method 4. Wind drift - opportunistic sources

Eggs that are blown ashore, following storms, may form 'wind drift'- or 'windrows' of eggs on beaches. Usually these eggs are alive and can be moved easily. This was the source of eggs used for two years of consecutive egg transfer experimentation in Southern British Columbia (Hay and Marliave 1988). Finding useful quantities of such eggs can be a problem. Hay and Marliave used a small aircraft to scout spotter planes and a network of local informants, mainly Fisheries and Oceans Canada Fishery Officers. Usually this source of eggs became available following intense storms - and there always are storms during herring spawning - but the available eggs usually are found only over a few hundred m. Each year in the Strait of Georgia the cumulative spawn may be several hundred km of spawn, and the perimeter of the Strait of Georgia is about 3700 km. Further, these windrows are difficult to see during a high tide. During high tides, herring egg windrows usually consist of a slurry of loose eggs and vegetation. When the tide recedes, the loose eggs and vegetation may accumulate into piles along the shore, sometimes reaching depths of 30 cm or more. Surprisingly, if these windrows are found soon after a storm, most of these eggs are alive. The exposure to air is not a problem. In fact, during our first trials at moving windrow eggs, we found that eggs left in air, but kept cool and damp, were much more likely to survive that those immersed in water in buckets and aquaria. In the two years of the egg transfer work we moved about 20 billion eggs each year – approximately equivalent to a spawning stock of about 20 metric tons.

6.3.5 Wet egg weight

This paragraph is more of an aside about a useful but rare statistic on wet egg weight. Remarkably, the estimate of the weight of a herring in nature is difficult to find. There may be only one grey reference by Hay and Miller (1982) who studied wind drift spawn in Georgia Strait. To estimate the quantities, they took sub-samples to determine the wet weight of a single, live, incubating egg. Their estimated weight, for a single egg, was 2.38 mg. This weight is much greater than the weight of an unfertilized egg from a female. At the time of spawning, when eggs are exposed to seawater, they take up water and expand their volume. Therefore egg weights estimated from fecundity analyses (i.e., the ovary weight divided by the fecundity weight) will be much less than the weight of a live, fertilized egg in nature. This estimate of egg weight from a Strait of Georgia herring may be roughly applicable to a Prince William Sound herring, but probably is not adequate for any detailed work in the future. Wet egg weight could

vary with salinity and therefore depth, because inter-tidal eggs may be exposed to different salinities than sub-tidal eggs. This simple statistic is essential for potential herring enhancement work. Therefore some additional data are advisable.

6.3.6 Recommendations about egg source(s)

In some ways, windrow eggs as a source for herring enhancement are ideal, because they can be taken without deleterious impacts on spawning habitat. If such a source of eggs is available each year, as it was in the Strait of Georgia, then it would be a good choice for enhancement. The major risks are (1) the sources of eggs are not predictable and the timing is unknown; (2) the location of windrows may be inconvenient for enhancement-related work; (3) collecting and relocating the windrow eggs is labor-intensive and requires vessels, such as barges, that can reach the shore – or areas close to the shore; (4) the loose eggs are more difficult to incubate that those firmly attached to substrate.

Even if wind-drift eggs were not available, then the access to eggs from operations like 'roe-on-kelp' operations may be the best. The collection of eggs would be almost identical to the processes used presently to encourage captive herring to lay their eggs on suspended kelp. There are many options that may be developed, however. One is the use of artificial substrate, such as the webbing from purse seines or trawls. Herring readily spawn on such material. One proposal, developed years ago in BC, was to suspend netting from logs, either in impoundments or on natural spawning areas. Then after the eggs have become well-fastened to the material, and have lost their stickiness, the netting could be gently rolled up on the logs so the logs could be towed to other locations.

6.4 Egg incubation

Probably this is the simplest enhancement activity. Under most conditions, herring eggs are robust. The exception can arise in laboratory settings where the eggs are artificially fertilized by killing and stripping females. Often such eggs may not be at the exact point of readiness, so low fertilization rates may follow. Normally, nearly all herring eggs in nature are fertilized. Rates less than 90 % are suspect.

6.4.1 Egg density

Hourston et al. (1984) report on hatching experiments of Pacific herring captured in southern BC. Although few quantitative data are provided, they noted that hatching rates were high for most of 14 different substrates (mainly naturally occurring macrophytes). They also examined egg deposition intensity and used females from three sources (different collections). The general conclusion was that the main factor affecting hatching success was egg intensity: hatching was lower at high intensity. They also noted however, that the measure of egg intensity varies with the properties of the substrate. They did not provide any advice about the optimal egg density – or egg layers – but they noted that the very high mortality of herring eggs found by Galkina (1971) was probably attributable to the high egg density (~ 20 layers).

6.4.2 Egg incubation duration

At ambient temperatures the duration of egg incubation will be about three weeks. The time can be estimated approximately by the equations of Alderdice and Velsen (1971) that relate temperature to the time required to hatch. Probably eggs should not be relocated until a few days after spawning, to allow them all to harden, and also allow the eggs and substrates to be colonized by the microscopic grazing community. This grazing community will control fungus on dead eggs. In naturally spawned eggs there always are some unfertilized eggs, even among healthy females, because a small proportion of eggs will not fertilize. For instance, fertilization will not occur if the micropyle is blocked. Unfertilized eggs will eventually die and probably become infected with fungus. Once well established, fungus can attach to healthy eggs and kill large numbers. Fungal outbreaks are rare among naturally spawned eggs deposited in suitable locations and normal conditions. The reason for this low infection rate is that the grazing community consumes fungus as it develops, thereby enhancing egg survival to hatching.

6.4.3 Estimates of survival to hatching

Unlike the eggs of many pelagic marine species, herring eggs have relatively high survival rates. Usually the exceptions are noted, and sometimes there may be instances of mass mortality, but in general these are rare. Such instances can occur when unusual conditions occur, such as freezing of eggs in the inter-tidal zone, or exposure to sunlight and desiccation in the inter-tidal zone. Sedimentation also can lower survival. Experimental exposure of incubating eggs to lower oxygen levels, when eggs were suspended below salmon netpens, also resulted in decreased survival.

In general, if incubating eggs from naturally spawned areas are protected from predation, then high survival rates from fertilization to hatching might be expected, probably at least 80% or more. The literature provides a mixed range of egg survival estimates but it is important to note that estimates of egg survival rates vary with location of the various studies. For instance, Norcross and Brown (2001) estimated natural egg survival to be about 25%. Rooper et al. (1999) estimated egg loss to predation and abiotic factors to be about 31% for an estimated survival rate of 69%. Palsson (1984), in Puget Sound, reported relatively high rates, but most of the Puget Sound spawning areas have relatively low densities of eggs, usually not more that a few hundred thousand per m^2. In contrast, Strait of Georgia egg densities are higher, often 500,000 eggs per m^2, or greater (Hay 2006). During the incubation period a loss of 50,000 eggs per square meter to local invertebrate predators (such as small snails, crabs, etc) would result in a 50% morality if original density was 100,000 eggs per m^2 but only 10% if the original density was 500,000 eggs per m^2.

6.5 Herring Larviculture

The term ‘larviculture’ is not common, but the implication is that it involves the feeding of captive larvae. Large research efforts have been devoted to this task, mainly because it was commonly believed that the feeding of young larvae of marine fishes was the key to understanding factors that controlled year class success. There is far too much literature on this topic to include here.

6.5.1 History in Europe and North America

Meyer (1878) reared herring eggs in the Baltic in an attempt to investigate methods for delaying development and hatch so that the eggs might be shipped to other countries for 'artificial raising'. Meyer successfully shipped live herring eggs to the Kiel laboratory but did not report on survival or growth rates. It is interesting that a translation of Meyers report was presented in an annual Report of The Commissioner, United States Commission of Fish and Fisheries, (Part 4) in 1878. The two main topics of the report were (i) an inquiry into the decrease of food-fishes and (ii) the propagation of food-fishes in the waters of the United States.

In experiments conducted in 1966 and 1967, Talbot and Johnson (1972) reared San Francisco Bay herring from eggs to more than two years of age. They observed that at 4 days after hatching, when their yolk sacs were almost depleted, larvae were too small to ingest newly hatched brine shrimp (*Artemia)* nauplii but the larvae were able to consume brine shrimp after 6 days. In June, when the herring had developed into juveniles, they were successfully fed live brine shrimp, but an attempt to feed some with pellets (Oregon Moist^{TM}) was not successful. Pellets sank to the bottom of the aquaria and were ignored by the juveniles. In November, at about ten months of age, the herring were successfully fed on frozen brine shrimp. Talbot and Johnson (1972) noted that metamorphosis, from the anguilliform larval shape to the normal herring shape, was complete at about 80 days of age and a total length of about 33 mm. They also noted that the sizes of the reared herring were slightly smaller than the herring of the same age that grew naturally in San Francisco Bay.

6.5.2 Larviculture – history in Norway

Norway has conducted a considerable amount of scientific research concerned with fish culture, both from a theoretical and applied perspective. Although there are many papers, reports and theses that are concerned with herring in particular, no research has specifically addressed the issue of herring culture for profit (farming) or the mass rearing of herring for stock enhancement. There are several areas of research that are directly applicable to the issue of herring enhancement in Prince William Sound, Alaska. Specifically, Norwegian research has addressed germane issues concerned with reproductive biology of herring, egg incubation and larval culture. Probably the most useful contribution concerns the extensive amount of work conducted using mesocosms, large containers, bags or enclosures suitable for rearing larvae and juveniles.

The earliest interest in larviculture in Norway was concerned with Atlantic cod. Over one hundred years ago Norway initiated a huge program to artificially spawn, incubate and grow Atlantic cod larvae for short periods prior to release. The work was eventually emulated by Scotland, Canada and the United States – and the potential benefits of the approach led other countries, such as Australia, to release larvae of other species. In retrospect, the popularity of the approach and the relatively large and expensive scale of operations seems remarkable because there was no definitive evidence that the cod larviculture was successful. There was, however, a lot of acrimonious debate about the issue, in Norway and elsewhere.

6.5.3 The Flödevigen experience

The chapter by Solemdal et al. (1984) provides a fascinating review of the history of the cod enhancement project and the Flödevigen laboratory which was pivotal in this work. It started in

1882 with private funds. The laboratory was constructed in 1884. For nearly ninety years, until 1971, the hatchery released yolk-sac larvae of cod, up to 400 million per year. After 20 years, private interests lost faith in the project and it was taken over by the government, leading to the establishment of the Flödevigen laboratory which continues to this day. In addition to cod, the laboratory released several other species including lobster. At the onset, the main biological proponent of the work was a prominent zoologist, G.O. Sars, who convinced a sea captain, Gunder Dannevig, to promote the development of this approach. He succeeded, both within Norway and internationally, and the laboratory was constructed and functioned. After a decade or two, however, the viability of the project was not confirmed and detractors began to speak out. These included other prominent Norwegian scientists some of whom subsequently achieved international fame, including Einar Lea, Johan Hjort, and others. These scientists pointed out that there was no convincing evidence of success of this larval rearing approach. In particular, Hjort believed that the key to larval survival was the available level of food for larvae after they resorb their yolk sac – so he did not endorse the release of yolk sac larvae. Instead they advocated a longer rearing period and release of older larger larvae. One early attempt to demonstrate the success of the project (of releasing yolk sac larvae) was to release them in a large enclosure that mimicked the natural environment.

Regardless of the criticism, the larviculture programs at Flödevigen and elsewhere in the world continued for decades and ended in Flödevigen in 1971. It is only within the last 25 years that clear objective analyses presented in peer reviewed literature has shown the futility of the endeavor – as a technique (of releasing *yolk sac* larvae) for enhancing Atlantic cod. There were some positive spin-offs from this work however, especially related to increased knowledge of the early life history of marine fish.

6.5.4 Perspective on herring larviculture in BC and elsewhere

Newly hatched brine shrimp (*Artemia***)** eggs, called 'nauplii', are easily raised and are widely used for experimental and commercial rearing of marine fish larvae. The successful use of brine shrimp for herring is relatively recent. At the annual meeting of the early Life History Section of the American Fisheries Society, held in Vancouver, BC in 1984, some European scientists privately expressed exasperation with the claims about larval fish survival made by some of the Norwegian participants – who claimed high rates of larval survival. Some even suspected results were wrong, even fabricated. We know now that high survival is possible – and it is related mainly to the quality of food. Herring larvae from BC and Alaska will feed on *Artemia* nauplii soon after hatching. Herring larval guts are transparent and the tiny bolus of food – usually discrete nauplii - can be seen and counted in the gut in live larvae. Larvae feed in lighted conditions but not in the dark (Alderdice and Velsen, 1971). When food is abundant larvae feed to satiation and their guts appear to be continuously full. In contrast, when the light is dim or during the night their guts are empty. This observation can be made after the lights are suddenly switched on in a darkened laboratory. It is a simple matter to discern whether larval herring are feeding simply by looking at them.

Blaxter (1968), and others experimented with varied diets in attempts to rear herring to juvenile stages. Blaxter reported an increase in survival using *Artemia* supplemented with barnacle nauplii, but larval survival in Blaxter's experiments was low relative to the much higher survival rates achieved in later years by Norwegian and Japanese researchers in the 1970's and

subsequent years. For instance, Blaxter (1968) reports that a combination of food types yielded higher survival rates than single-food diets, but this higher rate was only about a 10% survival rate from hatching to metamorphosis, and a 3-4% survival rate from fertilization to metamorphosis.

6.5.5 Experimental larval culture at the Pacific Biological Station, Nanaimo, BC

At the Pacific Biological Station in Nanaimo, BC, we were able to access high quality eggs, either from artificial fertilization or from natural sources. Laboratory incubation for the period before feeding was rarely a problem, even with varying temperature and salinity. Hatching rates and production of viable larvae were high, usually exceeding 95%. (See comments elsewhere on factors affecting fertilization rates and incubation survival). Maintaining active, feeding larvae through to the absorption of their yolk sac was simple. Virtually all larvae started feeding successfully and consumed food until their guts were full. Early growth rates (estimated by analyses of length and dry weight) were rapid. After about five days, larval feeding rates began to decline, and usually by 10-20 days after first feeding, most had stopped feeding. At these early stages death by starvation to the 'point of no return' (or when starvation was irreversible), occurred in all larvae in about 20 days. As the era of experimental physiology and ecology in the 1970's and 1980's was ending at the Pacific Biological Station, we tried a food enrichment product, 'Super Selco^{TM}', recommended by Jeff Marliave of the Vancouver Public Aquarium. Newly hatched *Artemia* nauplii were soaked in the 'fish based' product for a few hours prior to presentation to the herring larvae. Survival of the larvae, when fed with this product, was excellent, and virtually all of the larvae fed with the enriched larval food continued to feed and grow.

6.5.6 Larval food culture

Technological aspects of *Artemia* enrichment have been described in various reports (i.e., Lavens and Sorgeloos 1996). Unfortunately, this aspect of larval herring husbandry has been overlooked in literature concerned with larval culture of herring. There is a lot of information and research activity about rotifer culture, as a first food for marine fish larvae. Probably this would be an optional consideration for culture of Prince William Sound herring larvae that, like BC herring, would be able to feed directly on *Artemia*. On the other hand, it is clear that plain (not enriched, see below) *Artemia* are not adequate as the sole food source for herring larvae, so the nutritional benefits of additional food items, such as rotifers, for first-feeding herring larvae might make the additional efforts of mass-rearing of rotifers worthwhile.

6.5.6.1 Enrichment of Artemia

Enrichment of *Artemi*a nauplii with a product like Super Selco^{TM} (Artemia Systems N.V., Baarode, Belgium) can result in remarkable improvements in larval survival.

6.5.6.2 Quantities

The correct feeding levels of *Artemia* may be important. After a day or two, the nauplii grow and utilize much of their yolk sac. The result is a prey item that is less nutritious and without the benefit of the exposure to Selco which, to be effective, seems to adhere to the nauplii, but the beneficial impact may be lost with time. Therefore it probably is

important to develop larval rearing systems or feeding levels that do not allow for the accumulation of uneaten, less nutritious, older *Artemia* nauplii as larval herring prey.

6.5.6.3 Contamination

It is necessary to avoid contamination of larval herring rearing containers with unhatched *Artemia* eggs. Many larger larvae will feed on these but they merely pass through the fish undigested, in the original form. The unhatched *Artemia* egg capsule is, therefore, an impediment for optimal feeding.

6.5.6.4 Water and flow

Larval herring need cool, well oxygenated water, so completely static systems are not appropriate for mass culture where the larvae or their food have a significant BOD (biological oxygen demand). Some form of flow-through system is required but it is important to eliminate the potential for entrainment within drains. Perhaps this is obvious, but the problem is simplified in large containers that have large openings and closures that permit slow water exchange. Such inlets and outlets can be covered with fine mesh screens (i.e., 350 μm) that allow slow passage of water without risk of larval impingement.

6.5.6.5 Algal growth

Some natural algal growth seems to be beneficial. We noticed that larvae seem to do well in 'green water' tanks but we have no explanation for this. Boehlert and Morgan (1985) noticed that Pacific herring larvae fed more effectively in 'murky water'. Pacific herring larvae seem to be mainly visual feeders so prey may be more visible to larvae in some conditions.

6.6 Field observations of larval feeding

It is difficult to detect or quantify larval feeding from field samples of larvae collected in nets, or from large outdoor enclosures, because herring larvae usually void their gut contents when impinged against a net (Hay 1981). However, larval feeding in nature can be observed directly at certain times. At night, larvae are attracted to lights so careful observation of larvae in field conditions is relatively simple if the physical conditions permit. Such observation is possible on the wharf at the Pacific Biological Station, Nanaimo, BC. When observed early in the evening, while they still retain food in their guts, some larvae can be examined directly. We observed that wild herring larvae take a variety of food. Probably the eggs of copepods and other invertebrates were the most common food but we noticed that sometimes they consumed inedible items such as pollen (especially the round pollen from conifers or maple trees) or small round air bubbles. Sometimes the buoyancy of the bubbles, in the larval guts, seemed to impair the swimming movement of larvae. The potential significance of these observations is that herring larvae appear to take a range of prey items, although they seem to select items that are approximately round and that have high visual contrast. In informal (and unpublished) experiments in the laboratory, we provided herring larvae with fresh barnacle nauplii that are almost transparent. Fresh barnacle nauplii were obtained by smashing the adult barnacles. We also used food dye to color the barnacle nauplii. Herring larvae took more dyed nauplii than the natural, transparent larvae.

Readers should regard the preceding paragraph as more of an informal (and hopefully useful) narrative and not a detailed set of specific recommendations for larval rearing. There are a number of sources for planning large scale larval food production. See, for example, an FAO report that provides detailed information on the mass production of live food (FAO 1996). There also is an earlier report on large-scale larval fish rearing (FAO 1986). This report, while now a bit dated, provides a number of practical, technical methods useful for rearing marine larvae.

7 Norwegian larviculture work and development of mesocosms

Norwegian science in the last one hundred years has contributed substantially to our understanding of herring reproductive biology. In terms of enhancement, the experimental work on mesocosms is the most relevant to the issue of herring enhancement in Prince William Sound. Mesocosms are relatively large cages or semi-natural enclosures of varying size. Usually fish larvae are reared with natural food (zooplankton) that also exists within the mesocosms, although in many experiments larval fish diets are supplemented with other foods, including wild-captured zooplankton, brine shrimp (*Artemia*) nauplii and pellets. Mesocosms are relevant to herring enhancement because some form of enclosure will be required for rearing larvae during the first months of their post-hatching life.

7.1 Semi-natural mesocosms

An enduring legacy of the Flödevigen work was the construction of large seawater ponds – small land-locked basins, several m above sea level. Water levels were controlled by pumping (and discharging) sea water from the pond into the adjacent ocean water. These enclosures or mesocosms, could hold thousands of larvae, including larval herring. An especially useful result from rearing larvae in these enclosures was the demonstration of high rates of survival, from egg to large larva – rates that exceeded fifty percent survival. In the 1960's and 1970's most laboratory researchers working with herring struggled with much lower survival rates. Norwegian experiments conducted in these large mesocosms demonstrated that herring larvae survival could indeed be reasonably high. Only brief details of the size and dimensions of the mesocosms are presented in published literature but Øiestad (1983) presents illustrations and these are copied in Fig. 3. Such semi-natural enclosures may be a useful approach for herring enhancement in Prince William Sound.

Øiestad (1982) provides a descriptive overview of the development of bags, ponds and mesocosms used in Norway. Mainly these were experiments to study herring larvae ecology and not mass rearing. Studies included the trophic habits of larvae and evaluation of the effects of larval predation on micro-zooplankton. Of the nine different studies summarized by Øiestad, most were mesocosms consisting of large plastic bags ranging in size from 4 m^3 - 2500 m^3 that were used for experimental durations of up to 125 days. Maximal experimental duration in the ponds was 180 days.

7.2 Mesocosms and larval fish growth and survival

In a 1982 review paper Øiestad (1982) made two basic conclusions about marine fish larvae reared in mesocosms. One is that they can survive at feeding densities that previously were believed to be too low. The second is the larval numbers were very sensitive to predation in the bags. Subsequently, Moksness and Øiestad (1987) reared herring larvae in basins for up to 4 months with smaller capelin larvae. Of 25,000 eggs introduced at the beginning of the tests, 7000 survived to an age of 30 days and 4400 survived to an age of 100 days. They noted that herring larvae began schooling at 50 days and metamorphosis occurred at 60 days when they were 34 mm long.

Wespestad and Moksness (1990) used the same enclosures to rear Pacific herring *Clupea pallasi*. A total of 4891 larvae survived for 63 days after hatching, for a daily survival rate of about 2.7%. Some mesocosm work was conducted with other species using much greater initial numbers. For instance Øiestad et al. (1985) placed about 2.5 million cod larvae into an enclosure. They estimated that after one month (in April) about 500,000 metamorphosed into larvae and depleted the natural food by mid-May. An interesting point for this review is that the basin could support such a large number of larvae (>>500,000) without additional feeding. They also found that the metamorphosed fish were able to feed on pellets and some wild copepods captured from areas outside the enclosure. Moksness (1990) also used the Flödevigen outdoor enclosure to rear cod larve to juveniles – which were later used to test the survival of artificially reared cod that were tagged and released at about 2 years of age. The conclusion was that the observed recapture rates were too low to consider larger scale rearing and subsequent release of cod as a technique to support the commercial fishery.

Kvenseth and Øiestad (1984) describe experiments raising cod *Gadus morhua* in very large outdoor or enclosed ponds (surface area of 22,000 m^2 and volume of 60,000 m^3) in western Norway. They also used hydrographic monitoring and serial sampling of phytoplankton, zooplankton and fish larvae. Automatic feeding systems were used in the ponds. Approaches such as this are necessary for rearing large numbers of larvae and juveniles, such as herring in Prince William Sound.

7.3 Juvenile rearing and Mesocosms

Houde and Berkeley (1979, 1982) investigated feeding and growth of age 0+ juvenile herring in 1300 m^3 enclosures called CEPEX (Controlled Ecosystem Populations Experiment) enclosures. These enclosures were in Saanich Inlet, in southern BC. These enclosures were 10 m in diameter and 23.5 m deep. One hundred juvenile herring, approximately 3 grams each, were introduced into the enclosures and reared for one month. Food was not added, but there was an abundant zooplankton fauna present in the bag at the beginning of the experiment. Periodic samples were collected to determine growth and feeding capacity. Growth rates in the CEPEX enclosures were slow relative to control fish maintained in smaller (2 m^3) tanks on an adjacent research barge. These 'control' fish that were fed regularly with wild zooplankton collected in plankton nets. The specific growth rate (weight) was low (0.7% per day) in the mesocosm fish but high in the control (barge) fish at 5.35% per day.

The papers by Houde and Berkeley (1979, 1982) provide some potentially useful information about feeding stomach evacuation rates. For example, the relationship between dry weight of food contents and herring (wet) weight was:

$F = 5.92 + 5.55W$

where F = dry wt of food (mg) and W = wet weight in (g). They also found that digestion was 95% complete after 15 h (at 16 ^{o}C). The ration of wild herring collected in the adjacent waters of Saanich Inlet (southern Strait of Georgia, BC) was about 0.037 g/d for a fish weighing 2.6 g – for a mean of 0.0142 g of food per g of fish (total wet weight) or a consumption of 4.9% of their *dry* body weight per day. These data could be roughly applicable to Prince William Sound herring although temperature differences in juvenile rearing habitat probably have considerable effects on feeding and evacuation rates. Subsequently, more comprehensive data sets on wild and captive juvenile herring feeding and evacuation rates have been estimated from Baltic herring (Arrhenius 1995, 1996; Arrhenius and Hansson 1996a, 1996b).

7.4 Rearing to the juvenile stage – how long, how big – the critical questions

Field studies of juvenile herring feeding in Prince William Sound provide evidence of food deprivation leading to poor survival over the winter. Preceding parts of this report have commented that a fundamental ecological requirement of enhancement is to avoid release of cultured fish before density-dependent processes begin.

Clearly, release of larvae is ineffective for herring enhancement. To be effective, enhancement must continue to the juvenile stage, but for how long? Suppose the enhanced juveniles were fed rapidly so they grew well. Once released would these bigger, fatter, enhanced individuals survive better than smaller, thinner, naturally raised juveniles? Perhaps, especially if they could feed well. The important question, however, is whether such enhanced fish would **add** to the population, or merely displace the small, naturally produced, herring juveniles? Although there is much uncertainty regarding these questions, it would seem logical that the longer the enhanced herring were raised, the bigger they would become. From the cost perspective, the sooner the juveniles were released, the better. From an ecosystem perspective, the later the release, the better. Somewhere between the end of the first summer/fall (say October) and the middle of the first winter (say February) would appear to be the optimal time of release. A winter release – or a release after most of the rich summer/fall feeding occurs, presumes that very little food would be available to any juvenile herring, enhanced or natural. Therefore it seems likely that this is a period when density-dependent competition for food would not be a key factor. However, it also would be advisable to ensure that any enhanced herring are not so large that they might prey on the naturally raised members of the same cohort. This comment is based on the Japanese experience that has seen astounding growth of well-fed juveniles (see comment about Japanese enhancement in later sections).

Therefore perhaps the key question for herring enhancement in Prince William Sound, if it were to proceed, is the duration of juvenile rearing.

8 Japanese experience with enhancement

Japanese research appears to have made substantial progress rearing marine fish larvae, including herring. For instance Kurata (1959) reports on many aspects of larval feeding ecology. Since then there have been technical developments that have resulted in extraordinarily high rates of larval survival. Japanese work in this field seems to have developed *in situ*, without much scientific communication with work going on elsewhere. Much of the recent work has direct and significant implications for potential herring enhancement work in Prince William Sound. This review of Japanese work was facilitated by direct communication with several key people with experience in this area. One potentially important paper, which is in press, was not available for inclusion here.

8.1 Lake Furen

Probably one of the best and most accessible reports on Japanese enhancement of herring is that of Morita (1985). At the end of a paper describing the demise and present status of the massive Hokkaido-Sakhalin herring stock, there is a three-page summary of Japanese experience with herring enhancement, as it was practiced up to 1985. Morita describes procedures that were used to gather spawning fish from a brackish lake, artificially spawn the eggs, incubate the eggs and raise the eggs and larvae on a combination of food organisms, including rotifers, and *Artemia* as well as small 'pellets' that were the sole food used after 73 days of feeding.

The reference by Morita (1985) to artificial food is interesting and worthy of further investigation. Unfortunately, there is nothing in the accessible literature that describes such food. Personal experience, however, would indicate that any artificial food (such as a pellet) would require a specific gravity similar to the rearing water – so that it stays in suspension. Herring, at any age, do not forage on the bottom, and in general they do not seem to strike at floating items.

8.2 High Lake Furen survival rates

Morita (1985) describes very high survival rates, estimated at nearly 50% after 100 days of rearing. *At this time the juveniles reached a mean length of about 70 mm (with a range of 40-90 mm).* The juvenile herring were released into Lake Furen, a small brackish lagoon on eastern Hokkaido. There is no subsequent mention of the fate of these juveniles but Kobayashi (2001) reports that in subsequent years (1993-2000) about 300,000 juveniles have been tagged (at a length of 6-8 cm) and released into Lake Furen each year. From there they enter into the Akkeshi Bay area of eastern Hokkaido. These tagged herring have been recaptured inside and outside of Lake Furen. Growth rates of herring from Lake Furen are very high, reaching 15.5 cm after one year and 21.0 cm at age 2, when they mature sexually. Also remarkable is the apparent homing of herring back to Lake Furen. After release some were recaptured more than 300 km to the west (Cape Erimo) and others about 100 km to the north (Cape Shiretoko). This indicates that some herring move away from the immediate vicinity of Lake Furen in adjacent coastal waters. Presumably some or most find their way back to Lake Furen for spawning. If so, cumulative recapture rates for tagged Lake Furen herring were sometimes high (12.5% for the 1995 cohort,

4% for the 1998 cohort) but in most years the recapture rate was about 1%. Kobayashi (2000) concludes that the attempt at enhancement has not been fully successful.

Suzuki and Fukunaga (2004) summarize the number of releases in the Akkeshi Bay area that range from 130,000 to 578,000 annually. The average length at the time of release is 68-69 mm. Some of these herring are recovered in the Akkeshi Fish market, so there is little doubt that these artificially reared herring juveniles survived and joined wild stocks. The maximal return rate of marked fish was 12% in 2000. Although there are some uncertainties regarding the computation of 'recovery rates', this short communication shows a striking relationship between the size of the release and the recapture rate (Fig 4).

8.3 Size-at-release and survival – implications for Prince William Sound

Figure 4 shows that rearing larvae and juvenile to a size of about 70-80 mm (compared to the shortest size of about 60 mm) seems to improve return rates, presumably by improving their survival - although the results also could be an improvement in the geographic fidelity as a consequence of longer enhancement duration. Regardless, if this relationship holds for Prince William Sound, and if enhanced larve grew there at approximately the same rates as they do in Japan, they probably would be much larger than naturally reared herring in Prince William Sound. Figure 5 shows the growth rate of wild herring larvae and juveniles from surveys in the Strait of Georgia. At 100 days of age they are smaller (about 50 mm) compared to the mean length of 70 mm for the Akkeshi Bay herring shown in Fig. 4.

The implications of this are not clear, and conclusions from such comparisons are speculative. Nevertheless it seems likely that enhanced herring grow much faster than naturally reared herring, similar to the results of Houde (1979, 1982). Although tentative, the potentially promising aspect of this (somewhat speculative) result is that enhanced fish may be able to survive well, once released. The worrisome aspect is that if naturally available food is limiting, as it seems to be for juveniles in Prince William Sound, enhanced herring may be able to out-compete smaller, wild herring.

8.4 Miyako Stock Enhancement Center at Miyako Bay

Okouchi and Nakagawa (2006) and Okouchi (2007 pers. comm.) describe similar herring rearing and release projects conducted at the Miyako Stock Enhancement Center at Miyako Bay, in western Honshu. At latitude of 39^{o}N, this is close to the southern limit of the range of herring in the western Pacific. As in the Lake Furen project, work involving rearing, tagging and release experiments has been conducted since 1984. Feeding technology never seems to have been an issue with Japanese researchers, perhaps because they just borrowed technologies developed for other species, such as the popular Sea Bream.

8.5 ALC marking

Okouchi (per comm. 2007) advised that it took six years of experimentation to develop the ALC **(**alizarin complexone) otolith marking technology. Since 1994 they have applied ALC otolith marks annually to large numbers of juveniles, ranging from 13-71 million fish although not all are herring.

The ALC otolith marks are applied early, to larvae, when they are immersed in a 20 ppm ALC medium for 24 hours (i.e., ALC must be added to their incubation water for 24 hours). (Begg et al. 2005, in an introductory/summary paper for an international symposium on otoliths, discuss recent advances in otolith technology. For more details of otolith marking, see other papers in the same volume.)

The main emphasis of the Miyako Bay experimentation has been the confirmation of homing. Over the years they found evidence that released herring migrate away from the area before they return to their general release area. Some recovery information from incidentally-captured fish is complicated because there are no corresponding data on fishing or catch rates, etc. In any event, a key conclusion is that if the total recaptures of marked herring are aggregated from six different spawning grounds in the Miyako Bay area (an indentation on the north-eastern shore of Honshu Island with approximate dimensions of about 5 km by 20 km), the fidelity rate (or homing rate) is 71.5%.

A curious aspect of this work was the experimental marking of eyed eggs (pre-hatch) with ALC markers. The mark was successful. This result is surprising because the otolith of larval fish is very small and the size of a chemical mark must be extraordinarily small. Also the egg capsule is thought to be impenetrable to many chemicals, perhaps including ALC. However, the results of the Japanese work are very interesting and potentially very useful. If such otolith marking can be applied at the late egg stage, then this would enable a number of experimental/research possibilities – that extend beyond enhancement-related research.

8.6 Evaluation of enhancement in Japan

Kitada and Kishino (2006) review four case studies of Japanese enhancement projects. They suggest that limited carrying capacity may limit the ultimate expansion of enhancement activities. They found evidence that in some programs, enhanced fish replaced wild fish – so they advise that enhancement programs should proceed cautiously. Their review did not comment on the Japanese herring projects.

This paper was not available for inclusion at the time of writing:
Sugaya, T., M. Sato, E. Yokoyama, Y. Nemoto, T. Fujita, H. Okouchi, K. Hamasaki and S. Kitada (2007 in review). Population genetic structure and variability of Pacific herring *Clupea pallasii* in the stocking area along the Pacific coast of northern Japan. Aquaculture.

9 Disease and the potential impact on enhancement

High incidence of disease in Prince William Sound herring has attracted considerable research attention (for example see Carls et al 1998; Hershberger et al 1999; Kocan et al 1996, 1997, 1999; Marty et al 1998, 2003; Meyers et al 1994). The two diseases of concern are the viral hemorrhagic septicemia virus (VHSV) and the parasitic fungus *Ichthyophonus hoferi.* The exact role of disease in the population decline of 1993 remains uncertain but VHSH appears to be implicated with poor recruitment (Marty et al. 1993). These diseases are ubiquitous in the marine environment, in Prince William Sound and elsewhere, but infection rates vary in time and space and disease outbreaks are unpredictable. The persistence of a high incidence of disease in Prince William Sound seems exceptional among herring populations but perhaps that may be a function of the intense scientific scrutiny of Prince William Sound herring. Perhaps if there were more detailed monitoring of other populations, they too would have high infection rates. However, severe epizootic incidences, leading to mass mortality, are known in other herring populations.

The recent problems and concern with disease of herring in Prince William Sound pose a significant issue for potential enhancement activity. Suggestions for solutions of directions are beyond the scope of this paper except to point out some elementary aspects of the problem. One is that disease outbreaks seem to be associated with density confinement, similar to that seen in spawn-on-kelp fishery operations (Hershberger et al. 1999). Therefore the collection and holding of pre-spawning adults, as possible egg sources for culture (for enhancement), could lead to unanticipated problems if disease erupted in the parental stock. It seems best to avoid such confinement, if possible. Such avoidance could be accomplished by the collection and use of naturally deposited eggs (on natural substrate) or from suspension of artificial substrate to collect eggs from naturally spawning herring. Such practice, however, defeats any attempts to have enhancement operations occur in pathogen free environments.

9.1 Should enhancement facilities be pathogen free?

A basic question for enhancement activity is whether the rearing habitat should be natural, using untreated marine water from Prince William Sound, or whether it should occur in pathogen-free laboratory-style settings. For many reasons it seems that rearing eggs, larvae and juveniles in a natural environment seems preferable. Larvae and juveniles would be exposed to disease and probably many would succumb to the disease. The survivors, however, might be those who have some resistance to the disease or have acquired some degree of immunity. The alternative is the rearing of many juveniles in a disease-free environment, perhaps for a period of six months, and then releasing these naïve fish to a disease-ridden environment. Based on the laboratory results described in many of the papers on disease (listed above) where naïve, laboratory reared herring juveniles are exposed to disease and then experience catastrophic mortality, it seems preferable to risk disease exposure as soon as possible during enhancement, with the hope that such early exposure to disease would preclude a later, and potentially devastating mortality loss by disease, following release.

The impact of disease on any proposed herring enhancement may depend on the timing of exposure and perhaps the duration of confinement. Based on the work to date it seems preferable to use natural rearing environments with possible early exposure of larvae or juveniles to pathogens. This is only a tentative conclusion, however, and if enhancement proceeds, it may

be useful to have a group of disease experts prepare specific protocols on the risks and impacts of disease at different herring life history stages, within enhancement facilities.

10 Issues of scale: size of a herring enhancement project

How many fish, produced through enhancement, would be required to make a significant difference in Prince William Sound spawning biomass? Any answer is speculative but Fig. 1b (from Moffit 2005) shows that recruitment in recent years has been about 200,000,000 (two hundred million) fish in several of the years since 1994, and usually lower. **Therefore, for a starting objective, an estimate of 20,000,000 (twenty million) additional herring recruits would seem like a reasonable objective – this number would be only about 10% of present recruitment levels which are considered to be low.**

10.1 How many eggs are required for enhancement?

The quantitative estimates in the following text are meant only to be illustrative and not definitive. Prior to any enhancement activity there must be an estimate of the required number of wild eggs that must be extracted from the natural environment. It is highly probable that this number will be very large, perhaps unacceptably large, if the starting number must withstand very high mortality in the cultured eggs, larvae or juveniles. Based on the Japanese experience, however, total survival rates may be as high as 30%, from eggs to young juveniles. Better estimates of mortality during mass rearing of Prince William Sound herring would require pilot-scale experiments. For the present, however, some approximations may be made based on existing information.

From Fig. 1b, we see that the approximate mean recruitment in recent years is about 200 million fish. An additional 20,000,000 (twenty million) fish produced through enhancement would provide a ten percent increase in recruitment. The estimation of the number of eggs required to produce twenty million recruits would be simple – ***if*** mortality between the egg stage and recruit stage were low or minimal. This is explained in the following section (10.1.1). The estimation of the numbers of required eggs, when mortality is considered, is much more difficult. Making such an estimate requires understanding of mortality at each life-history stage, from egg to larva, larva to juvenile, and from juvenile to new recruit, at age 3 (or 36 months).

10.1.1 Relative fecundity: the number and weight of spawning fish required to produce twenty million recruits, assuming no mortality

One metric ton of spawning herring produces about 100,000,000 eggs (10^8/mt). This estimate is based on the observation that the mean relative fecundity of herring females, throughout most of their range from California to the Gulf of Alaska, is about 200 eggs/g (Hay 1985). This estimate of relative fecundity tends to hold over a broad range of sizes, from the smallest newly recruiting females to the larger, older females. Larger females have relatively larger ovaries (often about 30% of their total weight at spawning) whereas smaller females tend to have relatively smaller

ovaries (often about 20% of the total weight at spawning). However, egg size also varies: larger females tend to have larger eggs and vice versa. Therefore the estimate of relative fecundity, of about 200 eggs/g is robust (probably accurate within ±10%) and useful for the calculations used here.

Herring populations have a nearly exact 50:50 sex ratio, and the age-specific weights of the sexes are approximately similar. Therefore the estimate of relative fecundity for female Pacific herring, of 200 eggs/g, can be adjusted to reflect the egg production of the total population (both sexes) and is about 100 eggs/g. Because this estimate is about 100, some readers may incorrectly assume that this estimate is only a rough approximation, say within an 'order of magnitude'. Actually, this estimate is much better than that and probably as accurate as the estimate of relative fecundity (explained above) and accurate within about 10%. Therefore the range of estimates of egg production for Prince William Sound herring probably varies between 90-110 eggs/g of spawning fish (both sexes included).

With perfect survival and assuming one gram of spawning fish produces 100 eggs (10^2 eggs), it follows that one kg of spawning herring produces 100,000 (10^5 eggs) and one metric ton (mt) produces 100,000,000 (10^8 eggs). Twenty million eggs (2×10^6) would require 200 kg of spawning fish (i.e., $20 \times 10^6/10^5$ egg/kg) or 0.2 mt.

Ardent proponents of enhancement may be encouraged by the estimate of 200 kg of spawning fish as a requirement for producing 20 million recruits. This estimate is unrealistic however because it is obvious that mortality at all early life stages cannot be ignored. However the estimation of mortality, over the three year period, between fertilization and recruitment, is not simple, as shown is the next section.

10.1.2 Stage-specific survival

For the purposes of estimating stage-specific mortality (or survival) in the following analyses, nine life-history stages are distinguished, of which five are adapted from the classification used by Norcross and Brown (2001). The survival model used by Norcross and Brown (2001) assumes that the survival to any specific stage is simply the product of the survival of previous stages. For instance, if **S** represents survival, then survival to age 1 would be:

$$\mathbf{S_{age\text{-}1} = (S_{egg})(S_{larva})(S_{fall\ juvenile})(S_{winter\ juvenile})}$$

The following analysis assumes that the age (and duration) of each stage is as follows:
(1) unfertilized eggs (age 0 days)
(2) fertilized eggs (age 0 days, duration 0.001 days)
(3) the egg or embryonic (pre-hatch) period (age 0-20 days)
(4) hatch and post-hatch period (age 21-30 days)
(5) the larval drift stage (age 31-179 days)
(6) fall-juveniles (up to 180 days of age)
(7) winter juveniles (between 181 and 365 days)
(8) age 2 juveniles (age 366 to 730 days)
(9) age 3 recruits (age 731-1095 days)

The ages and durations of these stages are approximate. Probably most investigators familiar with herring biology could argue that there are other stage classifications that are preferable, and they may be correct. However the list used here is designed to expand on the information and extend the survival model provided by Norcross and Brown (2001). This list extends the 'post-hatch' stage to a slightly longer period, to 10 days, which may be slightly longer than that implied by Norcross and Brown (2001). Also, some additional stages are added and the rational for each stage is discussed briefly.

10.1.2.1 The unfertilized and unfertilized egg stages

The distinction between the 'unfertilized egg' and 'fertilized' egg stages provides a simple way of estimating fertilization success (at about 99% successful). Some may argue that this estimate is too high, but it matches what I have observed in nature – but not in laboratories. Fertilization rates in laboratories are often much lower, especially when artificial substrates are used for eggs and when eggs have been surgically removed from females. In any event, recognizing this as a distinct stage allows for clarity of this estimation and assumption. Minor changes in the assumptions about the rates of fertilization have little impact on estimates of overall survival.

10.1.2.2 Egg stage

Egg survival in nature, from fertilization to hatching, appears to be affected by a combination of biotic and abiotic factors. Survival estimates from the literature vary widely but several sources report measured survival rates of about 50% (Haegele 1993; Haegele and Schweigert 1989, 1991; Rooper et al. 1998, 1999). In Prince William Sound, Norcross and Brown (2001) indicate a survival range between 24% and 45%. Factors affecting survival include predation and weather, with mortality associated with storm action and dehydration. The duration of this stage in Prince William Sound is about 20 days.

10.1.2.3 Hatch and post-hatch period

This is not necessarily a distinct stage, but it is described by Norcross and Brown (2001) as a period when some abnormalities can be detected in live larvae. It also is a period when larvae exhaust their yolk sac (about 5 days post-hatching) and begin to feed (5-10 days post-hatching). Brown and Norcross estimate survival to be between 50% and 100% during this period. Probably they assumed that this stage was shorter than 10 days, because these are high estimates of survival at this stage. Regardless, for the purposes of the estimates in this report, the minimum survival is assumed to be 50% and maximum survival is 100% during this period.

10.1.2.4 Larval drift stage

This is a period when herring larvae feed voraciously and grow rapidly. It also is a period of intense mortality and may reach 10% per day (Arai and Hay 1982, and others) – so after a period of about 40 days, total survival would reduce the initial number to

about 1% of the starting number. This estimate is based on research in other areas, but such low survival rates have been observed in many marine species. Therefore natural herring larval populations may decline by about 99% during this time. This is consistent with the Norcross and Brown (2001) estimates of 1% (minimum) and 7% (maximum) survival during the larval drift stage.

10.1.2.5 Fall juvenile stage

Post metamorphic survival to the juvenile stage in nature is presumably mainly determined by predation, although predation rates could be impacted by disease and food availability. Once the fish have reached the juvenile stage, approximately beginning in the early and middle months of their first summer, then feeding becomes important to allow them to survive through their first winter when availability is limited (Norcross et al. 2001). Norcross and Brown show survival rates between 2% and 21% during this period, although the duration of the period they use is not explicitly described. In the present paper this stage is assumed to be relatively long (120 days) so relatively low survival rates would be expected.

10.1.2.6 Winter juvenile stage

Norcross and Brown (2001) show a range of site-specific rates for this stage, which is assumed here to occur approximately for six months, from about November to April (although Norcross et al. consider this stage to occur between October and March). They report total survival ranges from 5% to 99% during this stage, but these are the extremes for sub-sections (individual bays) within Prince William Sound. The high and low annual extremes for the data aggregated among the different areas would raise the minimum and lower the maximum estimates of survival during this period. For the purposes of this report I estimated the maximum average survival simply as the approximate average of the ranges of the annual estimates for the years reported: the 1995-1996 range is 39-86%, the 1996-1997 range is 18-86%, and the 1997-1998 range is 39-64%. The annual means from these estimates are 62%, 52% and 50% respectively. Therefore a minimum of 50% and a maximum of 62% survival during this period may provide useful annual summaries of these data, although a minimum survival rate of 50% during this period seems high – even if it is based roughly on the available data. Better estimates might be made from the available data if the survival estimates were weighted approximately by the relative numbers of juveniles occurring in each of the areas examined.

10.1.2.7 Age 2 and age 3 stages (older juvenile and pre-recruit years)

These stages are not well understood. Based on observations in the Strait of Georgia (BC) most juveniles migrate out of the area during the second summer, approximately between the ages of 12-18 months, although in some of the most remote bays and inlets, it seems that some herring are non-migratory and resident in the same general areas throughout the year. It is not clear if there is a similar mix of migratory and non-migratory herring in Prince William Sound. Further, it is not clear whether some areas of Prince William Sound might be more likely to retain non-migratory herring. In any event, the survival of these stages is poorly understood in Prince William Sound and all

other Pacific herring populations. For the purposes of this report I assumed that a maximum survival rate might be about 50% in each year of these two stages. A minimum estimate of 5% (one-tenth of the maximum survival rate) was arbitrarily chosen – although it is the same as the minimum survival rate for the earlier juvenile stages reported by Norcross and Brown (2001).

10.1.3 Estimates of survival from egg to recruit

The stage-specific survival rates for each of the eight stages described above is shown in Table 3. Table 3 also shows an estimate of the approximate weight of individuals at the conclusion of each stage. Following the model of Norcross and Brown (2001), the survival at each stage is the product of the minimum and maximum survival rates of the previous stages. A problem with this approach, however, is that it is unlikely that specific cohorts would encounter conditions leading to either consistently low or consistently high rates as they develop through each stage. A more realistic estimate might be better represented by the mean or median estimates. Therefore Table 3 shows the mean mortality as the midpoint between the minimal and maximal rate for each stage.

The estimated numbers and biomass of each stage is shown in Table 4. A starting number of one hundred million eggs (10^8) is used – as a proxy for the approximate egg production of one mt of spawning fish. The estimates of the minimum and maximum number of survivors at the end of the winter juvenile stage is nearly identical with those presented by Norcross and Brown (2001). (Note that Norcross and Brown began their calculations with ten million (10^6) instead of the one hundred million used in Table 4 of this paper – so the estimated numbers-at-stage here are 10 times greater than they show.

Table 4 also shows an average estimate of stage-specific survival, that might be more realistic than either the products of the consecutive minimum estimates for each stage or consecutive maximum for each stage. Table 4 shows an estimate of about 117,000 survivors at the end of the fall juvenile stage, and about 65,000 at the end of the first winter. As a very rough approximation, these calculations indicate that about 1% survive during the first year of life.

10.1.4 Estimation of pre-release survival rates in an enhancement project

Table 5 shows the same stage-specific stages as Table 3, but also shows the estimated survival rates that might be encountered in an enhancement project. For each stage a minimum and maximum estimate are shown. It must be understood that the estimate of survival used in these calculations (50% at each major stage) is little more than a guess. *These survival estimates are much higher than those* occurring in natural populations (see Table 3) but the cumulative survival is lower than the estimates reported in Japanese herring enhancement research (estimates at about 50% in the Lake Furen project – see Section 8.1). With the assumption of a starting number of one hundred million eggs (10^8), the minimal estimates of survival to the end of the fall juvenile stage was about 6.12 million (or also about 6% survival) and the maximal maximum estimates of survival was 57.18 million (or about 57% survival).
The end of the fall juvenile stage probably is a reasonable time to consider release of enhanced juveniles.

10.1.5 Estimation of post-release survival rates in an enhancement project

Table 6 shows the estimated numbers and survival of herring reared in an enhancement project and released at approximately 6 months (180 days) of age. The range of pre-release survival estimates up to this stage was estimated to be between 6% and 57% corresponding to about 6 million and 57 million survivors based on a starting number of one hundred million eggs. Once released these herring juveniles could encounter survival rates applicable to wild herring. These are shown in Table 6 as estimates of the minimum, maximum and average probability (p) of survival for three stages (winter juveniles, age 2 and age 3). Like some of the previous estimates, these estimates are only 'guesses' about probable survival rates. ***In general, survival rates of adult (post-recruit) herring are usually greater than 50% per year so an estimate of a maximal survival of 50% and a minimal survival of 5% (used in Table 6) may be reasonable, and perhaps even conservative.*** Such estimates however, assume that total annual survival is not affected by intense fishing or other forms of mortality. Table 6 also shows an 'average' estimate of survival, which is simply the arithmetic mean of the high and low estimates.

10.1.6 The numbers of eggs required to produce 20 million recruits

The three survival scenarios in Table 6 (best case, worst case and average case) show that, of a starting number of 100 million eggs, the worst, best, and average case scenarios would yield a total survival of 733 million, 8.8 million and 1.3 million fish respectively.

Probably, for the purposes of approximate estimation of survival of enhanced fish to the age 3 recruit stage, an estimate of about 1% survival of starting eggs may be reasonable. Therefore production of 20 million recruits would require about 2 billion eggs (i.e., 100 times 20 million). ***Based on the estimated relative fecundity of 100 egg/g of spawning fish, production of 20 million age 3 recruits would require a starting number of about 2 billion eggs – or the egg production corresponding to a spawning biomass of 20 mt of herring.***

If such projected survival estimates (1% from the egg stat to the recruit stage of enhanced herring enhancement for the first six months of life) are reasonable, then the estimate of 20 mt of spawning herring may not be a formidable barrier to initiation of an enhancement project. For instance, this quantity of fish probably is less than many single purse catches – or the numbers used in commercial spawn-on-kelp operations.

10.1.7 Enhancement impacts on spawn deposition

In terms of the estimated area of spawning habitat lost from the use of 2 billion eggs, this area could be the equivalent of 20 km of shoreline spawn (assuming a mean density of 100,000 eggs/m^2 (a very light density) and a mean width of 1 m (very narrow width). If the mean spawn width were 10 m (a more realistic width estimate) then the equivalent shoreline distance of 2 km would be required. Using estimates of higher spawn densities of 1,000,000 eggs/m^2 (probably a relatively high but not uncommon egg density) then the shoreline distances corresponding to 2 billion eggs would be between 200 m and 2 km respectively - if the mean spawn widths were

1m and 10 m, respectively. Probably under most conditions of a spawn deposition, use of 2 billion eggs for enhancement would be about 400 m (i.e., mean density of about 500,000/m^2 and mean width of 10 m).

It is essential to stress that the estimates used in some of the preceding calculations are guesses and many are not based on real data. Some could be misleading or wrong and such error could have a major impact on the estimates of total survival used here. The assumption of a total survival of about 1% could be wrong by a factor of 10. Probably the distribution of error estimates is not symmetrical – so there would be a greater change of the real number being closer to 0.1% survival than 10% survival. Therefore it would be incorrect to assume that the estimates derived in this report are robust. Instead, they are merely intended as guides and should be subject to re-examination and revision if an enhancement project were initiated.

10.2 Implications of larviculture mortality for duration of herring enhancement

These preceding estimates are intended to be illustrative although hopefully the survival and mortality estimates are roughly realistic. The previous examples show that if enhancement is a realistic option, then the duration of larval and juvenile culture periods may be vital. A potentially important observation is the low survival rates associated with the larval drift stage that Norcross and Brown (2001) estimated to have a maximum stage-specific survival rate of 7%.

Ignoring, for the moment, the earlier comments about the futility of release of enhanced fish prior to periods of intense density-dependent mortality, it may be informative to consider short enhancement durations, say of about two months, corresponding to the approximate end of the larval stage (Table 3). How many eggs would be required to produce 20 million recruits if the release occurred at 60 days of age?

One way to approach this is to assume that the products of a month-long enhancement (i.e., feeding larvae) would, once released into the wild, have the same survival potential as wild-reared herring larvae. From Table 6 we see that the survival of enhanced herring, to the end of the 'post-hatch' stage, varies between 24%-89% with average mortality at 57%. Then if these surviving larvae, at an age of about 30 days were released to the wild and experienced the same mortality estimates as wild larvae at each stage, then the 'average' survival to age 3 would be 0.00019 (the products of the age-specific survival estimates for each stage subsequent to release). If so, how many herring would be required to produce 20 million age 3 recruits if the survival rate were 0.00019? Using the relative fecundity estimate of 10^8 eggs/mt, the answer is about 1.05×10^{11} - or roughly about 1000 mt. Clearly, in the present context of the herring issues in Prince William Sound, this would be an unacceptably large number of herring to commit to an enhancement project. This estimate of required eggs would decrease substantially if survival were assumed to be maximum throughout all life stages following release – but such an assumption is not warranted for the purposes of these calculations. ***The conclusion is that if an enhancement project were to proceed, the duration of culturing should exceed the first month of life of a herring.***

Maximal natural survival rates during the larval drift stage have been shown to be low at 7% (Norcross and Brown 2001) so it seems clear that this may be a life history stage where an enhancement project could provide higher survival estimates. This, and the next stage -'fall juveniles' - also may be a stage where some density-dependent process may affect survival. ***Therefore, it seems clea r that an enhancement project should avoid rele ase times prior to the 'winter-juvenile' stage.***

10.3 Other estimates of survival

Some readers may object to the way these estimates of mortality were estimated. Probably many valid objections could be raised. Perhaps a better way would be to ignore life history stages and estimate the probable survival directly between eggs to recruits. In general, we might make a reasonable guess about the number of eggs deposited (from spawn surveys). Age-structured stock assessment analysis can provide annual estimates of the numbers of recruits. For the purposes of this report however, where the intention is only to establish some approximate guidelines that might help focus pilot scale experiments, we might assume that the total recent egg production of the Prince William Sound spawning biomass (say about 20,000 mt or about 2 x 10^4 mt) multiplied by the approximate relative fecundity (10^8 eggs/mt of SSB, Hay 1985) would yield an estimate of total annual egg production of 2 x 10^{12} eggs. The approximate survival from egg to recruit, assuming an annual average recruitment of about 200 million fish (Fig. 1b) would be about 0.0001 ([2 x 10^8 age 3 recruits]/ [2 x 10^{12} eggs] reduces to about $1/10^4$) - or one recruit surviving from ten thousand eggs. If mortality were constant over time then the daily survival rate would be in excess of 99% per day – or a mortality rate less than 1% per day. Clearly this daily survival rate is much higher than some observed field estimates. For instance Arai and Hay (1982) calculated mortality to be about 10% per day for yolk sac larvae (daily 'survival' rates would be about 90%) so mortality must decrease in older, larger size groups. The implication for a possible herring enhancement project is that mortality is variable during early development (a fact widely known) so that stage-specific estimates of mortality, even rough estimates, are preferable to assumptions that mortality rates are constant during the period between egg incubation and recruitment.

It is a problem, however, to measure actual mortality rates – especially among juveniles. In the Strait of Georgia annual surveys of juveniles, made in September of each year, estimated juvenile density by surface area and volume. When extrapolated to the whole area of the Strait, the results always were under-estimated – the total estimated number of age 0+ juveniles was less than the numbers of age three fish recruiting to the adult populations. The reason for these low estimates in not clear but in part it is related to the widespread distribution of juveniles. They extend their range throughout all areas of the Strait of Georgia and even in Johnstone Strait, especially in tidally active areas where spawning does not occur.

10.4 Implications of the high survival rates seen in Japanese research

The Japanese experience suggests that high survival of cultured eggs, larvae and juveniles could be possible. They report annual survival rates of about 30%, from eggs to juveniles. If so, then the required number of eggs for enhancement probably would be relatively small. The estimated

number of eggs required to increase present recruitment levels by 10% (i.e., an additional 20,000,000 recruits) would require the total egg production of only 200 kg of spawning fish – assuming that mortality were zero between egg fertilization and recruitment. Assuming that the 30% survival estimates approaching the Japanese rates might be reached, then the total biomass of spawning fish required as parental stock would then require about 666 kg or 0.6 mt – which might be rounded off to about 1000 kg or 1 mt. Of course this estimate does not allow for post-release mortality between the juvenile and adult stage. Also, this is based on an improvement of only 10%. Higher expectations would require more donor eggs.

10.5 Juvenile survival – is this the vital question?

Some key biological issues related to enhancement concern the mortality during the juvenile periods and the factors affecting recruitment. This latter point has been investigated for more than 100 years – and although there has been progress there still is much uncertainty. This presents both a problem and a challenge for enhancement work. Clearly it would be comforting to see the Prince William Sound herring population resume its former levels of abundance – and that is the basis for the enhancement concept. As the viability of an enhancement project is investigated, perhaps through pilot-scale field investigations, supplemented with laboratory tests, and perhaps retrospective and field analyses of herring growth and survival, there may be other opportunities for valuable scientific by-products.

There is an excellent opportunity to examine some key factors that might affect Prince William Sound herring recruitment. A specific issue concerns the concept of 'self-recruitment' to sub-components of the Prince William Sound population. Are there separate spawning groups (populations?) in Prince William Sound as suggested by O'Connell et al. (1998)? If so do they contribute recruits to the total population, in proportion to their spawning biomass? Are there some areas that contribute more or fewer recruits than other areas? Is larval and juvenile survival the same in all areas? What is the (genetically) effective population size of Prince William Sound? Is it much smaller than the numbers of spawners, based on the assumption that all eggs have a roughly equal probability of survival? Genetic work on Prince William Sound herring by O'Connell et al. (1998) and work on other species by Hauser et al. (2002) indicate that effective population size may be surprisingly low, perhaps orders of magnitude smaller than the numbers of spawners. If so, what is the implication for enhancement work that will, of necessity, produce a lot of young fish from a small component of the gene pool? It would be wise to re-examine the genetic structure of Prince William Sound herring, especially in light of work by Kitada et al. (2000) that suggests previous estimates of effective population size may have been under-estimated.

11 Criteria for enhancement decisions in Prince William Sound

Walters and Martell (2004), in a dedicated chapter on generic 'marine enhancement programs', explain three aspects of enhancement: (1) critical steps in program design; (2) monitoring and experimental requirements; (3) things that can go wrong. Within each of these categories they discuss criteria that can be used to evaluate the efficacy of potential enhancement programs, such

as the one under consideration for herring in Prince William Sound. These three main topics are examined in detail at the conclusion of this report but perhaps the paramount issue concerned with Prince William Sound herring enhancement is the time of release of cultured organisms relative to herring life history stages when density-dependence mechanisms limit population size. If density-dependent mechanisms restrict population size at life history stages that develop AFTER the time of release, then it is clear that such releases will not enhance the population size – and it is possible that there could be a negative impact by displacing naturally-produced 'wild' fish or by altering the genetic structure of the population.

11.1 Critical steps in program design

Each of the main points that follow are taken or adapted from Chapter 12 (Marine Enhancement Programs) from Walters and Martell (2004). Beneath each point is a '***Comment***' that attempts to interpret the existing situation, or information, relative to the potential for herring enhancement in Prince William Sound.

11.1.1 Make management priorities and trade-offs clear and acceptable

Comment: *Has there been a serious evaluation of possible resource trade-offs?* The cause of the herring collapse is not clear, nor is the explanation for the continued high incidence of disease. Probably the closures of the sac-roe fishery are evidence that conservation of the herring stock is a paramount concern for the management of herring. It is less clear if hard management decisions will follow if it became clear that part of the problem with the low herring abundance was related to fisheries programs for other species, such as the large pink-salmon hatchery system. This critical step asks 'what if the Prince William Sound herring stock cannot co-exist at high levels of abundance with other stocks'? Perhaps the population has now adjusted to a new ecological regime related to other fisheries or other anthropogenic factors. Maybe there is another, more fundamental explanation related to predator pits (i.e., Bakun and Weeks 2006).

Another management priority that needs clarification is the duration of herring enhancement, especially if it proves successful. Will managers be satisfied to cease enhancement activity if and when herring abundance increases?

11.1.2 Demonstrate recruitment overfishing or unsuccessfully rearing in the wild

Ensure stock assessments to show that the target stock is recruitment overfished or can no longer successfully rear in the wild.

Comment: *This step is fully met.* Annual stock assessments are done annually. There is no fishery, so there is no concern with recruitment-overfishing, unless herring are taken in significant quantities and bycatch (or killed by collateral damage) in other fisheries. This seems unlikely.

11.1.3 Show that enhanced fish can successfully recruit in the wild

Comment: *This has been shown by Japanese work.* It is highly probable that this step will be fully met.

11.1.4 Show that total abundance is increased by the enhancement contribution

Comment: *This step has NOT yet been shown by Japanese work.* Although the enhancement methods used in Prince William Sound may resemble those used in Japan, the objectives are not necessarily the same. The best way to meet this objective is to extend the culture time as long as necessary to reduce, or eliminate, density-dependent competition with wild juveniles.

11.1.5 Prevent continued overfishing

Ensure that fishery regulations are adequate to prevent continued overfishing of the wild population (unless there has been a policy decision to 'write-off' the wild population.

Comment: *This step is not applicable at the present time.* The fishery is closed. This step is only relevant if and when the stock 'recovered' to a level that supported a fishery. If that happened however, presumably the enhancement efforts would cease. If they did not end, but continued, then management rationale for enhancement would have changed – from a 'conservation and restoration' exercise to a 'production' exercise.

11.1.6 Show that the hatchery production system is sustainable over time, if it is to be permanent

Comment: *This step is not applicable at the present time.* The fishery is closed so enhancement is being considered for purposes of restoration, not production.

11.2 Monitoring and experimental requirements

Comment: Two key monitoring requirements exist. The first is to conduct broad marking programs to assess the survival of enhanced and wild herring. Probably the Japanese ALC marking procedure may be the most reasonable approach.

The other basic monitoring requirement is ongoing genetic analyses to ensure that the possible addition of recruits, from relatively few spawners, does not compromise the genetic integrity of Prince William Sound herring. In the case of Prince William Sound, there may be some uncertainty about the effective population size, as determined from microsatellite DNA analyses (O'Connell et al. 1998).

11.3 Things that can go wrong

11.3.1 Failure to produce fish that successfully recruit to the spawning population

***Comment*:** Japanese work indicates that cultured herring can compete and spawn. It is essential, however, to have a marking system for released fish. The Japanese work should provide good protocols for this. Also, this field is developing rapidly (see review by Niva et al. 2005).

11.3.2 Direct exploitation of wild fish to provide hatchery seed stock

***Comment*:** This is a real, but relatively small concern with the assumption that, following Japanese practices, there can be relatively good survival from hatching to the juvenile stage.

11.3.3 Post-release competition between hatchery and remaining juvenile fish

***Comment*:** This may be the most pressing concern. Monitoring and research should attempt to determine the optimal release time. Based on the information in this report, later releases of larger juveniles may reduce possible competition for scarce food resources in the late fall and early winter.

11.3.4 Increase in predation and disease risk for remaining wild fish

***Comment*:** This is a major concern, given the present high incidence of disease in Prince William Sound herring. It is especially troubling that the viral disease (VHS) tends to break out in crowed conditions.

11.3.5 Selection under enhancement conditions for traits that are inappropriate

***Comment*:** This is only a concern if enhancement activities had a long duration.

11.3.6 Attraction of fishing effort by unregulated fisheries

***Comment*:** Probably this is not an issue.

12 Facilities, Operations, Research and Costs

12.1 Facilities

It is obvious that the cost would vary with the size and scope of any enhancement operation. Probably equally important would be the location(s) of operations. Remote locations without convenient access to support facilities would cost more.

12.1.1 Salmon hatchery costs as a model?

Although one of the key recommendations in this report is for a cautious, modest start (if there is to be a start), in some ways it is simpler to estimate the cost of a full-blown enhancement project that would raise 20 million, or more, juveniles annually. It seems probable that the total costs may be roughly similar to the total costs of similar hatchery production of pink salmon in Prince William Sound that releases over 600 million pink salmon fry at a weight of about 0.5 g each (Cross et al. 2005). Probably the weights of released herring juveniles would be roughly similar, but perhaps lower. Estimates from Table 6 (and see text in Section 10.1.5) indicate an average post-release survival of about 5% (based on the 'average' estimate). If so, the required number of released juveniles (at age of 6 months) would be twenty times greater than the expected number of recruits (at age 3 years). Therefore production of 20 million age 3 recruits could require release of a number twenty times greater – or 400 million. If so, this would require that herring enhancement operations might be on approximately the same operational scale as the Prince William Sound pink salmon hatcheries.

Although pink salmon and herring rearing facilities are different (fresh water versus marine, etc.), a comparison of the costs of potential herring hatcheries probably would be roughly similar (say within an order of magnitude) to salmon hatcheries. Both types of operations require some expensive capital investment. Both would require periods of labor-intensive work and therefore need a combination of seasonal and full-time staff. Both would require significant expenditure for food. Both require pre- and post-release monitoring. The major difference with herring operations (at the initial stages) is that there would be a greater proportion of time spent on research to establish protocols for egg collection, larval food preparation and early feeding, disease monitoring, juvenile feeding, and *especially* experimental mass marking. Unfortunately, simple estimates of the costs of salmon hatcheries were not available as a potential guide to the total costs. Also, it is one thing to estimate annual operating costs and another to factor in the initial capital costs of facilities. For the purposes of estimating the costs of herring enhancement, both capital and operational costs must be considered. Nevertheless the total costs, if pro-rated over several years, would be many millions of dollars, perhaps tens of millions.

12.2 Pilot scale cost estimation

Costs of pilot scale operations would depend greatly on the approach but would be considerably less than full-scale implementation. As mentioned above, if the decision were to rear herring in pathogen-free facilities, in laboratory-like settings, then costs may be substantial. Even rearing a

few million juveniles would require extensive facilities. On the other hand, rearing eggs, larvae and juveniles in natural or semi-natural settings could be considerably less expensive.

12.2.1 Egg acquisition

The costs of collecting eggs on real or artificial substrate would be relatively modest. Assuming that this process would require charter of vessels and staff for a period of about 6-8 weeks (this length of time is required for preparation and set-up, etc.), the costs probably would be in the tens of thousands of dollars, but probably less than one hundred thousand dollars.

12.2.2 Larval rearing and tanks, cages or mesocosms

The costs of larval rearing would depend greatly on the site. If it were possible to establish large semi-natural mesocosms, perhaps similar to that used in Flödevigen, Norway, then the costs of expensive tank facilities might be avoided. Alternatively, perhaps there is somewhere in Prince William Sound where some portion of the shoreline, such as a small bay or inlet, might be sequestered for herring enhancement. This would require some form of screening to reduce or eliminate key larval herring predators such as jellyfish, arrow worms (chaetognaths), some predatory zooplankton and juvenile fishes. Such a location also would need to be amenable to food supplementation, with artificially reared rotifers and/or *Atremia* nauplii. Yet another possibility would be lagoon-type locations, even with lower salinity water, similar to some of the Japanese facilities – where the larval and juvenile herring are reared in brackish lagoons (which they refer to as 'lakes'). A possible advantage of such lower-salinity areas may be a reduction in marine predatory fish that avoid low-salinity areas. The early life history stages of herring, however, usually are very tolerant of lower salinities, to 15 ppt (or lower.

12.2.3 Juvenile density – in nature and in enhancement facilities

If suitable semi-natural mesocosm facilities could be located, this might result in considerable cost reduction. Alternatively the costs of tank facilities could be substantial. The key question is how much area of volume would be required? (The following attempt to address this is very speculative and perhaps there may be better ways).

Suppose the initial number of herring juveniles in Prince William Sound is 100 times greater than the approximate number of average recruits. If the annual number of juveniles is about 200 million or 2×10^8 (see Fig. 1b) then this estimate would be 20 billion - or 2×10^{10}. This would require 99% mortality between the youngest juvenile stage and the age of recruits, about 2 years and 10 months later. This estimate of young (age ~2 months) juveniles is about 100 times less than the number of eggs (2×10^{12}) deposited by a spawning stock biomass of 20,000 metric tons (i.e., 2×10^4 mt with a relative fecundity of 10^8eggs/mt = 10^{12} eggs). So perhaps this estimate is roughly realistic. Now suppose these 20 billion juveniles are confined mainly to the nearshore water of Prince William Sound, between the inter-tidal zone and a depth of 10 m. From Table 2, the estimated area of such water is 709 km^2 or about 7×10^8 m^2. If the average depth were 5 m, then the total volume of this shallow 'juvenile' habitat would be about 35×10^8 m^3 or (3.5×10^9 m^3). Therefore the average volume of water per juvenile would

be estimated as the total volume of the habitat (3.5×10^9 m^3) divided by the numbers of juveniles (2×10^{10}). The result is 0.175 m^3 per juvenile, or 175 liters per juvenile.

Juvenile herring begin to school at a young age and it is obvious that they do not occupy all potential areas of available habitat. Therefore their density in nature must be considerably greater. For the purposes of estimating the required volume of containers used for enhancement, we might begin with an assumption that each juvenile requires between 1 and 10 liters each. Then, if the starting number of juveniles in a pilot-scale facility were one million (10^6), the required volume would be between 10^6 and 10^7 liters – or 1000-10,000 cubic m (10^3 -10^4 m^3). At a minimum this would correspond to a cubic tank with dimensions of 10 m on each side (depth x length x width). More realistically it could be a large container (or the cumulative volume of many containers) with a depth of one m, that would extend 30 m (i.e., about 100 feet) for both length and width. This would be a substantial volume of water, roughly equivalent to a large swimming pool. Based on the calculations above, which are acknowledged to be crude, such a pool would provide the minimum volume for one million juveniles. Such tanks or containers would be expensive. As a first approach, some modification of cages used in modern fish-farms might be used, if they could be lined with fine-mesh screen material. If so, the costs of a single facility would probably be in the 'tens of thousands' of dollars or perhaps $100,000 or more, but this is a guess. If such a single cage/container provided a minimum rearing volume, then it would take ten such cages to provide a rearing volume, assuming 10 liters per juvenile, to reach the 'maximum' volume estimated above. Therefore, for a pilot-scale facility rearing one million juveniles, it may take the equivalent of between one and ten specialized netpens, each of which may cost $100,000 or more. A full scale enhancement project may require many of these. It is vital, however, to appreciate that these are very rough estimates. Their main purpose may serve only as a guide to developing more accurate estimates.

12.2.4 Food costs

Aside from the cost of rearing facilities, there also would be substantial food costs. The costs of food may be estimated, approximately by determining the total weight of juveniles reared prior to release, and assuming a conservative conversion efficiency. Suppose, for instance, that in a pilot-scale facility, one million juveniles were reared to a weight of about 0.5 g. Then the total fish weight would be 5 million g (or 5000 kg or 5 mt). If the conversion efficiency (adjusting for the loss of uneaten food) was 10%, then it may require about 50 tons of food to raise one million juveniles. The approximate cost of pellet food used for salmonids is about $2000/ton (Chris Beattie, Product Manager, Skretting Canada, pers. comm.). Therefore the cost of feeding one million juveniles to a release weight of 0.5 g would be about $100,000. This estimate could be lower by a factor of two (or more) depending on the size or time of release and the actual conversion efficiency. Therefore if a minimum estimate were about 2 mt, then total feed costs would be about $50,000-$100,000 for one million enhanced juveniles, and ten times greater (or much more) than that of a full-scale project raising hundreds of millions of juveniles.

12.2.5 Fishing – to recover marked fish

If the present fishery is closed, how will sufficient recruit fish be captured to allow for monitoring of marked fish? Presumably, some fishing operations would be required to do this. If so, perhaps the sale of captured fish could be a source of revenue to offset the costs of the experimental culture. If not, then the cost of a dedicated vessel charter and other expenses would probably require several hundred thousand dollars per year.

12.2.6 Staff

Probably a staff of 5-10 would be sufficient for a pilot scale facility, but this would vary with the location and types of facility. Probably salary and benefits would cost $500,000-$1,000,000 per year.

12.2.7 Mass-marking research

A key issue is marking and mark recovery. Probably research grants could be used to start this and preliminary work could begin with modest funding (< $100,000) but soon would require substantially more, perhaps $500,000 or more. Full technological development of mass marking procedures, which is essential, might be very expensive. Therefore a complementary research program, at the initial stages of a pilot-scale project, could require $100,000-$500,000.

12.2.8 Strategic planning, cooperative and collaborative research

Prior to start up of any field activity, it would be essential to develop robust strategic and research plans that, for instance, could investigate different options for various facilities. A specific requirement may be development of cooperative or collaborative relationships with Japanese agencies and researchers. This would require both travel and hospitality budgets, although activities such as reciprocal trips to Japan to investigate methodology, etc., might be required to produce deliverables in the form of informative methodological reports. Probably any serious attempt at enhancement, even at the pilot scale, would require substantial funding for several major projects a year. An allocation of at least $400,000-$500,000 per year, during the early years of the project, seems essential.

12.2.9 Discretionary funding

Aside from the anticipated costs (facilities, egg acquisition, food, staff, etc) there could be substantial unpredictable costs that require discretionary funding. Probably this should be at least 10-20% of the total allocation.

12.3 Estimate of total costs for pilot-scale project

The breakdown of costs is as follows (with numbers representing dollars, in thousands):

egg acquisition	$50	-	$100
rearing facilities	$100	-	$500
food	$50	-	$100
fishing	$50	-	$100
staff	$500	-	$1000

strategic planning, research	$400	-	$500
discretionary	$200	-	$400
Total	$1350	-	$2700

Therefore how much would a pilot-scale project cost? Based on the speculation above it might be approximately between $1.3 and $3.0 million dollars. This cost would be required to initiate what might be one of the largest marine enhancement projects of its kind and certainly larger than anything previously attempted for herring. Two important caveats about these cost estimates are as follows. (1) The estimates are based on very preliminary and incomplete information. They require considerable refinement, and perhaps correction, prior to the initiation of any enhancement activity. (2) The cost of a pilot scale project, raising about one million juveniles, would not be 10% of a larger, full-scale program to rear and release, for instance, 20 million juveniles. There are economies of scale that would be considerable so a project that would be tens times the size of the pilot scale work would not require that research effort, although monitoring work would increase.

13 Summary and Recommendations

Prior to initiation of any enhancement activity there should be review of the existing circumstances to ensure that enhancement is warranted and that it is the only way to proceed. Based on the guidelines presented by Walters and Martell (2004) this could be done by a distinct, separate project that evaluates the condition of the present stock, and the methods used to evaluate the present stock. This could include an external review of assessment procedures and key biological aspects used in the assessments.

Enhancement activity, if it were to proceed, should begin slowly, with pilot scale activity. As much as possible the work should try to be developed so that results will have multiple benefits. For instance, Japanese work on enhancement, while still of questionable value as a means of improving recruitment, has made some valuable contributions to the understanding of herring biology, particularly homing and migration.

The time of release is a critical aspect of any marine fish enhancement work. There is general agreement that it is futile to release enhanced fish at a size or age that is still subject to density-dependent effects. This can happen if their survival is determined by the carrying capacity of the habitat they require when released. The probable implication for Prince William Sound herring is that enhancement would be required to maintain herring until the end of their first summer or growing season. In this way, they would not compete with naturally reared herring.

The scale of enhancement operations will depend on survival rates between the time of fertilization and the time of release, which could be at an age of about 6 months, or possibly longer. Based on survival and mortality estimates from the scientific literature, the numbers of eggs required to produce enough juveniles to impact recruitment could be formidable, but this would depend on the scale of operations and the duration of the enhancement program. The most troubling scenarios could require the equivalent of many tons of spawning herring and require significant quantities of eggs, so there could be deleterious impacts to natural spawning areas as eggs were collected and

transferred. On the other hand, the very high survival rates seen in Japanese work, if emulated in Prince William Sound, would require only modest use of naturally deposited eggs or spawners.

The duration of captivity, between the time of first feeding and release, is a key issue requiring further investigation. There is a trade-off between rearing large numbers for a short duration (early release) versus rearing of smaller numbers for a longer duration.

The 'large number, short duration' option could:

(i) have adverse impact on natural spawn during the collection of herring spawn,
(ii) require large amounts of larval food such as rotifers and *Artemia*,
(iii) encounter higher post-release mortality through predation,
(iv) present high risk of competition for food with natural herring,
(v) be less expensive in terms of required rearing facilities and food,
(vi) be technologically easier,
(vii) have a lower probability of success.

The 'small number, long duration' option could:

(i) have small impacts on donor sites during spawn collection,
(ii) require moderate amounts of food for larvae but significantly more food for juveniles,
(iii) encounter lower post-release mortality through predation,
(iv) present low risk of competition for food with natural herring,
(v) be more expensive in terms of staff and facilities,
(vi) be more technologically challenging,
(vii) have a higher probability of success.

The technological requirements for enhancement are best determined through pilot scale experiments. A large challenge would be the housing and feeding of millions (or billions) of juvenile herring. Some rearing in mesocosms could be tried. The Norwegian experience with large semi-natural mesocosms might provide examples of useful prototypes.

Supplemental feeding with a food source like *Artemia* will be required. Mass rearing of *Artemia*, and other potential fish foods, is both an art and a science. It will require time to set up facilities and have technicians learn the procedures.

Mass marking programs are an essential part of enhancement. It appears that the Japanese experience with ALC otolith marks is successful. Prince William Sound herring enhancement initiatives should build on the Japanese achievements.

Scientific cooperation and collaboration with Japanese agencies and researchers would provide valuable technical information for the initial stages of enhancement.

The role of disease in any enhancement activity in Prince William Sound is uncertain. Disease could be a serious problem. A basic decision will be required about the types of facilities used and the exposure of cultured fish to disease. The choice will be between: (i) rearing fish in pathogen-free facilities (if possible) before releasing them to the wild, (where they might experience severe disease-related mortality, or (ii) letting the larval and juvenile stages be exposed to disease, knowing that some mortality of enhanced fish will occur prior to release, but subsequent mortality in the wild may be lower. It seems preferable to have exposure to disease early in the life of herring, with the hope

that the survivors may acquire some resistance. In any event, this is a specific issue that requires more attention from disease experts.

A qualifier: a decision to 'investigate' enhancement is not a commitment to 'conduct' enhancement.

A decision to investigate the feasibility of enhancement does not necessarily mean that the EVOS Trustee Council is committed to the concept or determined to engage in enhancement activity. Instead, the intention is to examine the implications of the concept, as it applies to herring in Prince William Sound. Full scale enhancement activity would require several years of preparation, mainly to develop and determine some technological issues, such as mass marking of young fish prior to release. Mass marking and other technological activities are fundamental pre-requisites of enhancement activity. Therefore, because the development of these technological issues will take time, it is important that some investigations begin immediately. It also is important to understand that these investigations also could result in a definitive conclusion the enhancement of herring is impractical or far too expensive.

We suggest a sequential three-phase plan that could lead to full scale enhancement within five years. Each phase consists of several concurrent steps of complementary activities. Phase I will consist of three activities, each of which could resulting a conclusion that enhancement of herring is not warranted, because of technological or biological issues. Therefore we reiterate: the first components of a restoration plan are to determine the technological and logistical feasibility of the plan. These steps will not necessarily lead to enhancement activity.

Phases and activity of a herring restoration plan.

Herring restoration in PWS could proceed in three distinct consecutive phases, each of which has several distinct but concurrent activities or 'steps'. The three phases and suggested durations are: (1) Justification, decision rules and feasibility – one year; (2) Pilot scale enhancement and methodology tests – four years; (3) Full scale enhancement – initiated in five years. Each phase would have several steps or activities that could be conducted concurrently within the duration of each phase. The text below provides some background and

Phase one - step one: Justification, decision rules and feasibility

1. First step: develop decision rules and reference points.

It is certain that any restoration or enhancement will be very expensive and, at the onset, the results will be uncertain until shown otherwise. Critics and skeptics of enhancement will point out that the requirement for enhancement must be clear and demonstrable. Therefore it follows that there must be clear criteria (or decision rules) related to the abundance or condition of the Prince William Sound herring population that should be established prior to any enhancement activity. These decision rules could be developed in a dedicated report that could be used as a guide to enhancement activity, in much the same way that decision rules are used to manage a fishery. The criteria used to support decision rules would be related to some estimate of total herring abundance, although other demographic/ecological, such as specific cohort sizes, or sequences of weak cohorts. Also some spatial attributes could be incorporated when decisions rules are being developed.

Ideally, decision rules would be developed that would provide clear benchmarks for when enhancement activity might be initiated, or suspended or stopped. For instance, at an extreme, if the herring population trend population trend to decline were to continue, with extirpation anticipated to occur within a decade, then it would be clear that enhancement should be initiated. Likewise, if the PWS herring population reached some pre-determined level of the estimated virgin population biomass, either before or subsequent to enhancement activity, then enhancement would not be

warranted. Such a pre-determined level would presumably be low, well below the lowest point natural variation expected for the population over a sustained period. Therefore the decision rule could also incorporate trends in changes in absolute abundance and the temporal durations of such trends.

This step requires that the decision about whether enhancement should, or should not proceed be based on specific criteria about the PWS population. Specifically, what metric(s) will determine whether enhancement is warranted? If the metric is based on abundance, then biological criteria must be defined about how low the population must go *before* enhancement is implemented. For instance, must the population decline to 10 percent, or 5 percent, or 1 percent of the pre-crash (in 1993) levels of abundance? The criteria, however also could be related to annual patterns of recruitment. For instance, if two, three, or more years of poor recruitment occurred consecutively then this might also be considered as rationale for enhancement. Further, in some special instances, the spatial distribution might be considered.

A related issue is the metric when enhancement is no longer warranted, or when the population has increased to a level that natural reproduction and survival are sustainable. This also can be defined as a metric, say when the population abundance is within the range of normal variation, or when any increments related to enhancement activity are not effective.

Yet another metric could consider the worst scenario, when the population may be headed for extinction. In thus case *conservation hatcheries* may be warranted. In the event of such a dire situation the artificial propagation of herring might not really be an 'enhancement activity' but rather an essential conservation activity. Regardless, it would be useful to develop criteria that would define abundance levels that would define the situation when conservation actions wound be warranted.

The development of metrics that would be used to initiate suspend or halt enhancement activity for herring in Prince William Sound is a vital pre-requisite to any action. The formulation of these metrics will requires input from several sources, representing different perspectives on the present situation. Primary sources of input would be from the Alaska Department of Fish and Game that conduct the annual age-structured assessments. Additional input could come from the commercial fishing community, coastal communities, plus the academic and the biological consulting community that has worked on herring issues in PWS. The mechanisms for developing these decision rules and metrics need careful consideration but probably the most efficient way would be to have one person (or team), under contract, lead a committee to prepare a report that investigates and defines the metrics and decision rules. Following this report, a workshop discussing the metrics and decision rules may be appropriate.

Synopsis: Write and define a contract to prepare a report that: (i) presens data on the past and present state of Prince William Sound herring, with comments on the strengths and weaknesses of the information; (ii) defines criteria, such as abundance levels, that would be a basis for initiating enhancement activity and suspending or stopping such activity following favorable responses of the population; (iii) defines criteria where possible extinction is a concern and that would warrant implementation of 'conservation hatcheries.

Phase one – step two: assessment and development of mass marking technology

An essential requirement for initiation of enhancement activity would be a means for the evaluation of success or failure – or measuring the relative survival of enhancement fish compared to wild,

naturally produced herring. Such evaluation requires that enhanced fish can be identified. Compared to salmonids, identification of hatchery fish is a challenge for marine fish species such as herring that have many more, smaller eggs and that lack precise natal homing. In salmonid hatcheries, the verification of the survival is seen through the return of released fish back to the point of release – a phenomenon of natal homing through olfaction. Most marine fish do not appear to have the same capability to home with the same precision, perhaps mainly because the olfactory characteristics of their spawning habitats in marine spawning coastal areas are less distinct. Also, and perhaps more important, the residence time for the early life history stages of marine fish in their natal habitats is much shorter (days or weeks) than salmonids that live in freshwater spawning habitats and juvenile nursery areas for months or years, prior to open sea migrations. Therefore compared to salmonids herring have less time to imprint and because they are much smaller (by a factor of about one thousand times), herring larvae have a much less developed physiological and anatomical capabilities that might support imprinting capability. In any event, they do not home with the same geographic precision as salmonids so natal homing cannot be used as a mechanism to verify successful enhancement.

Mass marking of enhanced herring appears to be the only potential method for evaluating success of enhancement. Mainly this is related to marking of herring eggs or larvae in PWS. For certain potential restoration approaches, however, mass marking of age 0+ juveniles may also be a requirement. The work in this step would involve a combination of laboratory and field work, supported by detailed technical reports showing methods, data, results and conclusions.

Ideally this work should investigate several different marking options relative to potential screening methods. This might include investigation of the implications of otolith marking substances such a Alizarin, than can be detected with relatively simple, visual-based florescent screening using microscopic analyses of otoliths. Another promising approach would be marking otoliths with specific elements or isotopes and screening using laser mass-spectrometry. Accurate cost estimates for such marking must be developed to reflect different potential enhancement scenarios. At one extreme the potential enhancement scenarios range from rearing a relatively large number of eggs and larvae for short periods (< 2 months) prior to release. At the other extreme, a smaller number would be reared for reared for longer periods (~6 month).

Synopsis: Write and define a contract to prepare a report that will provide definitive approaches and/or methodology to mass marking. This report would include detailed review and analysis of the Japanese work and experience with mass marking of herring.
The report(s) should comment on the success rates for establishing marks and the costs related to different marking scenarios, at both ends of the process (marking and reading the marks at later stages).

Phase one – step three: Recapture and mark-detection methodology – a pre-application statistical guide concerned with issues of scale.

Mass marking of enhanced fish is an essential requirement to demonstrate the efficacy of any enhancement or restoration work. A complementary activity is determining the numbers of marked fish and recapture rates that must be made to demonstrate the capacity for survival of enhanced (marked) fish. When mass marking is considered for Prince William Sound, some key issues will be related to the numbers of marked fish that are released and the numbers that can be subsequently recaptured and screened. There may be significant costs related to the recapture and screening of marked fish. These costs will vary according to the numbers released, the estimated post-release survival and the efforts related to recapture. The costs of recapture and screening will be,

approximately, inversely related to the numbers of release. For instance if a relatively high proportion of the total population can be marked and released, the effort related to recovery and screening is reduced. If the number of marked and released herring is proportionally small relative to the numbers of wild fish, then the efforts related to recapture and screening could be substantial and impractical.

For instance, if the proportion of marked fish in the population is only 0.001 (one in a thousand) then estimating the survival rates of enhanced fish with require examination of hundreds of thousands, or millions of fish. Probably this is impractical. However with an average recruitment of about twenty million herring per year in PWS, having a mark frequency of one fish in a thousand will require that twenty thousand marked fish survive to age three. If the mark frequency were higher, say one per hundred, then over two hundred thousand would have to survive to age three. Even if the frequency of marked herring were one per hundred at age three, the screening effort to assess the survival of marked fish would be considerable, requiring examination of thousands of fish – just to get single-digit estimates of survival, with wide (i.e., unreliable) confidence limits. Similarly a frequency of one in ten herring surviving to age thee would require survival of two million fish to age three. However, it seems probably that the required screening to assess survival, if ten percent of the population were marked, would be possible.

In each of these simplistic three mark-rate scenarios (0.001, 0.01 and 0.1 mark frequencies) substantial post-release mortality would require that the actual number of marked herring be much greater than the actual number estimated to be alive at age three. Probably the numbers of marked herring, prior to release, would be much greater by a factor of ten or a hundred. For example, assuming a one percent survival for each mark-rate scenario (0.001, 0.01 and 0.1 mark frequencies) the release numbers would have to be: two million fish, twenty million and two hundred million herring – released after an initial rearing period. Two hundred million herring would represent the progeny of roughly about 2 tons of herring, based on the approximate relative fecundity of about 10^8 eggs per tons of spawning herring. Therefore acquisition of sufficient eggs is not a problem with rearing such a number because this is a relatively small amount relative to the total population, even at present low levels of abundance. Instead the main issue of concern would be the cost and effort related to rearing such a large number of young herring prior to release.

Synopsis. There is a need for a dedicated report that comments on the feasibility of marking and different mark-recapture rates. Some relatively simple modelling and statistical analyses should investigate the options and financial costs of several release-recapture scenarios and relate this to the cost of rearing herring, prior to release.

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Williams, E.H., and T.J. Quinn, II. 2000. Pacific herring *Clupea pallasi* recruitment in the Bering Sea and north-east Pacific Ocean, I: relationships among populations. Fisheries Oceanography 9: 285-299.

Williams, E.H., and T.J. Quinn, II. 2000. Pacific herring *Clupea pallasi* recruitment in the Bering Sea and north-east Pacific Ocean, II: relationships to environmental variables and among populations. Fisheries Oceanography 9:285-299.

Woodby, D., D.Carlile, S. Siddeek, F. Funk, J.H. Clark and L. Hulbert. 2005. Commercial fisheries of Alaska. Alaska Department of Fish and Game. Special Publication No. 05-09.

(a)

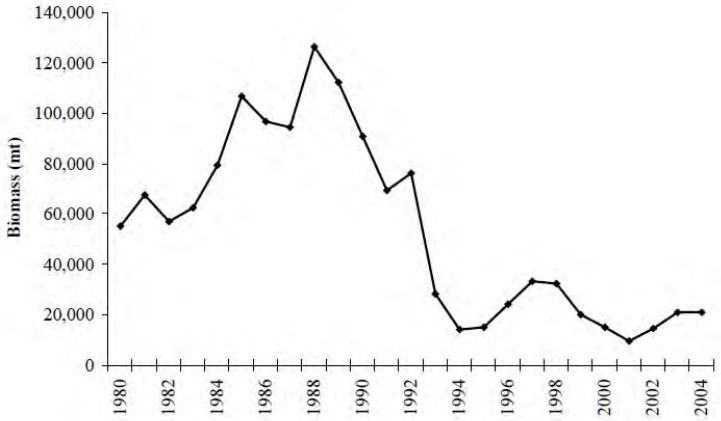


Figure 50. Prefishery run biomass (metric tons) of adult Pacific herring in Prince William Sound, 1980-2004. The biomass values are calculated from the age-structured model used to produce the 2005 projections.

(b)

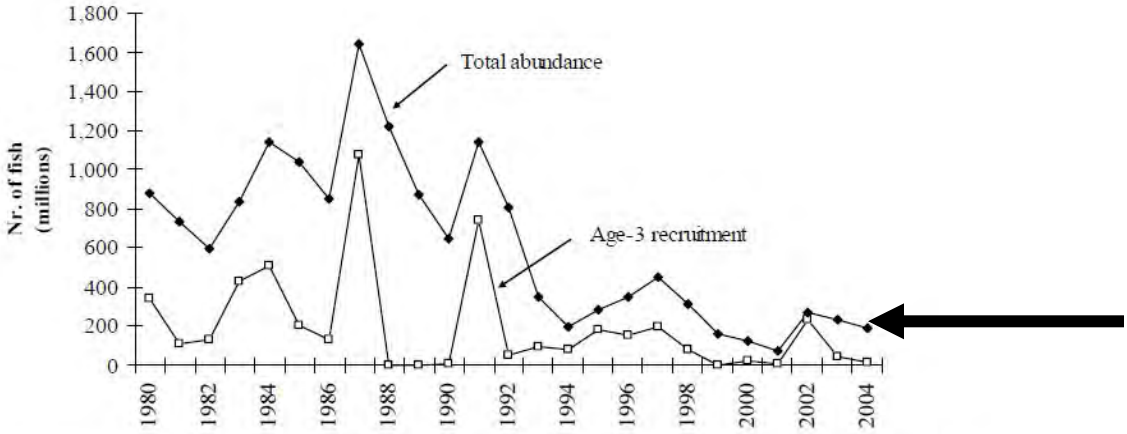


Figure 49. Age-3 recruitment and total prefishery abundance of Pacific herring in Prince William Sound, 1980-2004. The abundance values are outputs of the age-structured model used to produce the 2005 projections.

Fig. 1. Herring abundance trends in Prince William Sound.

(a) (Copied from Moffit 2005). Prefishery run biomass (metric tons) of adult Pacific herring in Prince William Sound, 1980-2004. The biomass values are calculated from the age-structured model used to produce the 2005 projections. (b) (Copied from Moffit 2005). Total numbers of herring (age 3 and older) and numbers of age 3 recruits in Prince William Sound. The arrow to the right shows the approximate present level of recruitment at about 200,000,000 fish/y.

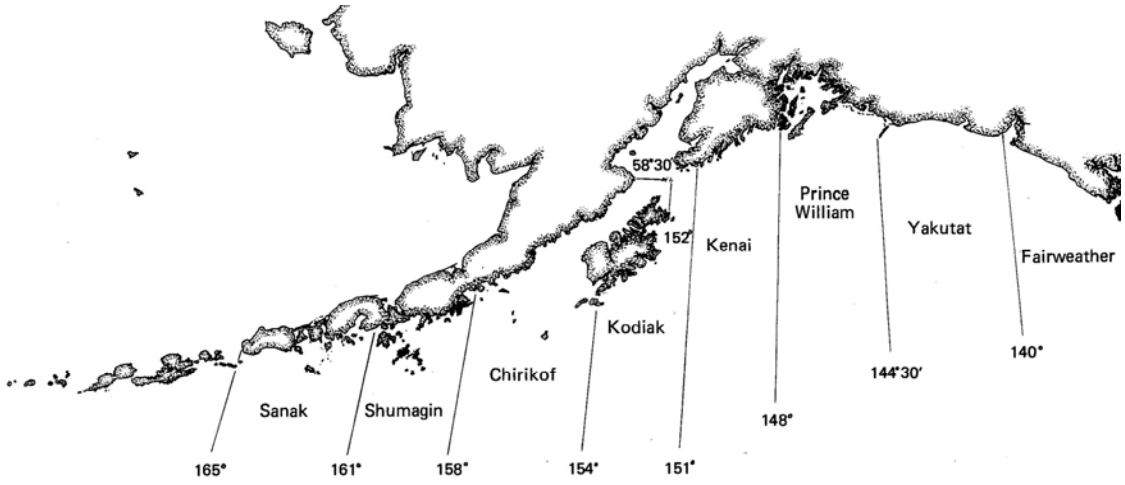


Fig. 2. The Gulf of Alaska showing different districts.

The Prince William district contains both the inside waters of Prince William Sound and the adjacent waters. (Copied from Ronholt et al. 1978). See text for explanation.

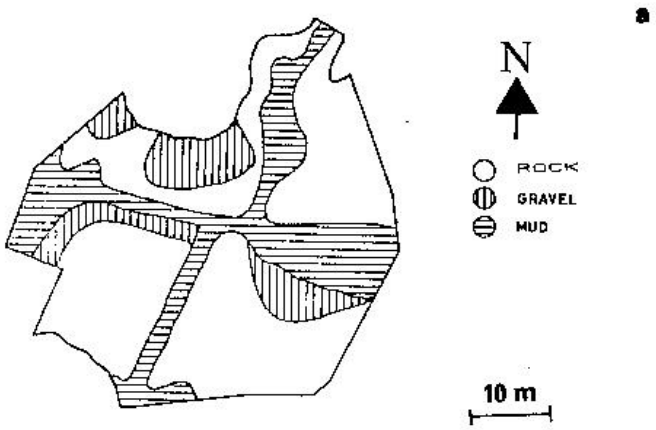


Fig. 1a. The basin, with main bottom types indicated.

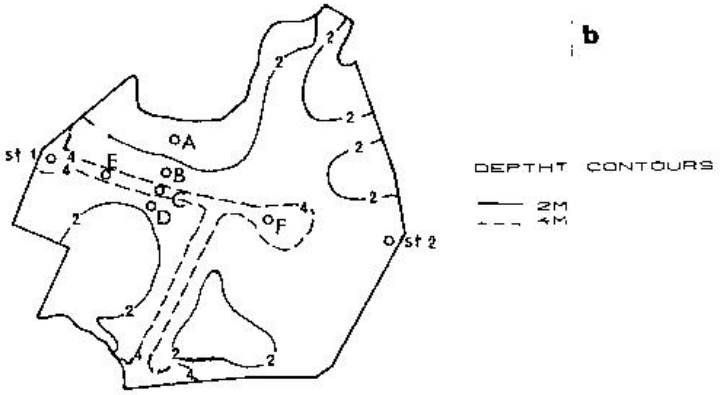


Fig. 1b. The basin, with depth contours and sampling stations indicated. 1: weekly monitoring of hydrography, nutrition salts, and phytoplankton; 1 and 2: weekly monitoring of zooplankton (pump sampling); 2 → 1: net sampling for fish larvae and zooplankton; A,B,C,D and 1,E,C,F,2: zooplankton sampling along transects (pump sampling).

Fig. 3. Illustration of outdoor mesocosms used for larval fish rearing in Flödevigen, Norway.

(Copied from Øiestad 1983). Outdoor enclosures such as this may be suitable for larval and juvenile rearing projects in Prince William Sound.

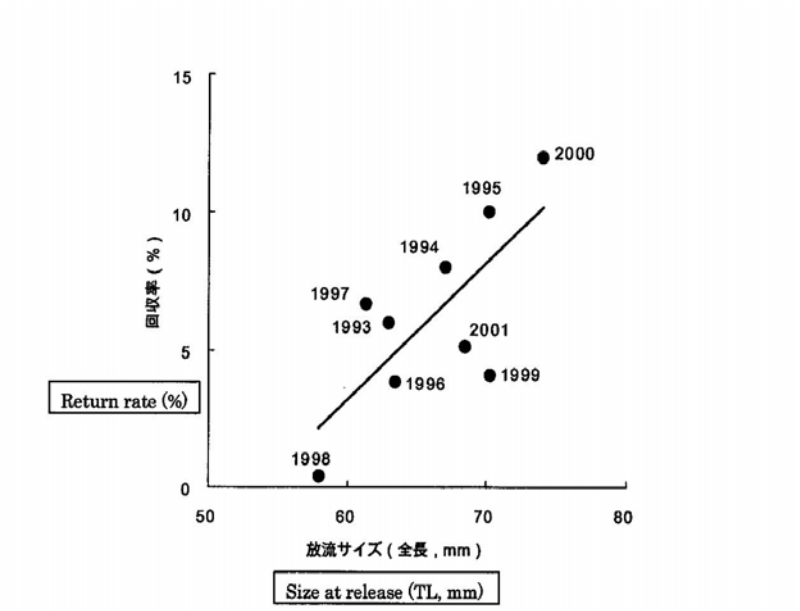
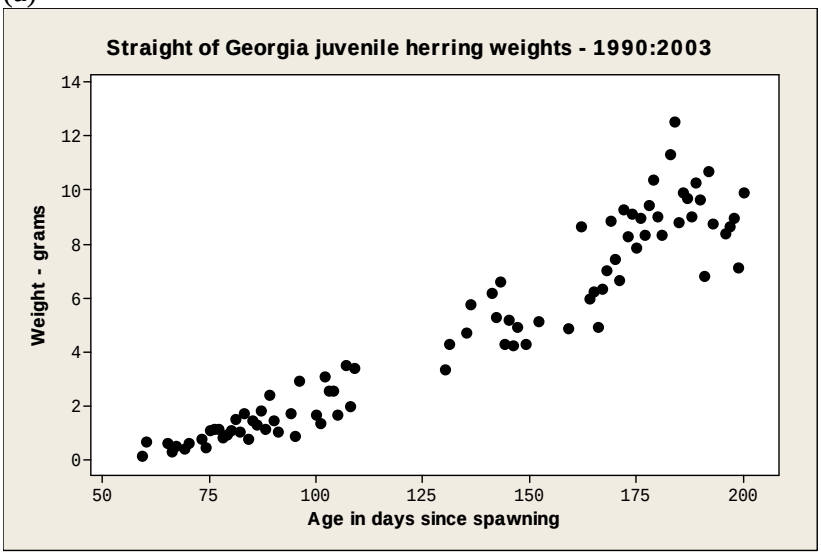


Fig. 4. Relationship between the size at release of age 0 herring juveniles.

The line shows the estimated percentage of returning spawners relative to the size of release (mm) to Akkeshi Bay, eastern Hokkaido. Copied from Suzuki and Fukunaga (2004). (See text for explanation).

(a)



(b)

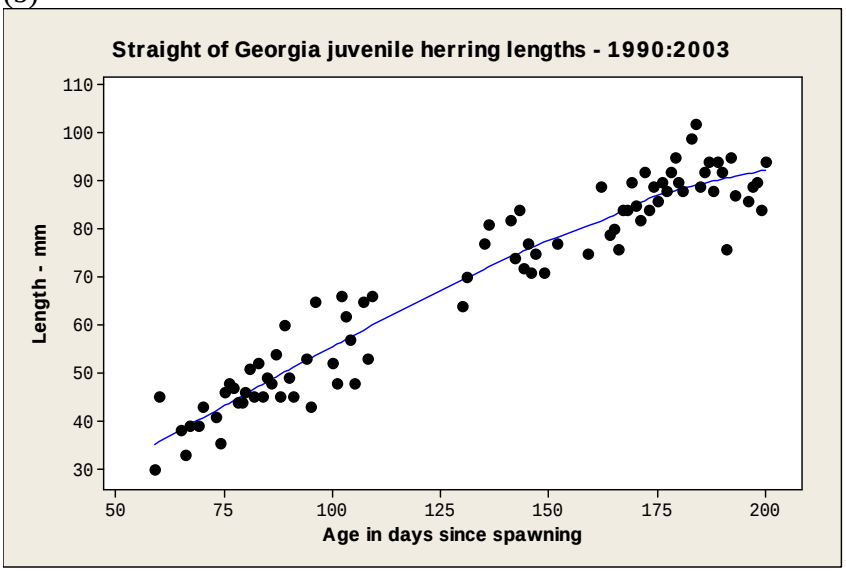


Fig. 5. (a) Weight (g) and (b) length (mm) by age (days) for juvenile herring in the Strait of Georgia. The data were aggregated over sample years from approximately 1990-2003.

Table 1. Simplified life history stages of Pacific herring in Prince William Sound.
The table shows 'within' cohort interactions in column A, as the cohort ages from eggs to adults – progressing from Row 1-6. 'PWS-Gulf' refers to possible adult herring migrations to adjacent shelf waters in the Gulf of Alaska. Columns B-D show 'between-cohort' interactions, with eth stop symbol indicating little or no interactions. The shaded boxes show the largest interactions. For instance age 1+ herring will complete for food with age 0+ juveniles (column-row B3). Column E shows interactions between different stages of herring and other species.

			Within Cohort		Between Cohorts	Between Cohorts	Between Cohorts		Between species
			A		B	C	D		E
	Stage				1+ Juvenile	2+ Pre-recruit	Adult		Other species
1	$Egg_{(y)}$ PWS		Oxygen and abiotic factors PWS 1				Eggs = SSB x 10^{8}- PWS 12		Predation, disease PWS 18
2	$Larvae_{(y)}$ PWS		Spring-Food Competition PWS 2		Spring-summer Predation PWS 7	Spring-summer Predation PWS 9	Spring-summer Predation PWS 13		Spring-summer Predation PWS 19
3	$0+ Juvenile_{(y)}$ PWS		Summer-fall-winter food competition PWS 3		Late summer – fall food competition PWS 8	Late summer – fall food competition PWS 10	Late summer – fall food competition PWS 14		Predation Late summer – Fall Food Competition PWS 20
4	$1+ Juvenile_{(y+1)}$ PWS-GULF		Summer-fall-winter food competition PWS-GULF 4			Food competition PWS-GULF 11	Food competition PWS-GULF 15		Predation food competition PWS-GULF 21
5	$2+ Pre-recruit_{(y+2)}$ PWS-GULF		Summer-Fall-winter food competition PWS-GULF 5				Food competition PWS-GULF 16		Predation food competition PWS-Gulf 22
6	$Adult_{(y+3+)}$ PWS-GULF		Predation food competition PWS-GULF 6				Food competition PWS-GULF 17		Predation food competition PWS-GULF 23

Table 2. Comparison of depth strata between Prince William Sound (PWS) and the Strait of Georgia (SOG). PWS data are from Table 1 (Okey, 1998) in Okey and Pauly, 1998. The SOG depth strata data were derived from GIS (Arcview©) analyses of BC Statistical areas for all areas of the Straight of Georgia (Statistical Areas 14-19, 28-29 and part of 13, but excluding all of Puget Sound). The SOG depth strata intervals were adjusted to match those presented for PWS.

Depth stratum (m)	PWS		SOG	
	Area (km^2)	%	Area (km^2)	%
intertidal (+ - 0)	300	3.31	215	2.37
0-10	709	7.83	597	6.57
10-20	709	7.83	312	3.43
20-100	2018	22.28	2591	28.53
>100	5325	58.76	5364	59.08
TOTAL	9059	100.00	9080	100.00

Table 3. Estimates of stage-specific survival.

For each of nine life history stages (first column) the table shows the estimated duration of the stage (in days) and approximate weight (grams) of each individual (at the conclusion of each stage). Based on information described in the text, the six columns to the right show the minimum, maximum and average estimates of survival (p). The underlined numbers show estimates taken from Norcross and Brown (Table 4) 2001. The survival rates shown in the last three columns are in exponential format (E), and are identical to the previous three columns that are shown in arithmetic format.

Life History Stage	Durations	Age at stage end	Approx. weight at each stage	Minimum age-specific survival	Maximim age-specific survival	Average age-specific survival	Minumum age-specific survival	Maximum age-specific survival	Average age-specific survival	Minumum age-specific survival	Maximum age-specific survival	Average age-specific survival
	days	*days*	*grams*	*p*	*p*	*p*	*rate*	*rate*	*rate*	*rate*	*rate*	*rate*
unfertilized eggs	0											
fertilized eggs	1	1	0.001	0.99	0.99	0.99	0.990000000	0.990000000	0.990000000	9.900E-01	9.900E-01	9.900E-01
eggs	20	20	0.001	0.24	0.45	0.345	0.237600000	0.445500000	0.341550000	2.376E-01	4.455E-01	3.416E-01
posthatch	10	30	0.02	0.5	1	0.75	0.118800000	0.445500000	0.256162500	1.188E-01	4.455E-01	2.562E-01
larval_drift	30	60	0.5	0.01	0.07	0.04	0.001188000	0.031185000	0.010246500	1.188E-03	3.119E-02	1.025E-02
fall_juveniles	120	180	8	0.02	0.21	0.115	0.000023760	0.006548850	0.001178348	2.376E-05	6.549E-03	1.178E-03
winter_juveniles	185	365	10	0.5	0.62	0.56	0.000011880	0.004060287	0.000659875	1.188E-05	4.060E-03	6.599E-04
age 2	365	730	40	0.05	0.5	0.275	0.000000594	0.002030144	0.000181466	5.940E-07	2.030E-03	1.815E-04
age 3	365	1095	120	0.05	0.5	0.275	0.000000030	0.001015072	0.000049903	2.970E-08	1.015E-03	4.990E-05

Table 4. Numbers and biomass at each life history stage in natural populations.

The first four columns show the same information as Table 3 (life history stage, duration, age at the end of the stage and the weight of individuals at the end of the stage). The table tracks the stage-specific survival of each stage according to three estimates of age-specific survival: minimum, maximum and average survival. The beginning number of eggs is one hundred million (10^8) – corresponding to the numbers of eggs produced by one mt of spawning herring. The underlined numbers, describing the minimum and maximum numbers of survivors, are identical to the estimates presented in Norcross and Brown (2001, Table 4) after adjusting for the 100-fold difference in starting numbers. The last six columns, showing estimated biomass (in grams and metric tons) show mixed responses. Relative to the starting biomass, which is the cumulative weight of all one hundred million individual eggs, the cohort biomass decreases under the minimum survival scenario, increases under the maximum survival scenario and fluctuates under the average survival scenario.

Life History Stage	Durations	Age at stage end	Approx. weight at each stage	Cohort number minimum survival	Cohort number maximum survival	Cohort number average survival	Cohort biomass - minimum survival	Cohort biomass - maximum survival	Cohort biomass - average survival	Cohort biomass - minimum survival	Cohort biomass - maximum survival	Cohort biomass - average survival
	days	*days*	*grams*	*number*	*number*	*number*	*grams*	*grams*	*grams*	*m tons*	*m tons*	*m tons*
unfertilized eggs				**100000000**	**100000000**	**100000000**						
fertilized eggs	1	1	0.001	99000000	99000000	99000000	99000	99000	99000	0.099	0.099	0.099
eggs	20	20	0.001	23760000	44550000	34155000	23760	44550	34155	0.024	0.045	0.034
posthatch	10	30	0.02	11880000	44550000	25616250	237600	891000	512325	0.238	0.891	0.512
larval_drift	30	60	0.5	118800	3118500	1024650	59400	1559250	512325	0.059	1.559	0.512
fall_juveniles	120	180	8	2376	654885	117835	19008	5239080	942678	0.019	5.239	0.943
winter_juveniles	185	365	10	1188	406029	65987	11880	4060287	659875	0.012	4.060	0.660
age 2	365	730	40	59	203014	18147	2376	8120574	725862	0.002	8.121	0.726
age 3	365	1095	120	3	101507	4990	356	12180861	598836	0.000	12.181	0.599

Table 5. Estimated survival in a hypothetical enhancement project.

The columns show the estimated minimum and maximum stage-specific survival rates in a hypothetical enhancement project. The life history stages follow those used in the Tables 3 and Table 4. A minimum and maximum survival estimate is estimated for each stage. These survival estimates, shown as probability of survival (p), are assumed to be much higher than those occurring in natural populations but the cumulative survival is lower than the estimates reported in Japanese herring enhancement research. These survival estimates are used to estimate the survival of one hundred million (10^8) eggs at the pre-fertilization stage to the 'fall juvenile' stage. The underlined numbers show the minimal estimates of survival (6.19 million or also about 6.19% survival) and maximal estimates of survival (57.18 million or about 57.18% survival) at the end of the fall juvenile stage, at an age of 6 months. Similar estimates are made for the end of the winter juvenile stage (shown in Italics). No further estimates are shown for survival in enhancement based on the assumption that release would occur at some time between during the winter juvenile stage – a stage when intra-specific density-dependent effects may be minimal.

Life History Stage	Duration *days*	Age at stage end *days*	Approx. weight at each stage *grams*	Minimum stage specific survival *p*	Minimum stage specific survival *numbers*	Minimum stage specific survival *numbers (millions)*	Maximum stage specific survival *p*	Maximum stage specific survival *numbers*	Maximum stage specific survival *numbers (millions)*	Biomass with minimum survival *grams*	Biomass with maximum survival *grams*	Biomass with minimum survival *m tons*	Biomass with maximum survival *m tons*
pre-fertilization					100,000,000			100,000,000					
fertilized	1	1	0.001	0.99	99,000,000	99	0.99	99,000,000	99	99,000	99,000	0.099	0.099
embryo	20	20	0.001	0.5	49,500,000	49.5	0.95	94,050,000	94.05	49,500	94,050	0.0495	0.09405
posthatch	10	30	0.02	0.5	24,700,000	24.7	0.95	89,347,500	89.3475	494,000	1,786,950	0.494	1.78695
larval_drift	30	60	0.5	0.5	12,375,000	12.375	0.8	71,478,000	71.478	6,187,500	35,739,000	6.1875	35.739
fall_juveniles	120	180	8	0.5	6,187,500	6.1875	0.8	57,182,400	57.1824	49,500,000	457,459,200	49.5	457.4592
winter_juveniles	*185*	*365*	*10*	*0.05*	*3,093,750*	*3.09375*	*0.99*	*56,610,570*	*56.61057*	*30,937,500*	*566,105,700*	*30.9375*	*566.1057*
age 2	365	730	40	—	—	—	—	—	—	—	—	—	—
age 3	365	1095	120	—	—	—	—	—	—	—	—	—	—

Table 6. Estimated survival following enhancement and release.

The estimated numbers and survival of herring reared in an enhancement project and released ('HEP Release') at approximately 6 months (180 days) of age. The life history stages follow those of Tables 3-5 but the estimates of stage-specific survival rates for wild herring (*prior to release*) are not used for these calculations but are included to illustrate how herring reared in an enhancement project may be subject to stage-specific survival rates, depending on the time of release. The estimated numbers of herring, surviving from an initial number of 100 million (from Table 5) is shown in the last three columns for the 'worst', 'best' and 'average' survival scenarios. In these scenarios the numbers of juveniles surviving to the point of release are about six million for the worst case scenario, 57 million for the best case scenario, and 31 million for the average case scenario. Once released these herring juveniles would then encounter survival rates applicable to wild herring, shown here as the minimum, maximum and average probability (p) of survival for three stages (winter juveniles, age 2 and age 3). The large ***bold Italic*** numbers at the lower right show the impact of these three 'post-release' survival scenarios imposed on three of the 'pre-release' survival scenarios that apply during enhancement.

Life History Stages	Durations	Age at stage end	Time of Release	Minimum age-specific survival	Maximim age-specific survival	Average age-specific survival	scenario minimum HEP and lowest natural survival	scenario maximum HEP and highest natural survival	scenario average and average natural survival
	days	*days*		*p*	*p*	*p*	*numbers*	*numbers*	*numbers*
unfertilized eggs							100,000,000	100,000,000	100,000,000
fertilized eggs	1	1		0.99	0.99	0.99	99,000,000	99,000,000	99,000,000
eggs	20	20		0.24	0.45	0.345	49,500,000	94,050,000	71,775,000
posthatch	10	30		0.5	1	0.75	24,700,000	89,347,500	57,023,750
larval_drift	30	60		0.01	0.07	0.04	12,375,000	71,478,000	41,926,500
fall_juveniles	120	180	***HEP Release***	0.02	0.21	0.115	6,187,500	57,182,400	31,684,950
winter_juveniles	185	365		***0.5***	***0.62***	***0.56***	3,093,750	35,453,088	17,743,572
age 2	365	730		***0.05***	***0.5***	***0.275***	154,690	17,726,544	4,879,482
age 3	365	1095		***0.05***	***0.5***	***0.275***	***733***	***8,863,272***	***1,341,858***

Appendix D – Herring Marking Workshop Proceedings

EVOS Trustee Council Herring tagging workshop

Workshop proceedings: tagging and marking techniques applicable to the restoration of herring in Prince William Sound

December 11 and 12, 2008
EVOSTC Office – Anchorage, Alaska

EVOSTC Herring Tagging Workshop

Table of Contents

Executive summary

Perspectives on herring tagging and the workshop

The difference between a 'tag' and a 'mark' is that tags have unique identity codes, marks do not. Marks are not distinguished from each other so one mark may be applied to many individuals. There is wide range of methodologies that can be used as marks or tags, and sometimes the terms 'tagging' and 'marking' can be used inter-changeably. For instance, both marking and tagging usually require the capture of fish, application of the mark or tag, release of the marked or tagged fish, and then recovery of the marked or tagged fish. These terms are used many times in this report, so to avoid tedious repetition the term 'tagging' is often used as a broader term to describe activities applicable to both tagging and marking.

Tagging fish can be interesting, fun and useful but sometimes it also can be expensive and ineffective. Tagging often is a high-profile activity winning approval from many quarters, especially if tagging operations are conspicuous. Usually advertisements are required for recovery of tagged fish. Therefore tagging work can promote the impression that management or research agencies are doing constructive things. The flip side of this rosy picture is that tagging programs can go badly wrong and be wasteful, especially if the methods and objectives are not well established or if monitoring actions are not fully engaged.

Some form of herring tagging or marking in Prince William Sound is essential if an enhancement program, through some form of supplemental production is initiated (i.e., a 'herring hatchery' – or some version of a hatchery). This requirement is well established in the scientific literature. It makes sense to develop protocols to evaluate expensive research activities.

A significant challenge for any herring tagging program will be obtaining sufficient recapture in the absence of a commercial herring fishery. There is irony in the present situation: the present interest in tagging herring is prompted by concern about their low abundance and failure to recover from a collapse in 1993-1994. If stocks levels were high enough to allow fishery, then a herring enhancement program – or a tagging program - would not be required. The recapture of tagged fish can only be done by systematic sampling of recruiting (or near-adult) fish, at about age 3-5. Based on the precedent of recent research in Japan, it seems likely that a relatively large number of young herring could be marked and released in Prince William Sound. It is much less certain if a sufficient number of marked fish could be recaptured in order to evaluate the viability of an enhancement project.

A two-day workshop held in the EVOSTC Anchorage offices on December 11-12, 2008, discussed potential options for marking and tagging Prince William Sound (PWS) herring. The workshop was attended by biologists and scientists with expertise in different types of fish marking and tagging and some who were knowledgeable about PWS herring assessments and biology. This review of tagging and marking methodology revealed that most conventional forms of fish tagging or marking have requirements that limit or preclude their use as a means to assess the efficacy of herring restoration projects. For instance, past work with external tags indicates a high mortality and tag loss issues. Internal coded-wire nose tags work well but require a fishery for sufficient tag detection and recapture. Fatty acid analysis may have some limited applications but it is expensive and unsuitable for the mass marking approaches required

for most types of enhancement. In theory genetic tags could work but probably would not receive permitting requirements, and, in any event would require a major, unprecedented development of sufficient herring broodstock – a challenge that probably exceeds that of the tag recaptures. Acoustic tags have very promising attributes but can only be applied to relatively large fish and at several hundred dollars per tag is not a candidate for mass marking. Nevertheless, some applications of acoustic tags may have substantial, although indirect, benefits for Prince William Sound herring enhancement.

The preceding comments should not be construed to conclude that the workshop was not successful. On the contrary, it was very successful n the sense that it assembled comprehensive and topical information on the general topics of marking and tagging fish, with an emphasis on herring. The workshop provided direction for the types of marking options that could be considered with potential herring restoration options. It also provided an opportunity to pull together a broad and comprehensive array of different approaches to marking, some of which may have potential applications for addressing specific research and management issues in Prince William Sound.

Why tagging and why now? Rationale for the workshop

The enhancement of herring in Prince William Sound PWS through human intervention, is one option for their potential restoration. The concept of 'enhancement' is broad and includes a wide range of potential activities. Nearly all herring enhancement initiatives will need some effort to evaluate the effectiveness of any enhancement activity. This involves the estimation of the relative survival of enhanced herring compared to survival without enhancement. The requirement for tagging or marking is well established as a necessary technical and scientific requirement for any enhancement project – on any fish species. There are different ways that herring can be tagged or marked. There also are different technical and logistic considerations associated with different types of tagging or marking.

Tagging could be an integral and substantial part of any enhancement activity. Initially tagging could be the most challenging, time-consuming and expensive part of herring enhancement. If done properly, however, there will be gains in knowledge and understanding that will provide considerable insight into PWS herring and ecological factors that affect them.

The workshop heard about six different types of marking and tagging methods or approaches:
(1) external tags, used in the past with adult-size fish;
(2) internal coded wire tags, used mainly on large juveniles and adult fish;
(2) otolith ('earbone') fluorescent dyes applied to very young fish
(3) natural tags, with emphasis on chemical fingerprints, mainly from otoliths
(4) acoustic tags, suitable for large juveniles or adults
(5) genetic tags, that require prior development of spawning broodstock
(6) fatty acid signatures, that reflect.

There are some real or perceived obstacles to implementation of marking or tagging. An important concern is ADF&G policy regarding the movement of wild fish and the release of cultured fish into the wild. Marking that involved the holding of fish for extended periods, prior to release, would need to develop protocols to satisfy these policy requirements. There also are

disease issues that would need to meet policy limits. There also would be challenges related to marshalling the human skills required for tagging work. The workshop showed that capabilities exist, mostly within the State of Alaska, but there would be challenges to pull these human resources together. Tagging work can be costly. Probably the cost of the tag application is small relative to other potential costs, especially tag recovery. The cost of examining captured fish to estimate the proportion of marked individuals will vary according to the proportion of fish tagged relative to the numbers of wild fish.

Conclusions and recommendations from the workshop

External tags on herring have high tag loss and may lead to increased injury and mortality. Coded wire nose tags (cwt) can work successfully on herring, but require a fishery for effective tag recovery. Acoustic tags seem assured to provide interesting results on herring movements in PWS the results are may be only indirectly beneficial to issues of herring enhancement. The potential development of genetic tags/marks may be plausible, but the methods would be dependent on the unprecedented development of herring 'broodstock' rearing. At best development of this approach would require years of expensive research. Even if successful, the approach probably would encounter resistance because of the release of genetically modified fish. Fatty acid analysis could have a role for analysis of specific issues related to herring enhancement (i.e., provision of external food) but such an application would require considerably more research and probably would be an expensive option.

The potential marking method that seems to have promise is the array of chemical dyes and marks. Such approaches have been successfully applied in Japan using the Alizarin Complexerone. However, this specific chemical may incur severe permitting problems if applied in the American waters. It is possible that permitting issues could be successfully addressed but there also are other chemical dyes and chemical marking agents, that have been used if similar fish-marking purposes, that may warrant careful consideration. There are several potential chemical approaches that may vary according to the ease of obtaining permitting approval and costs, both for the purchase and application of the mark and the cost of potential recovery.

A specific recommendation is the conditional endorsement of acoustic tagging, with the caveat that the initial involvement should be limited. Arrays of acoustic receivers have been installed in PWS and there may be opportunities to leverage costs with other organization, so the present time is an excellent opportunity to pursue this approach. Acoustic tagging methods, that have not been conducted previously with herring, but they have been used on other small species of fish species. It seems probable that useful information on herring ecology and migratory movements could be revealed by acoustic tagging. Acoustic tagging information, however, may have limited application to many issues related to herring enhancement.

Chapter One - Workshop Overview and Report Goals

The workshop attempted to review available methods for marking and tagging and recovery of marked or tagged herring in Prince William Sound, Alaska, in support of possible use of various enhancement options. The Prince William Sound herring population remains depressed and some form of enhancement is under consideration. Before any type of enhancement activity can be considered, there is a requirement to establish procedures and methodologies that can evaluate the survival of enhanced fish and the efficacy of different enhancement options. Such evaluation will require a marking program. However there are many different types of marking options that have different advantages and disadvantages. Herring are fragile fish so some potential marking methods may be unsuitable. There is virtually no experience with either large-scale herring enhancement or marking of herring in Alaska of the United States but there is a knowledgeable and capable scientific community that can provide essential information. This group of experts assembled at the workshop presented and reviewed seven different approaches and technologies for possible use relative to enhancement of PWS herring. Two additional workshop goals were to examine permitting requirements and sampling methodologies – specifically issues concerning the number of released and recovered fish that would be required for scientifically defensible results. The workshop began with two reviews of herring: one on their general biology in the eastern Pacific. The other commented on their present status within PWS. The collected contributions from presenters are assembled as individual chapters in this report. These contributions are deliberately short and were prepared in response to suggested guidelines that asked each person to address certain basic questions. Contributors were requested to prepare short bibliographies with key references.

Content and organization of the report

The report is divided into chapters. Chapter one provides background information for the workshop as report. Chapter two presents a summary and brief discussion of the individual contributions. Chapter three presents synthesis that discusses the key issues raised I the workshop. Chapter three also presents some recommendations and suggested guidelines about tagging and marking Prince William Sound herring. Each of the remaining chapters (4-13) is a separate contribution presented at the workshop. Each of these chapters is reproduced here as it was prepared by the authors except for occasional minor editing and adjustment of headings and sub-headings have been adjusted to follow the same format so that they could be presented in the collected Table of Contents.

Limitations and potential applications of the report

This report, however, does not present a definitive conclusion or recommendations because many biological, technical and procedural uncertainties remain. Nevertheless, the information in this report will provide a review of the advantages and disadvantages of various marking and tagging approaches. This information can be used and applied as other aspects of potential enhancement are examined, revealed and perhaps developed. Therefore this report presents a ‘state-of-the-art’ assembly of vital information that is a pre-requisite for any future enhancement activity.

Workshop Agenda

December 11

9:00 – 9:15 Opening Remarks and Introductions – Jen Schorr (EVOS Trustee ouncil)

9:15 - 10:00 Herring Overview - Why this workshop, why now? - Jeep Rice (NOAA)
Herring behavior and biology – Doug Hay (Nearshore Consulting)
Herring in Prince William Sound – Steve Moffitt (ADF&G)

10:15 – 12:00 Tags – External and Internal
Traditional/Historical – Doug Hay
Coded Wire Tags and other tags – Geraldine Vander Haegen (Northwest Marine Technology)
Questions and Answers

1:00 – 1:30 Acoustic Tags- Will they work, what life stages?
Andy Seitz (UAF) and Brenda Norcross (UAF)

1:30 – 5:00 Otolith Marking (or other hard parts) – Successful applications but can it work for PWS herring and at what scale?
Sampling theory of mass marking – Pete Hagen (NOAA)
Overview of marking options – Dion Oxman (ADF&G)
Chemical Analysis – Growth Pattern Analysis – Andrew Munro (University of Adelaide)
Instrumentation and recovery of marks – Ken Severin (UAF)
Questions and Answers

12 December

9:00 – 10:00 Permitting- hurdles
Federal Permits for chemical markers – Pete Hagen (NOAA)
ADF&G Fish Transport – issues and strategies – Chris Habicht (ADF&G)
Questions and Answers

10:00 – 11:00 Genetic Marking – practical, stable?
Jeff Olsen – (USFWS)
Jeff Guyon – (NOAA)
Chris Habicht
Questions and Answers

11:15 – 12:15 Fatty Acid Signatures – Stable over time?
Ron Heintz – (NOAA)
Ted Otis – (ADF&G)
Questions and Answers

1:15 – 3:00 Group Discussion – How do technologies compare, which appear the most feasible: Recommendations – Other Comments?

3:15 – 4:00 More Discussion – Next Steps for a particular technology?

4 : 15-4:30 Deliverables – Doug Hay

4:30 Closing remarks

Report Goals

This report has fourteen chapters. Chapters two and three condense the proceedings of the two-day workshop into a much shorter *synopsis*. This condensation requires the sacrifice of some information presented at the workshop. Readers interested in detail about tagging and marking methodology should consult individual chapters that follow.

This introductory section attempts to provide a succinct *synthesis* and commentary of the information to a form that is can be linked to the issues related to restoration or enhancement of herring in Prince William Sound (PWS) with emphasis on different life history stages of herring. The synthesis consists of a commentary plus three simple matrix tables. The tables examine different tagging and marking technologies according to generalized life history stages of herring (i.e., egg, larvae, juveniles and adults).

In late 2007 and throughout 2008 on-going discussions about herring enhancement in PWS have tentatively identified a number of potential options, of which one was called 'supplemental production'. Supplemental production would involve the artificial rearing of herring for subsequent release to the natural environment. One approach to this could involve some form of a herring 'hatchery' although this terminology could be misleading. There are a number of different ways that herring can be supplemented, and the concept of a land-based herring hatchery, similar to traditional salmonid hatcheries, is only one of a many different future scenarios that could develop (Hay 2008). Common to all potential approaches to supplemental production, however, is the requirement for assessment and verification of success (or failure). Such verification requires the development of a marking or tagging program. The other potential restoration activities described in the herring restoration plan also would require monitoring and evaluation that could be accomplished by tagging or marking. Therefore this synthesis also presents an abridged list of these potential restoration activities and for each considers the potential and limitations of marking or tagging.

This report summarizes eleven presentations. Seven were concerned with marking or tagging methodology. Two were concerned with herring biology and assessment. Three others were concerned the procedural issues: specifically (i) issues about the numbers of tags that must be released and recovered to provide useful results; (ii) permitting or legal aspects of all aspects of tagging, including genetic implications; (iii) technical aspects and instrumentation aspects, especially as they relate to chemical analyses of herring.

Strengths and weaknesses of the workshop

A strong part of the workshop was in the collective expertise of participants and their willingness to provide detailed, candid summaries about the advantages and disadvantages of the different technological application. The speakers understood their subject area and were apprised of the recent scientific literature in their fields. Therefore this workshop represented a reasonable assemblage of the state of the art of fish tagging and marking, at least as it would apply to herring. Another strong aspect of the workshop was the preparation and organization that was done by the EVOSTC staff and colleagues within ADFG, NOAA and other agencies. They deserve credit for their careful preparation. It also helped that all of the participants provided written and detailed contributions on time

An unavoidable weakness of the workshop was the limited familiarity of some participants with details of herring biology. Some sections of the contributions may not be wholly applicable to herring issues in PWS. On the other hand, the greater breadth of experience with other species also provided the potential for useful perspectives that may apply to PWS. For example, the rational for much of the scientific work undertaken by Andrew Munro in Australia was remarkable similar (but not identical) to issues concerning herring in PWS.

Definitions and concepts

The following terms and concepts are used in the introductory text, and in the individual reports. The text below is an attempt to explain some of the usage. These definitions and usage varies among sources, however, and are presented here as guides and not definitive definitions. Therefore readers are advised that different authors take put different emphasis on some terms.

Tags versus marks

A ‘tag’ is usually a device that is attached or inserted into fish that has a unique identification code, usually a number or a combination of a number or letter, or a bar code. The essential characteristic of a tag is that, when recovered, the identity of an *individual* fish can be determined. Usually this also provides an opportunity to assemble other information, such as the date and location when the tag was applied and the fish released, etc. A ‘mark’ usually is simply an external or internal modification of a fish that allows it to be distinguished from fish with no marks (natural). *The key distinction between a mark and a tag is that marks do not allow for identification at the individual level.* An example of a mark is a traditional ‘fin-clip’ – usually a small ventral fin or an adipose identified any attribute that identifies a fish. (Fin-clip procedures have been discontinued in recent years). A more vivid example of a mark is the traditional ‘brand’ applied to cattle or other domestic animals. In contrast, if the animals also had a tattoo, with a unique individual number, then the tattoo would be a ‘tag’. It follows that tag is also a mark, but not vice versa.

Some types of marks may occur naturally. For instance, the parasitic composition has been used to distinguish – or attempt to distinguish among populations. Similarly, the naturally occurring chemical composition of bones, especially otoliths, as a means of distinguishing different

populations, is a rapidly growing field of scientific activity. Similarly, fatty acid analysis may also have potential applications.

Definitions and distinctions can become a bit fuzzy, however, when new and different approaches are considered, especially novel genetic approaches, where unique genetic configurations may be developed and used.

Internal and external tags or marks.

In practice there is a bewildering array of different types of marks and tags. Marks can include *internal* chemical modification, usually as a dye or other substance that is taken up by bones or otoliths (earbones) that can be later identified, usually in a laboratory. Internal tags usually are some form of metal or plastic insertions into a fish. Early herring tagging work began with small metal bands, each with a unique number, inserted into the body cavity of herring. Marks also can involve chemical or physical changes to the *external* appearance to a fish. The classic 'Petersen' disk was a small plastic disk, with a unique printed number, attached to the dorsal fins of fish.

Active and passive tags.

Some types of tags, such as radio or acoustic tags, are *active* and emit signals that allows for their detection. (Radio tags work in freshwater but not in seawater). The acoustic (or radio) signal is unique, providing identification of individual fish. In contrast, most other tags or marks are *passive*. For example an internal metal tag with a number or bar code but be detected when placed in a magnetic field – usually a hand-held detector device. There also are tags that are passive until stimulated by an external – at which time they can emit a signal. The tags of special interest for PWS are acoustic tags. They are relatively large so cannot be applied to the early life stages (larvae and small juveniles) of herring and they are relatively expensive but they may have potential application for other aspects of enhancement.

Release and recovery.

After fish are marked or tagged they usually are *released* into the natural environment. Normally such a release event is recorded according to date, time and location, etc. This is a simple, straightforward concept, although the tagging or marking date may *precede* the release date, especially if herring are marked in the egg or larval stages.

A substantial challenge for any tagging or marking project is the *recovery* (or recapture) of the tagged or marked fish at some time of place following release. In most marine fishes recovery of tags or marks occurs during a fishery and usually the tagged or marked fish is dead. (Some types of tags, however, can be detected while attached to a live fish, in a natural environment – see text in next sub-heading).

In PWS the herring fishery is suspended because of low spawning biomass. Therefore recovery of tagged or marked herring will present special difficulties for recovery of tagged or marked

fish. The dilemma concerns the numbers of marked herring that must be released. If there were an active fishery, that captured the maximum quota – which usually is between ten and twenty percent of the spawning biomass (approximately one in every five or ten fish) then tagging and marking projects can be effective with only a relatively small number of releases. On the other hand, if there is no fishery, the only way that tags can be recovered would be by special, research samples (if permitted). Suppose, for instance, such research samples were allowed to capture a total of 10 tons of herring to look for recaptures. The total herring present (2008) biomass of PWS herring is about 20,000 tons. Therefore in very general terms, this quantity would represent about only about 1 in 2000 fish in PWS would be screened for tags. It follows that if there is no fishery to provide for recaptures, a high number of tagged or marked herring must be released. The issue(s) of the numbers of released tags versus the numbers of recaptures represents a special logistical concern that warrants special attention. For this reason, a preliminary examination of the basic issues was included in the workshop (see report by Pete Hagen).

Meta-populations, populations, local populations, sub-populations, and stocks.

There are no universal definitions for these terms that would satisfy all biologists. However some of these terms are used (and probably mis-used) frequently - in this report and in elsewhere herring general literature. The following paragraphs present a brief review of definitions of terms that occur in this report. (Many biologists are passionate about this terminology and nuances of the concepts they represent.)

The simplest concept is that there is single population of herring in Prince William Sound. The biological implication is that all herring in PWS are part of an integrated biological unit and that there are no barriers to interbreeding among any different regions within PWS. The basic assumption also would be that there is little or no immigration of emigration of herring into, or out of PWS.

The fact that there are different spawning areas within PWS has led some scientists to speculate, and others to conclude, that there two or more biologically distinct populations in PWS. The biological implication is that such small units are reproductively isolated and do not interbreed. If so, then each unit could have distinct biological characteristics and population dynamics. Each would warrant distinct population assessment and unique management. Mainly this view has been discounted in recent years as increasing genetic (and other) evidence indicates substantial genetic interchange among adjacent herring populations – not only within PWS but perhaps more broadly with and among other herring populations in the eastern Pacific, as well as in other parts of the world.

It seems most probably that if there are different components to PWS herring, then they could constitute '*local populations*' or '*sub-populations*' that collectively make up a '*meta-population*' which is an aggregate of smaller units (that could be called sub-populations or populations). Interested readers could examine Hanski and Gaggiotti (2004) for more elaboration of these terms.

It is problematical whether the PWS should be referred to as a meta-population. A PWS metapopulation would be an aggregate of local populations within PWS. Alternately PWS

herring could be part of a larger herring meta-population that extends geographically throughout a broad range in the Gulf of Alaska, including Kodiak, Sitka, and elsewhere, even BC and Washington State, but not the Bering Sea (see Hay et al. 2008, and references therein). Probably the most ardent advocates of meta-population theory would demand that PWS should be considered as a local population, or cluster of several local populations that are part of a larger meta-population that occupies a large geographical range in the north-east Pacific. For the purposes of this report, however, PWS will simply be called a 'population' that might consist of one or more 'local populations' that are recognizable mainly by the geographic location, timing and temporal (among year) continuity of spawning.

Hatchery, spawn, egg, milt, larvae, broodstock

These terms occur in the some of the presentations and are mainly are not simple, but often are considered in the context of the life history of Pacific salmon. For herring there are some differences worthy of comment. The term *'spawn'* , when applied to herring, usually means the naturally deposited eggs or milt in the water. The scientific literature usually refers to artificially spawned herring to mean the physical removal of eggs and artificial fertilization. A key difference between herring and salmon is that herring eggs are very adhesive and stick to a substrate within a few seconds after contact. Usually once stuck, these eggs remain in position until hatched. A *hatchery* for herring could be roughly similar to that of salmonids except the duration of the egg stage is much shorter (2-3 weeks) and the hatched larve are small (< 1 cm long and weighing only few mg), roughly 1/1000 the size of salmonids. The larval stage lasts for a month or two and larvae require live food. Some of the following papers suggest marking procedures that would require marking live females before eggs are released, in an attempt to have a mark taken up by the eggs while still within the ovary. Such a procedure would require the rearing of herring to the adult stage, through to sexual maturity, and to be used as a source of eggs for hatchery work. These reared fish could then be called 'broodstock'.

Chapter Two - Summaries and commentaries

This Chapter presents a condensed version of all workshop presentations and provides a post-workshop commentary on the contributions (presented as eleven distinct chapters, beginning with Chapter four). These commentaries below are not intended as critical reviews of each contribution Instead the purpose of the summary and commentary is to provide a brief review and a short commentary that provides a context for each presentation: specifically, what are the merits and limitations of each tagging or marking approach, as it may apply to issues concerned with enhancement of herring in PWS.

The sequence of the following summaries and commentaries is arranged to cover three natural groupings of reports:

(1) Biological and management reviews (two reports);
(2) Marking and tagging methods (eight report);
(3) Logistic, legal/permitting and technical issues (three reports).

Summary 1: Herring behavior and biology
(There is no corresponding Chapter for this summary)

Doug Hay, Nearshore Research, Nanaimo, BC

Herring occur in all oceans of the northern hemisphere. The largest stocks, often exceeding a million tons, occur in areas that have large continental shelves. In general, the northeastern Pacific has small continental shelves and maximal herring stock sizes are much smaller than most other areas. The exception is the Bering Sea where maximum herring stock sizes can be very large.

All major herring stocks in the world fluctuate: in some stocks the maximal abundance sometimes exceeds more than 100 times minimal abundance levels. Fluctuations often are associated with overfishing, but it is clear that most populations would fluctuate even in the absence of fishing. It also is clear that most herring stocks recover from overfishing. A notable exception is the large Hokkaido-Sakhalin stock that crashed in the early 1950's. Maximal abundance was probably much greater than one million tonnes, but since the 1950's the total abundance has only been a small fraction of that. To date, however, there is not a clear understanding of what causes herring stocks to fluctuate or why the Hokkaido-Sakhalin stock has not recovered.

Herring have several distinct life history stages. The extreme earliest part of the life cycle is as an unfertilized egg – or 'oocyte' within the ovary of a female. Eggs begin developing within the ovary in the late fall and early winter. The oocytes reach maximal size several weeks prior to spawning, which usually occurs in spring months. Most spawning in PWS seems to occur between late March and May. Spawned eggs are very sticky, and are usually deposited on seaweeds in shallow inter-tidal or sub-tidal water, usually with a maximum depth of about 10 meters. Incubation time is temperature dependent and usually takes 2-4 weeks. Newly hatched larvae live off their yolk sacs for about 4-5 days then begin feeding on micro-zooplankton: usually eggs or nauplii of copepods. Young herring larvae occupy the upper parts of the water

column, usually the top 20 meters, where they are part of the plankton community. At this time they may be advected by water currents to considerable distances away (10-100+ km) from their incubation sites.

Larval mortalities are very high (~10 percent per day) during the early life stages but they grow rapidly and enter a 'juvenile' stage after several months. During their first year f life they are known as 'age 0+ herring. At this time they develop silver pigmentation and begin to resemble adult herring although their maximal size during their first year of life usually id less than 10 cm. There is considerable uncertainty about factors affecting the distribution or survival of age 0+ juveniles but there is string evidence of starvation by some during the winter in PWS.

Age 0+ juveniles seem to spend all of the time within PWS. They grow larger during the second summer of life and it seems that they also spend their second summer within the Sound. In other parts of the North Pacific, the age 1+ juveniles can sometimes be found on open shelf waters, especially during the later part of their second year of life. The distribution of herring juveniles the winter is an important issue, especially if some forms of enhancement are considered. It appears to be is this life stage that might benefit from some forms of intervention in attempts to restore herring to PWS. It follows that carefully design juvenile tagging programs could provide substantial information that could assist intervention and enhancement efforts.

As herring enter their third year of life many will begin sexual maturation. In general, the males tend to mature earliest by age and also are mature earlier with in spawning season. The age 3 and 4 year classes often make up a large part of the total population. Herring spawn only once a year but every year after they first reach sexual maturity. Most live to be about 8-10 years old, but some persist to age 15 or greater.

An important issue for all herring life history stages in PWS is the extent to which adult herring leave the inside waters and venture to shelf waters. In all other parts of their range summer feeding on the shelf is the norm, so it seems probable that PWS herring might do the same. This is an issue that might be addressed with some types of tagging work.

Commentary 1:

There is a vast scientific literature on Pacific herring *(Clupea pallasi)* and Atlantic herring *(Clupea harengus)* herring. There also is a substantial literature on herring in PWS. A recent report by Rice and Carls provides an overview of many aspects of herring biology (See Rice and Carls, 2007). A more general source of scientific herring literature is summarized in the 2001 Wakefield Symposium on Herring (Funk et al. 2001).

Summary 2: Pacific Herring Stock Status in Prince William Sound

Steve Moffitt,
ADF&G,
Commercial Fisheries Research, Cordova

Commercial herring fisheries in PWS began in the early 1900s when herring markets were for fish oil, fertilizer, fish meal; pickled fish, dry salted fish, or halibut bait. Peak catches reached

60,000 tons the 1930s. Herring roe fisheries began in the late 1960s and developed into separate fisheries for sac roe: spawn-on-kelp, and bait. Present management objectives attempt to provide for an optimum sustained yield equitable allocation among all user groups. A minimum threshold spawning stock biomass (SSB) of 22,000 tons (20,020 metric tons), set at 25% of the average unfished biomass, is required for fisheries to open. Exploitation rates can vary from 0 to 20% when the predicted SSB is between 22,000 and 42,500 tons (38,220 metric tons). Herring in all locations of PWS are assumed to be one stock but ADF&G uses a precautionary approach to account for possible local stock structure where each spawning concentration is considered as a possible separate stock group.

Stock assessment program and Current stock status

ADF&G has conducted stock assessments in PWS since 1969. Initially aerial and beach surveys provided data to estimate biomass and have continued almost without interruption. Biological data has been collected since 1973. Dive surveys to estimate spawning biomass began with feasibility studies in 1983 and 1984 and continued in 1988-1992. Following a sharp 1993 decline in abundance ADF&G and the Prince William Sound Science Center (PWSSC) conducted cooperative acoustics surveys in the late fall. Also, spring acoustics surveys, conducted immediately before spawning commences have been conducted every year since 1995. ADF&G began using an age structured analysis (ASA) assessment model in 1993. Subsequently the model was adjusted to account for disease mortality and hydroacoustics assessment data. The ASA model indicates abundance in 2009 are below the threshold level (22,000 tons) and all fisheries have been closed for 2009.

Decline and lack of recovery

PWS herring declined sharply between 1992 and 1993 but the exact timing of the decline is in contention. AD&FG's 1993 projection ~134,500 tons of SSB but spring assessment work prior the purse seine fishery detected few schools no purse seine sac roe harvests occurred in fish in 1993. By 1996-1998 the SSB recovered slightly and all fisheries were opened. SSB biomass declined again in the spring of 1999. No commercial fishery harvests have opened since then.
Reviews of hypotheses for the decline and lack of recovery indicate that outbreak of viral hemorrhagic septicemia virus (VHSV) are implicated, and perhaps exacerbated by a large SSB in poor condition in 1993. The *Exxon Valdez* oil spill may have had indirect effects. Since the 1993 decline, PWS herring appear to undergo disease outbreaks and abundance declines about every 4 years. but reasons for possible continued disease effects on the PWS population are unknown.

Commentary 2

The general methods of herring stock assessment conducted in PWS are consistent with those used in other areas, both within the North-eastern pacific and in Atlantic herring populations. There are a few notable differences. One is that in PWS total spawn abundance is quantified in units of 'mile-days' rather than the simpler sum of total spawn lengths (miles) within each season. Although this procedure might lead to some significant differences in total estimates of spawning biomass (relative to that that might be obtained by used the spawn quantification methods used elsewhere) the differences would not account for the changes in abundance sine

1993-1994. The decline in abundance is real and not a reflection of stock assessment methods which are credible.

Further, since the development of the herring roe fishery, the management system appears to have substantial built-in conservation measures (i.e., the 20 percent maximal harvest rate, etc). This management approach would compare favorably with many other systems used for other herring populations.

Summary 3: External tags –review of British Columbia programs

Doug Hay
Nearshore Research, Nanaimo, BC

The belly tagging methods used in BC were mainly limited to the early 'reduction fisheries' when herring were reduced to meal and oil. Catch rates in this fishery, that occurred from Washington State to Alaska, were very high and not sustainable – the fishery collapsed, coast-wide, in the 1960's. Tags were recovered win the processing plants. Often the quality of recovery information was poor wit uncertain recovery origin and date. On the other hand there were many thousands of recoveries, so considerable information was gained form this work.

Commentary 3.

The external Floy tag studies, conducted within coastal British Columbia, also provided a lot of interesting information about herring movements and herring. There was probably considerable tag loss, and concern that the application of the tag, which was an insertion into the flesh, led to injury with increased vulnerability to disease and predation. Nevertheless work that extended over nearly a decade provided more information on herring movements. Perhaps the most important contribution to the work was to show that herring spawning 'fidelity' was not as geographically exact as that of most salmonids – and there is reasonable doubt about whether it really occurs at all.

Summary 4: Coded wire tags, implant elastomer marks and alpha tags

Geraldine Vander Haegen,
Northwest Marine Technology, Tumwater, Wa

Coded wire tags have been used in Norway, Main and British Columbia. The attached report presents useful and detailed information about the advantages and disadvantages of these tags plus approximate estimates of cost. A great advantage to these tags is their small size and the demonstration that they can be successfully applied to herring. They cannot be used for eggs or larvae but there is potential to used coded wire tags on juveniles, perhaps age 0+ juveniles.

Commentary 4

A particular concern about coded wire tags is the technology for recovery required that magnetic detectors must be very close to fish – usually within inches. In a fishery that takes millions of fish it is probably impractical to screen every captured fish. There are about 5000-10 000 fish

per ton of adult spawning herring – so a fishery that took 200 tons would take over one million fish. Usually most roe fisheries will take a few thousand tons, so in most fisheries many millions of herring are captured. This seems to defy the possibility of screening each fish. However, there are now ingenious systems for rapid screening of fish on processing lines. Therefore if there were a commercial herring roe fishery in PWS, and if tag detectors could be set up in the processing plants, then recovery of coded wire tags may be feasible. The key considerations would be the costs of tag application and recovery – and the number of tags that would have to be applied and recovered for a meaningful program. Geraldine's report provides many useful and detailed estimates of such costs.

There is a brief section in the report that did not receive much discussion in the workshop but may be potentially interesting. The visible implant elastomer (VIE) are internal colored tags that are visible externally. The elastomer material is biocompatible and carries no known human health hazards. The tags may be applied to small fish, have high retention rates and with deleterious impact on the well-being of the fish. The main drawback is the difficulty of detection in ambient conditions, especially if there has been substantial growth of the tagged fish during the period between release and recovery. The visible implant alpha (VIAlpha) tag is a small, internally-implanted, fluorescent tag with an alphanumeric code but remain externally visible for easy recovery. The tags are implanted in transparent tissue (adipose eyelids, fin membranes, clear boney tissue) with syringe-like injectors, and are available in several colors and in two sizes: standard - 1.0 x 2.5 mm and large 1.5 x 3.5 mm. Tag material does not irritate the tissue at the implant site and s not deleterious to the fish. The tags may become obscured if the implanted tissue becomes pigmented.

Summary 5: Otoliths marks – two related chapters

Overview of marking options
Dion Oxman (ADF&G, Juneau, Ak,

Chemical Analysis – Growth Pattern Analysis
Andrew Munroe,
University of Adelaide, Adelaide, Australia

The two reports by are concerned with otoliths – as structure that can be used to receive artificial marks or reveal natural marks. Although prepared independently, the two papers are complementary. Dion Oxman provided a clear, systematic and concise review of five approaches. Andrew Munroe discussed the similar topics and emphasized the advantages, disadvantages of each and discussed the particular implications for herring. The reference sections for both papers are rich and cite many recent papers.

Otolith marks also can be created by stress treatments. For example, short thermal shocks are known to induce distinct rings on the otoliths of many species, but it is unclear if this can be done in herring.

Natural variability in otolith shape and microstructures has been used to distinguish among different populations of fish, including herring – and this might be possible for artificially reared Pacific herring. One specific concern, however, is the temporal consistency of such variation, especially among different cohorts. If consistency in otolith shape varied among years, either in

enhanced herring, wild herring or both, then to be useful, the otolith structure would need to be examined, and described each year.

A special class of otolith activities is the analysis of fine-scale chemical structure of otoliths, usually the elemental composition of the otolith at different periods of a fish's growth. Depending on the species, it is often possible to trace the chemical history – and infer the ecological history – from spatial variation in the otolith. Specifically, the ratios of elements changes, as the fish changes habitats from the larval stage to the adult stage.

A special aspect of otolith microchemistry could is the potential for the addition of specific elements (of 'rare earths') that would provide a unique chemical signature or 'fingerprint'. Such artificial additions are usually provided in the holding water, and taken up by the fish through the gills. There was keen interest from some of the workshop participants about whether such chemical additions could be provided through food – because one of the potential restoration options was the promotion of the food supply to wild herring populations in specific areas. If additional food could be spiked with a unique but innocuous chemical fingerprint, this would assist with the evaluation of the efficacy of this approach.

Commentary 5

It is probable that some form of otolith marking is essential if herring supplementation is considered. Japanese herring enhancement work used alizerine complexerone to mark the otoliths of very young larvae. The workshop, however, identified a number of other potential marking agents that warrant consideration: oxytetracycline, calcein and strontium chloride. The advantages and disadvantages of each are considered in the detailed reports.

There is some uncertainly if the dyes are effective is applied to egg stages, but this is not a vital question at this time because most marking could be done at the larval or early juvenile stage.

One of the main uncertainties about the evaluation of otoliths marks is the cost of analyses, the numbers of fish that would need to be marked and the numbers of samples (or recoveries) that would be required to assess the survival of marked fish. (See also the summary and commentary for Chapter 7, on instrumentation). The use of chemical dyes or markers must first examine potential concerns related to permitting.

Summary 6: Acoustic Tags - Will they work? What life stages?

Andy Seitz, University of Alaska, Fairbanks
Brenda Norcross, University of Alaska, Fairbanks

Acoustic tags emit acoustic pulses that encode an ID numbers that are recorded by acoustic receivers when a marked fish is within range – usually a few hundred meters. Tags vary in size but are becoming progressively smaller with time The smallest can implanted in fish as small as 12 cm – equivalent to age 0+ herring in the late summer or fall of their first year of life. These tags may provide a new tool for examination of migration patterns and other life history

questions on adult Pacific herring. However, herring may be susceptible to handling stress. Preliminary work is underway that will examine the feasibility of implanting acoustic tags in Pacific herring under in laboratory conditions.

Commentary 6

Acoustic tags are relatively expensive (at several hundred dollars each) and would be limited to the large juvenile and adults stages of herring. The key application would be information about the movements of herring within PWS and between the PWS and outside shelf waters. The possible (or 'probable') use of the shelf waters as summer feeding areas is a fundamental aspect of PWS herring that begs to be resolved. Throughout their range in the eastern Pacific, most herring populations use the productive shelf waters for feeding, and return to inside, nearshore waters such as those within PWS, for over-wintering and spawning. This issue is fundamental to the question of herring enhancement in PWS because it is essential to understand if the limitations to herring population growth occur both within and outside of the Sound. Utilization of acoustic tags could address that issue.

It may be an opportunistic time to consider application of acoustic tag technology because they will be employed to examine movements of other species, especially salmonids, within PWS and adjacent waters. One of the major costs related to acoustic tags is the deployment of acoustic receiver. Through cooperative and collaborative research called POST (Pacific Ocean Shelf Tracking) an array of 10 receivers was installed across the mouth of Port Gravina. The array consists of 10 VR3 acoustic receivers spaced ~800m apart and moored at depths ranging from 43-130 m. Te PWS Science Center has installed an additional eight receivers in and around pinnacles near the POST array.

Summary 7: Fatty Acid Signatures – Stable over time?

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Ted Otis, ADF&G

Can fatty acid signatures (FAS) could make effective markers for some types of research and monitoring activities? The answer to this question was addresses in this informative review. The review points out the uncertainly about the temporal stability of FAS in certain tissues, such as heart muscle. On one end of the scientific debate, FAS's are seen to be definitive and under genetic control. On the other, FAS's are considered to represent prevailing trophic conditions (i.e. herring are what they eat).

Commentary 7

Perhaps remarkably, the first conclusion from the review of FAS's as potential marks, is that they are not practical. In part, that conclusion may be over-stated. There may well be a role for some fatty acid analysis. For instance, in any situation, such as a hatchery, where herring are reared on an artificial diet, the reared fish will have a different FA than wild or naturally-fed fish, although the retention of the unique FAS is unlikely to persist over time – but probably differences could be detected between release-hatchery fish and wild fish over a period of weeks or months, especially during the winter, when feeding activity is diminished. It follows that that

there may be potential to use FAS's to identify and distinguish between hatchery-reared and wild juveniles.

Another potential application is related to one of the potential herring restoration options of providing food to wild juveniles, especially age 0+ herring in certain bays where previous biological studies have shown that many reach a period of irreversible starvation during the winter. Supplemental feeding has been suggested as a potential solution to preventing or limiting death by starvation in some bays. It follows that examination of fatty acid signature of the artificially-provided food, period samples of the fish (age 0+ juveniles) plus suitable control groups, would provide evidence of the whether the supplemental food was being consumed by herring.

The review did not consider the potential for using fatty acid signatures as marks under all of the seven potential restoration options – particularly the suggestion that supplemental food could be provided to age 0+ juveniles in order to reduce the rate of over-wintering starvation. As the present time this option is more of a concept than a well-defined procedure. The technically feasibility if such additions has not been established. The apparent starvation of some age0+ herring, during the winter period, may not be a phenomenon unique to herring. Also, winter starvation may (or may not) be an explanation for the strength of subsequent recruitment, although it seems logical that it might be. Recent work on herring juveniles in BC and elsewhere indicates that cohort strength appears to be established by the fall of the first year of life (Hay et al 2003, Schweigert et al., in review). If the same processes also occurred in PWS then supplemental feeding over the winter, after the summer period, may be futile. However a potentially important difference between PWS and other areas, is the possible role of hatchery-reared salmon in the reduction if available food for age 0+ juveniles. It is plausible that PWS herring juveniles are put more at risk of death by starvation if their food supply during earlier months has been compromised by the enhancement of competitor species, such as pink salmon juveniles. If so, the provision of supplemental food, if technically feasible, may be a useful approach and analyses of FAS's could be very useful.

Summary 8: Genetic Marking Strategies

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Chris Habicht, ADF&G, Juneau
Jeffrey Olsen, USFWS, Juneau
There are two distinct parts to this report – so there are two summaries and two commentaries.

Summary 8

This contribution reviewed the genetic structure of PWS herring discussed the potential use of natural and transfected genetic marks to track the supplemented fish. Results of genetic studies conducted to date provide equivocal results: there is evidence both of limited spatial structure within PWS but, like some reports form other populations, the genetic variation may not be temporally stable. Relative to most salmonid species, herring exhibit relatively small degrees of genetic variation on over broad geographic distances. This implication is that there is considerable mixing of herring among different populations – even between relatively distant populations in different parts of the Gulf of Alaska.

Commentary 8

The report on PWS herring genetics provides an overview of population genetic theory and describes PWS herring as a 'metapopulation', following the application of this term in many previous scientific papers. It is essential to point out, however, that metapopulation theory – especially as it might apply to Pacific herring – is still in a period of refinement and there are other possible interpretations for the observed genetic variation seen in herring. A key issue is the geographic scale that is application to the designation of a herring population as a 'metapopulation' or 'sub-population'. Most Atlantic herring populations are much larger than those in the Pacific and occur over a much greater geographic range. For example the Norwegian spring spawning herring consists of many millions of tons and ranges from Southern Norway to the Barents Sea – a distance exceeding that between the coast of Washington State and PWS. Clearly the ecology of these Norwegian herring differs from that of the eastern Pacific herring so caution must be taken when comparing population structure among populations that wary in size and range by orders of magnitude.

Summary 9: Genetic marking methods

The genetics report provides two interesting suggestions for the potential application of genetic marks for herring: insert a novel gene or to alter the frequency of a naturally occurring gene. Transgenic techniques could be used to add a new unique genetic mark to hatchery-raised fish so that they (or their progeny) will bear the mark. Procedures for adding a molecular mark have been developed for other fish species. A second method for genetically marking fish would use controlled breeding to alter the frequency of an existing genetic marker (i.e. a microsatellite allele) in hatchery fish relative to the wild fish.

Commentary 9

Both approaches would raise concerns from a number of sources. A major policy concern would be the release of genetically modified fish. Even if the approach is technically feasible, there would probably be strenuous objections from a number of sources, including organizations such as the American Fisheries Society.

There are some additional and formidable technical and logistic concerns. One is the requirement for the development of a herring broodstock. It does appear that the rearing of viable herring broodstock has not yet been achieved. Herring were reared to sexual maturity in pilot (unpublished) experiments at the Pacific biological Station in Nanaimo BC, but the development of females was not synchronous, and varied widely among females. All fish were reared in tanks supplied with natural running seawater from Departure Bay in the Strait of Georgia. Tank temperatures and photoperiods were nearly identical to natural, ambient conditions. Some herring originating from a population that normally matured and spawned in early March were mature in mid-December. In the few instances where a ripe male and female were available at the same time, the eggs were not viable: fertilization appeared to be normal, but the eggs died during early development. The simple conclusion from this pilot work is that rearing a viable broodstock of herring is a formidable task, requiring substantial effort and access to expensive fish holding facilities.

On the positive side, a genetics approach to marking could provide a relatively high number of genetically modified or genetically unique fish. For instance, with perfect survival from egg to age-3 recruit, would require the progeny from only 100 kg of spawning adults to produce 100,000,000 recruits. Such a number would represent a very strong cohort in PWS. Even allowing for significant mortality, the required size of the broodstock probably could be developed. For instance there would be only between 500-1000 adult herring in 100 kg of captive spawning broodstock fish.

Therefore the most formidable concerns with a genetics approach would be the acceptability of the process. Almost certainly there would be vigorous resistance from the related to public perception, concerns within the scientific community and concerns from government and regulatory agencies. The problem of developing a captive broodstock would require advancements that have not yet been achieved but probably have not been seriously attempted on a broad scale. Even if these concerns were addressed, there would still be an issue related to the cost of screening fish, in order to assess the relative survival of hatchery-released fish.

Summary 10: Sampling considerations of a mass marking program to evaluate herring enhancement efforts

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The essence of any mark and release program will be an estimation of the survival of the marked fish. To do this there must be some prior understanding of the approximate number of fish that are likely to occur in the wild, the numbers of marked fish that have been released to the wild and the numbers of total fish that will be recaptured. In theory this seems simple but in practice, the ratios of marked fish to wild fish are very important. This Chapter explains how confidence intervals (CI) change as a function of the proportion of the marked fish in a sampled population. It advocates the statistical and methodological advantages of having pre-determined assessment and calculations of assumptions and factors affecting estimates of confidence levels.

Incorporation of quality control processes is another consideration for establishing sampling criteria in a mass marking program is. In practice this involves factors such as the quantitative assessment of tag- or mark-induced mortality, tag or mark retention and error associated with tag or mark recognition in recaptured fish. The absence of commercial fishing for PWS herring presents special challenges for potential tag recapture in PWS. Further, in contrast to salmonid species, the population structure and migration routesof herring are not well defined. Therefore it will important sampling effort is can be adjusted in order to accurately estimate the success of the enhancement effort.

Commentary 10

If any enhancement-related project proceeds to the point where marking or tagging is considered, it will be necessary to first conduct some relatively simple modelling studies. Such modelling would consider and comment of the issues of the numbers of marked fish that must be released, the corresponding the numbers of recaptured order to evaluate the success of marking – or enhancement. As pointed out in Chapter 6, it also will be essential to include potential quality

control issues related to tagging mortality, tag shedding, mark recognition and the potential for behavioral issues (migration, homing etc) that potentially affect affect spatial distributions or released and wild fish.

Summary 11. Instrumentation and Recovery of Marks on Fish Hard Parts (Particularly Otoliths)

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This chapter comments on factor natural and induced marks on otoliths and points out analytical issues from the perspective of a laboratory scientist. The chapter provides interesting perspectives on the analytical advantages and disadvantages of between natural marks and induced marks that must be read on a 'presence' or 'absence' basis. Disadvantages of applied marks could arise from fish handling exposing fish to injury, disease susceptibility or mortality. Further there are increased costs and associated with the application of marks. Analysis of natural marks avoids such risks. The explanation for the natural marks is lacking. Presumably in marine fish otolith chemistry is affected by water chemistry. Therefore differences in otolith marks among fish are likely to be subtle because marine water is relatively homogeneous over broad geographic areas. Consequently analysis of natural marks requires more precision and care with sample preparation.

Complications arise if the desired mark on an otolith occurs in only a very specific location. Instruments vary in their analytical precision. Consequently the spatial resolutions of analyses varies with instrumentation and can affect the interpretation of analytical results. Also instruments vary in their detectability of some elements.

Analytical time can often be shorter with well prepared samples – for example it may take only taking only seconds to positively identify a well prepared Sr marked otolith, but it can take many minutes to positively confirm that identify a poorly prepared specimen lacks a mark. Also, warm-up times vary among different instruments

Commentary 11

This Chapter points out the desirability of including a practical laboratory analyst in any potential future experimental design. In particular, information on the sensitivity and costs of analysis are essential. It is especially important that the implications of cost of laboratory analysis be addressed relative to the statistical issues (see Summary 10 – or Chapter Six). The Chapter also points out clearly that extra time (or cost) related to sample preparation may prove to be cost-effective if it reduced the time (cost) of laboratory analyses.

Summary 12: Alaska Department of Fish and Game Fish Transport – genetic issues and strategies

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Six important statements from this chapter are as follows:

1. An Alaska Department of Fish and Game (ADFG) Fish Transport Permit (FTP) is required for any transport of fish or eggs within or into the State.
2. An FTP is required to take wild fish or eggs into a culture facility and to release eggs or fish into the wild.
3. There would be genetic concerns associated with the issuance of an FTP for research, restoration, or enhancement of PWS herring.
4. The ADF&G Division of Commercial Fisheries has a Genetic Policy that was written with Pacific salmon in mind (http://www.genetics.cf.adfg.state.ak.us/policy/genepol.pdf), but its tenets also apply well to Pacific herring.
5. This policy places primary emphasis on the protection of wild stocks to ensure that the actions proposed do not harm wild stocks.
6. If the actions proposed have any potential to harm wild stocks, the genetic review determines if the likely benefits from the proposed actions are likely to outweigh the potential harm.

Commentary 12

This chapter provides a very useful, succinct explanation of policy issues that could arise with some types of herring enhancement activities would not prompt genetic concerns. The main exception would be the use of genetic tags where the released fish may be genetically dissimilar to wild fish (see chapter on genetic marks).

A different but partially related issue would be the potential for disease transfer, associated with fish transfers or release of cultured fish. This issues was not addressed I the workshop but would ne an additional consideration for many enhancement-related activities.

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Chapter three - Synthesis, comparisons and recommendations

Life-History versus marking methodology

The first set of connections is to relate the seven tagging-marking methodologies to eight herring life history stages. Further, for each stage there is both a potential stage– or range of stages – for potential tag-mark 'application' and a different stage (or range) for potential tag recovery. These linkages are shown in Table 1.

Table 1 does not show 'natural marks' because, *a priori*, it seems improbable that most types of enhancement, such as artificial rearing of eggs are larvae, would induce such a mark, but the possibility cannot be ruled out. Although the analyses of 'natural marks' received a considerable amount of attention in several of the chapters, 'natural marks are not included in Table 1.

Application-recovery of chemical and dyes (Rows 2-4 and Columns 1-2 in Table 1)

It is uncertain if chemical dyes or chemicals would effectively mark the otoliths of pre-hatched embryos. To do so the dye would need to pass through the egg capsule (chorion) and then be taken up by very small otoliths. Even if the dye were taken up it seems probably that the mark would be very tiny, and correspondingly difficult to detecting recaptured fish as later life stages.

Following the Japanese experience (See the 2007 EVOSTC white paper on the feasibility of herring enhancement) it is much more certain that dyes can be taken up by larvae or young juveniles and then effectively detected at later life stages.

Application-recovery of external tags (Rows 4-8, Columns 5-6 in Table 1).

Probably the both the application and recovery of external tags could not occur earlier than age 0+ juveniles, application and recovery could occur at all later life stages. External tags, however, have not proved to be successful for herring because of high tagging mortality and tag shedding. They would not be recommended for the monitoring of a herring enhancement program in PWS.

Application-recovery of internal coded wire (cwt) tags (Rows 4-8, Columns 7-8 in Table 1).

Coded wire tags (cwt) have been successfully applied to herring. It is plausible that they could be applied to PWS herring. The major drawback concerns issues of recovery. Normally tagged herring can be recovered in processing plants, although such recovery can be expensive. The problem with PWS herring is that there is no commercial fishery. Even if smaller, 'research' samples were taken, it seems improbably that such small catches could encounter sufficient herring to make the use of CWT successful. (See Commentary 6).

Application-recovery of acoustic tags (Rows 4-8, Columns 7-8 in Table 1).

It is probable that adult herring, and perhaps large juveniles, can successfully live with surgically-inserted acoustic tags. It also seems probably that some herring would be detected by

the array of receivers that will be installed within PWS and adjacent waters. In short, acoustic tags may present a good opportunity to tag adults (or large juvenile) herring and learn something about their movements.

The main difficult with consideration of using acoustic tags on PWS herring is that it is a stretch to justify the activity as having a direct connection to enhancement. At best information from such work could provide very useful information about the ecology and migrations of PWS herring a,d such information, in turn, could indirectly support enhancement.

Therefore probably a cautious recommendation to support such activity is warranted. The 'caution' aspect of such a recommendation would be to ensure that the results of such work are reported in a timely fashion.

Application-recovery of genetic fingerprints (Row 1, Columns 1-14 in Table 1).

Is the oocyte a stage for application of genetic marks? The 'oocyte' stage refers to the unfertilized eggs within an ovary. Although it may be debatable, some may argued that it is within the ovary, when genetic marks could be applied within an ovary. (Others may argue, perhaps correctly, that it within the female adult stage that a genetic mark is applied.) Regardless such an application would occur only with females that are part of a distinct broodstock, and not from females extracted from the wild population.

Regardless of the stage of the application of a genetic mark, the recovery of genetic mark could occur, theoretically, at nearly any stage, from an egg to an adult (see Column 12). In practice, if genetic marks were applied to herring in an attempt to increase recruitment, then the age-3 (or age-4) recruit stage would be best time to look.

Application-recovery of fatty acid signature (Row 1, Columns 1-14 in Table 1).

In theory, fatty acids signatures could be applied and recovered at by time but this is a strain on the terminology. The main problem with fatty acids is that they seem to lack temporal stability. Over short periods, however, there may be useful application of fatty acids, especially if there were attempts to provide artificial food to over-wintering juveniles – which is a suggestion made for one potential herring enhancement-restoration option.(see commentary 7). A considerable concern, however, is the relatively steep cost of laboratory analyses. If any potential applications were considered, they would be best applied to tightly focused objectives, preferable conducted at small spatial scales

Table 1. Simplified life history stages of Pacific herring in Prince William Sound .

The left column shows the life history stages of herring progressing from an unfertilized egg – or 'oocyte' within a female to a spawning fish. For each of the seven types of marking or tagging methods the applicability is shown both for the application (App) of tags and the potential for recovery (Rec). The boxes labeled 'm' (for 'maybe') indicate uncertainty. Boxes labeled 'yes' indicate that the tags probably can be applied, or recovered, at that stage. The blank boxes represent combinations that probably are not biologically feasible. Note: The 'yes' boxes do not consider either logistic feasibility (in terms of numbers released or recovered) or legal acceptability. The row and column numbers are used for reference in the text.

	Stage	Chemical dye		Chemical otolith		external tag		internal nose cwt		acoustic		genetic		fatty acid	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
		App	Rec	App	Rec	App	Rec	App	Rec	App	Rec	App	Rec	App	Rec
1	Oocyte -within female											yes	yes	yes	yes
2	Egg(y)	m*	na	m*	na								yes	yes	yes
3	Larvae(y)	yes	m1*	yes	m1*								yes	yes	yes
4	0+ Juvenile(y)	yes	yes	yes	yes	m2*	yes	m2*	yes	m2*	m2*		yes	yes	yes
5	1+ Juvenile(y+1)		yes	yes	yes	yes	yes	yes	yes	yes	yes		yes	yes	yes
6	2+ Pre-recruit(y+2)		yes		yes	yes	yes	yes	yes	yes	yes		yes	yes	yes
7	Adult(y+3+)		yes		yes	yes	yes	yes	yes	yes	yes		yes	yes	yes
8	Broodstock		na		na	yes	yes	yes	yes	yes	yes		yes	yes	yes

*m1** *Eggs of very young larvae may have otoliths that are too small for effective marking*

*M2** *The smallest 0+ juveniles may be too small to maintain a nose tag or acoustic tag.*

Comparisons among approaches

Table 2 provides a more detailed summary of each tagging of marking option that links each approach to the contributor. Table 2, when considered relative to summaries and commentaries in Chapter two, plus the preceding analysis in Table 1, serves to reduce the feasible – or useful - number of tagging and marking options – based mainly on technical criteria.

For instance, external tags seem to be unacceptable because of high tag loss and induced mortality. Coded wire tags can work successfully, but require a fishery for effective tag recovery. Acoustic tags would appear to be promising, but the results, although immensely useful for illuminating issues of general herring biology, may be only parenthetical to issues of herring restoration or enhancement. Genetic tags/marks are plausible, but would be dependent on the unprecedented development of herring 'broodstock' rearing. Also the resistance to release of genetically modified herring would probably be insurmountable. Fatty acid analysis may have a role for analysis of specific issues (i.e., provision of external food) but such an application would require considerably more research and it would probably be expensive.

The potential marking that does seem to have some promise is the array of chemical dyes and marks (Table 3). It is not clear, however, which approach would be best, although the simplest approaches, which involve a chemical dye mark applies to the otolith at an early life history stage, seem to be the most expensive and the least expensive. Such approaches have been successfully applied in Japan using the Alizarin Complexerone. During discussions of this approach in the workshop it was clear that the advantages and disadvantages of each specific dye or chemical vary. Some of more expensive than others and the permitting issues vary according to each substance. The discussions also seemed to reach a consensus that although the regulatory barriers for the use of chemical dyes, while formidable, were not necessarily impenetrable.

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Table 2. Summary of the types of marks and tags shown by each life history stage and according to each presentation the workshop. The boxes labeled 'm' (for 'maybe') indicate uncertainty. Boxes labeled 'yes' indicate that the tags probably can be applied, or recovered, at each life-history stage. The 'yes' boxes do not consider either logistic feasibility (in terms of numbers released or recovered) or legal acceptability.

		Life history stage - application						
Workshop Presenter	**Type of tag or mark**	**egg**	**larvae**	**larvae**	**0+ J**	**1+J**	**R**	**A**
Doug Hay	external Floy tags,				m	y	y	y
	internal belly tags					y	y	y
Vander Haegen	coded wire tags				m	y	y	y
Andy Seitz	acoustic tags				m	y	y	y
Dion Oxman	review of otolith marking	m	y	y	m			
Andrew Munro	chemical analysis of otoliths	m	y	y	m			
Olsen/Guyon	genetic marking	y						y

Table 3. Summary of the otolith marking methods that might be applicable for mass-marking of PWS herring shown each life history stage. The boxes labeled 'm' (for 'maybe') indicate uncertainty. Boxes labeled 'yes' indicate that the tags probably can be applied, or recovered, at each life-history stage. The 'yes' boxes do not consider either logistic feasibility (in terms of numbers released or recovered) or legal acceptability.

		Life history stage - application						
Workshop Presenter	**Type of tag or mark**	**egg**	**larvae**	**larvae**	**0+ J**	**1+J**	**R**	**A**
1. Stress marks – developing unique dark rings on otoliths								
	thermal marks, dry marks		m	y	y			
2. visible markers -fluorescent chemicals								
	alizerine complexerone	m	m	y	y	y		
	oxytetracycline	m	m	y	y	y		
	calcein	m	m	y	y	y		
	strontium chloride	m	m	y	y	y		
3. invisible markers								
	added elements – rare earths	m	m	y	y	y		
4. natural markers								
	otolith shape			m	y	y	y	y
	microstuctural		m	m	y	y	y	y
	elemental fingerprints		m	m	y	y	y	y
5. Transgenerational marking		y						

A review of real or perceived obstacles to implementation of marking or tagging

1. Public perception.

Negative public perception could probably be a major obstacle issue if a genetically modified fish were released, even if the modifications were limited to the selectively neutral microsatellite alleles.

2. Regulatory and permitting obstacles.

The ADF&G has strict policies regarding the movement of wild fish and the release of cultured fish into the wild. Marking that required the holding of fish for extended periods, would need to develop methods to satisfy these policy criteria. There also are disease issues that would need to meet policy limits. It seems unlikely that present regulatory agencies (State or Federal) would permit the released of genetically modified fish, regardless of how innocuous the modification.

3. Technical knowledge and capability

The knowledge and capability to institute any of the tagging methods exists, and most of it already is within the state of Alaska. The challenge would be to marshal the collective expertise to commit to working with a herring marking project. It seems unlikely that a major marking or tagging program could get underway without some considerable support by a major research agency that has experience and capability with similar projects. Small contracting organizations or individual contractors probably would not be able to efficiently summon all of the available skill sets required for this work. Skill sets would include in-depth knowledge of fish husbandry, physiology and disease, nutrition, fish genetics (even if the project were not concerned with genetic marks), elements of physical and biological oceanography, plus a grasp of the statistical issues related to determining the numbers of released and recaptures needed for valid work.

Even if there were a clear choice about optimal types of tags, there are many uncertainties about how the technological approach marking or tagging program could proceed. For instance, the use of chemical marks would require relatively long-term holding and rearing of young herring juveniles. The types, locations and scales of such facilities are uncertain.

4. Technical limitations – a recovery' dilemma

The vexing issue about marking of PWS herring is that the recovery of marked individuals would be very limited unless there is a commercial fishery. Ironically, if there were sufficient spawning stock biomass to warrant a fishery, then a herring enhancement program would not be required. It is not clear if there is potential for a satisfactory 'work-around'. Such a solution would depend on getting permission to sample a sufficient number of spawning fish to assess the survival of marked fish. This issue requires careful examination.

5. Costs.

Cost estimates of tagging programs can be estimates once the numbers of potential approaches is reduced. However, the cost of tag applications are probably small relative to potential costs of developing herring holding and rearing facilities, regardless of the physical form of such facilities could have. Further, the cost of examining captured fish to estimate the proportion of marked individuals will vary according to the proportion of fish tagged relative to the numbers of wild fish.

Chapter Four - Prince William Sound Pacific herring stock status

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Prince William Sound Pacific herring regulatory management plan

This section is intended to summarize the Prince William Sound Pacific herring *Clupea pallasii* management plan and associated regulations that may influence the health of the resource. This section does not review regulations that relate to items such as the size of the sign required on a pound structure.

The PWS management area (Registration Area E) is described in 5 AAC 27.300 as follows: "The Prince William Sound Area has as its western boundary a line extending south from Cape Fairfield, as its eastern boundary a line extending south from Cape Suckling and as its southern boundary 59° N. lat." (Figure 1).

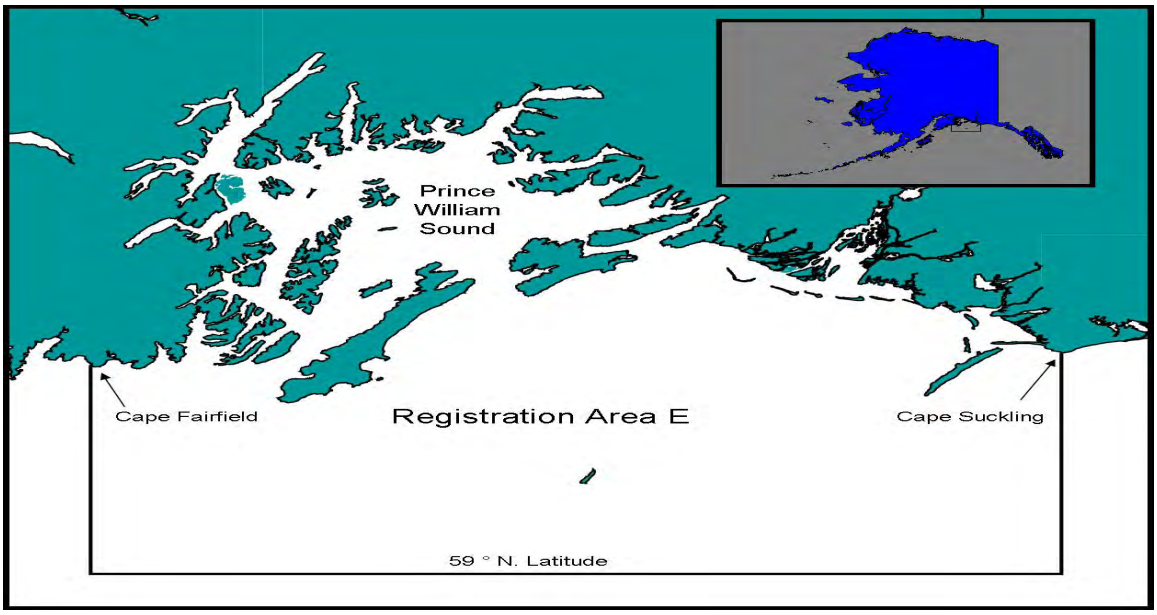

Figure 1. Pacific herring Registration Area E (5 AAC 27.300 Description of Prince William Sound Area).

Prince William Sound has a history of commercial exploitation of Pacific herring dating back to the early 1900s. (Rounsefell and Dahlgren 1932). Commercial markets in the 1920s through the 1940s were for fish oil, fertilizer, or fish meal; pickled fish, dry salted fish, or halibut bait. Significant harvests of Pacific herring (~60,000 tons peak) occurred in the late 1930s with the development of the reduction fishery (Pirtle et al. 1970).

The modern era of herring exploitation in PWS began with increased demand for herring roe from Japanese markets in the late 1960s. By the 1980 there were five separate fisheries for

herring in PWS including two fisheries for sac roe: 1) spring purse seine sac roe, and 2) spring drift gillnet sac roe; two fisheries for spawn-on-kelp: 1) spring wild harvest of spawn-on-kelp, and 2) spring impoundment or "pound" spawn-on-kelp; and finally a fall/winter food and bait fishery. (Randall et al.1981).

Fishing seasons are set in regulation for the food and bait and sac roe fisheries; however, fishery open periods are established using the emergency order authority delegated to the Alaska Department of Fish and Game (ADF&G). The management year for herring is from 1 July through 30 June, so the first fishery that occurs in a management year is the fall/winter food and bait fishery. Spawn-on-kelp fisheries do not have a season in regulation and open periods are established by emergency order.

The PWS Herring Management Plan, 5 AAC 27.365, has as objectives to 1) provide for an optimum sustained yield and 2) provide an equitable allocation among all user groups. The fishery is managed for a minimum spawning biomass of 22,000 tons (20,020 metric tons); no fisheries will open if stock assessments indicate the predicted biomass will be below this threshold. The threshold is set at 25% of the average unfished biomass should allow fairly quick recoveries from perturbations (Funk and Rowell 1995). The management plan allows for an exploitation rates from 0 to 20% when the predicted biomass is between 22,000 and 42,500 tons (38,220 metric tons). The exploitation rate can be adjusted based on the anticipated age class strength. The department may allow a maximum exploitation rate of 20% when the projected spawning biomass exceeds 42,500 tons. The threshold (22,000 tons) and maximum exploitation rate (20%) policy is a compromise between maximizing yield and providing stable yields through time (Funk and Rowell 1995). For management purposes, herring in all locations of PWS are assumed to be one stock.

Although the regulatory management plan considers all herring in PWS to consist of one stock (5 AAC 27.365), ADF&G uses a precautionary approach to account for possible local stock structure. When the sac roe fisheries began in the late 1960s, ADF&G had little stock structure information. Therefore, a precautionary approach was used to manage the fishery and each spawning concentration was assumed to be a separate stock group. Management strategies and ideas about the stock structure developed with the fisheries.

The projected prefishery run biomass is based on the final spawning biomass estimate from the previous year, cohort analysis, and projected recruitment. The plan allocates the projected available herring surplus among the five herring fisheries (Table 1).

The spawn-on-kelp fisheries are not harvesting fish, so the quota percentages are adjusted to spawn-on-kelp product from the actual fish biomass (Morstad and Baker 1995). Of the four spring fisheries in PWS, only the wild spawn-on-kelp harvest is open entry. For the remaining spring fisheries there are 104 permanent and 2 interim purse seine sac roe permits, 24 drift gillnet sac roe permits, and 128 herring pound permits in PWS. The fall/winter food and bait fishery is open entry; however, there are vessel restrictions.

Table 1. Percentage of the guideline harvest level allocated to each of the five fisheries for Pacific herring in Prince William Sound.

Fishery	Percentage of the guideline harvest level
Purse seine sac roe fishery (spring)	58.1%
Gillnet sac roe fishery (spring)	3.4%
Food and bait fishery (fall/winter)	16.3%
Spawn-on-kelp not in pounds (spring)	8.0%
Spawn-on-kelp in pounds (spring)	14.2%

Stock assessment program

ADF&G has completed Pacific herring stock assessments in PWS since harvesting herring for roe and harvesting roe-on-kelp began in 1969. Population trends were initially monitored with aerial surveys and beach surveys to estimate biomass and the linear extent of beach used for spawning (Brady 1987), and have continued almost without interruption. Age, sex, and size data has been collected from most fisheries and spawning aggregations since 1973 (e.g., Baker et al. 1991). Dive surveys to estimate spawning biomass began with feasibility studies in 1983 and 1984 and continued in 1988-1992 (Brown and Baker 1998) and 1994-1997 (Willette et al. 1998). In 1975, the department began conducting winter hydroacoustics surveys to evaluate stock status; however, these were generally not very successful (e.g., Randall et al. 1983). Following the decline in herring abundance in 1993, ADF&G in cooperation with the Prince William Sound Science Center (PWSSC) resumed acoustics surveys in the late fall (e.g., Thomas and Thorne 2003). However, because herring are more aggregated and stationary immediately before spawning commences, spring (March/April) acoustics surveys have been conducted every year since 1995.

ADF&G began using an age structured analysis (ASA) model to forecast the size of the prefishery run biomass in 1993 (Funk 1994). The model provides a best fit to the time series of historical data including purse seine harvests, purse seine harvest age compositions, spawning escapement age compositions, spawn deposition survey spawning biomass estimates, and aerial survey miles of spawn estimates. After the population level problems with disease became evident in 1993, the model was adjusted to account for disease mortality (e.g., Quinn et al. 2001; Marty et al. 2004). Subsequently, the ASA model was adjusted to include the hydroacoustics assessment data directly into the model (Hulson et al. 2008).

Current stock status

The current biomass trends are tracked with three measures of abundance: 1) aerial survey biomass estimates, 2) aerial survey mile-days of spawn, and 3) hydroacoustics survey estimates

of the prespawning biomass. The aerial survey biomass estimates are not used in the ASA model and won't be discussed further. Mile-days of spawn are the sum of the daily survey estimates of the linear shoreline extent of milt in the water (Brady 1987). The historical time series (1973-2007) of mile-days of spawn were recalculated in 2007 after all maps were digitized. The data are available on the PWS Herring Portal (http://www.pwsherringportal.org/Home.htm). The acoustics estimate trends generally follow those shown by the aerial survey mile-days of spawn indices (1997-2008; $r^2 = 0.578$; p=0.004).

The 2009 ASA model output for the historical time series of abundance and biomass estimates are below the threshold level of 22,000 tons and all fisheries have been closed for 2009 (Figure 2).

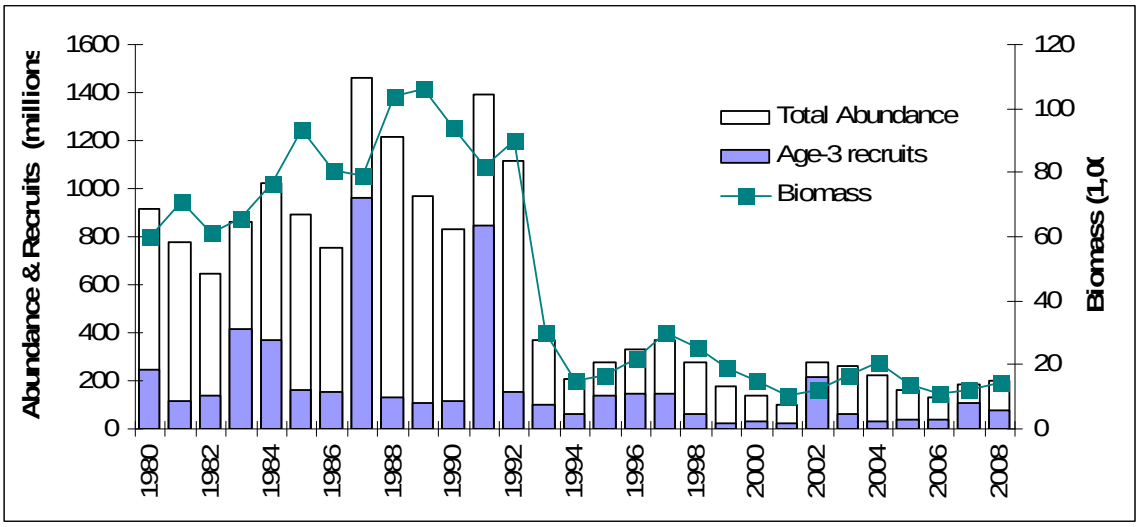

Figure 2. Total abundance, age-3 recruitment, and estimated prefishery run biomass from the 2008 version of the ASA model for Prince William Sound herring.

Decline and lack of recovery

The Prince William Sound herring biomass declined significantly between 1992 and 1993, although the timing for the beginning of the decline is in contention (Hulson et al. 2008; Thorne and Thomas 2008). The department projection for 1993 was ~134,500 tons of adult spawning herring (Funk 1994); however, spring assessment work prior the purse seine fishery found few schools and the purse seine sac roe fishery did not harvest any fish in 1993. The biomass recovered slightly and all fisheries were opened in 1996-1997 and 1997-1998. The biomass declined again in the spring of 1999, and only a few tons of fish were introduced into pounds in 1999. No commercial fishery harvests have been opened since 1999.

Hypothesis for the decline have been reviewed several times, most recently in Carls and Rice 2006. The available evidence suggests that the decline can best be explained by an outbreak of viral hemorrhagic septicemia virus (VHSV) in a large biomass in poor condition. No available evidence suggests the *Exxon Valdez* oil spill was a direct cause after 1989, but it may have contributed indirectly because the lack of fishing in 1989 increased the size of the biomass at a time of declining zooplankton abundance (Carls and Rice 2006).

Since the decline, the PWS herring have had disease outbreaks that appear to have contributed to population level declines about every 4 years (Marty et al. 2004). The inclusion of the age stratified disease information in the ASA model leads to better model fits than using other models. However, the reasons for possible continued disease effects on the PWS population are unknown.

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Chapter Five - Internal belly tags and external anchor tags

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Tagging work in BC from 1936 to 1967 used internal belly tags (Fig. 1). Tags were recovered from processing plants at the end of the fishing season so the dates of recapture was unknown, except for the year. More recent experiments (1979-1992), with external anchor tags, were often able to recover specific dates of recapture. Hay et al. (1999) used tagging data to comment on geographic fidelity and homing to previously used spawning sites (Daniel, K., McCarter P.B. and Hay, D. 2001).

The tags

The belly tags used from 1936-1967 were nickel or silver-plated iron rectangles with rounded ends (19 mm long, 4 mm wide and 1.6 mm thick) that were inserted into the body cavity through a small incision (Hart and Tester 1937).

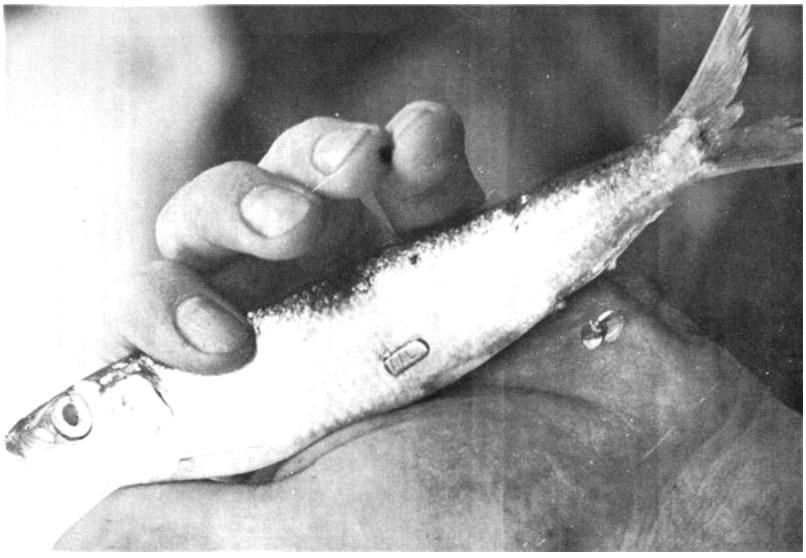

Fig. 1. Photograph of a partially inserted metal ‘belly tag’.

The Floy© anchor tags (Fig. 2) used from 1979-1992 were made of a plastic tube attached to a monofilament T-shaped end that was inserted into the dorsal musculature (Hay, 1981). Both the belly tags and anchor tags had individual coding numbers so recoveries could be traced back to the date and location of release. Laboratory control studies indicated a relatively high rate of tag loss and mortality, perhaps associated with injury from tagging (Hay, 1981).

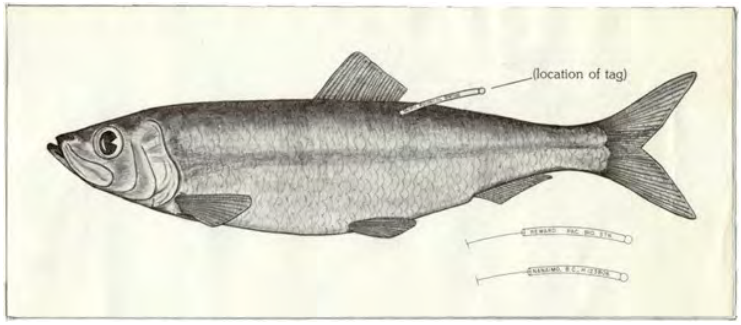

Fig. 2 Drawing of a herring with an attached 'anchor tag'. The drawings at the lower right show tags before insertion.

Spatial analysis of recaptures

There are four hierarchical levels of geographic areas on the BC coast. The largest are the six 'Regions': Queen Charlotte Islands (QCI), North Coast Rupert District (NC), Central Coast (CC), Johnstone Strait (JS), Strait of Georgia (SOG) and West Coast of Vancouver Island (WCVI). Regions can be divided into approximately smaller 'Statistical Area', and these can be further divided into 'sections'. The finest geographic grouping used for the analyses was a 'location'.

Releases and recapture

Between 1936 and 1991, a total of 1,595,249 tags were released in a total of 955 different capture and release sessions (Hay et al. 2001). Over 500 000 anchor tags were released between 1978-1991. Approximately 85% of the 955 tag release sessions resulted in some eventual tag returns and the overall mean recovery rate of the 1.6 million was 2.68%, but this varied annually from a low of about 0.5% to a maximum of over 11%. For both belly and anchor tags, about 76% were released between February and April, during the spawning season and about 15% of the belly tags were released in the summer months. The year of recapture is known for nearly all belly tags returns but the month and day is unknown for most. Most anchor tags (> 85%) were recovered in March and April. A total of 42 767 tags were recaptured, including 37 326 belly tags and 5 441 anchor tags. Of these recaptures, however, about 9400 were made within the same year as the release.

There are different levels of 'precision' about tag recovery information, particularly with respect to exact date and location of the recovery. The exact location and date of release is known for all

tag releases but the accuracy and precision of tag recovery data varies. For nearly all belly tagging data, we know only the 'season' or year of recovery although from review of historical catch data it is clear that most fisheries, hence tag recaptures, were made between November and March. In the reduction fishery the metal tags accumulated in the reduction chambers in processing plants and were not necessarily recovered for individual catches. Therefore recovered belly tags may have come from several different Statistical areas or many different Sections, although for most the Region was known. In contrast, exact recovery dates were recorded for most anchor tags.

Rates of tag recovery

Hay et al. (1999) compared the location of each tag recovery to the area of release at each of the four different geographic scales or domains: Region, Statistical Area, Section and Location. The relationship between fidelity and geographic size of the domain used for analysis (areas of release or recovery in km^2) is shown in Fig. 3. Estimates of fidelity were made for tags at large for one or more years (Hay et al. 1999). In general, estimated fidelity rates depend on geographic scale used in the analysis: large areas have high fidelity rates and vice versa. Exceptionally large areas, such as the entire BC coast, the Gulf of Alaska etc., as areas of release and recovery would have fidelity that would approach one. In contrast, fidelity of very small geographic units (e.g. Locations or smaller) will approach zero.

A question of biological interest is the spatial scale at which fidelity begins to increase above zero, and when it approaches one. Within BC the scale of a 'Regions', with an approximate area of 5000-10 000 km^2 has a fidelity rate of about 80-90%. At the other extremes, there are almost no Sections, with areas < 100 km^2 that have detectable fidelity above 0. There are, however, a number of sections with fidelity estimates between 10%-80% that are approximately 200-500 km^2 sea-surface area.

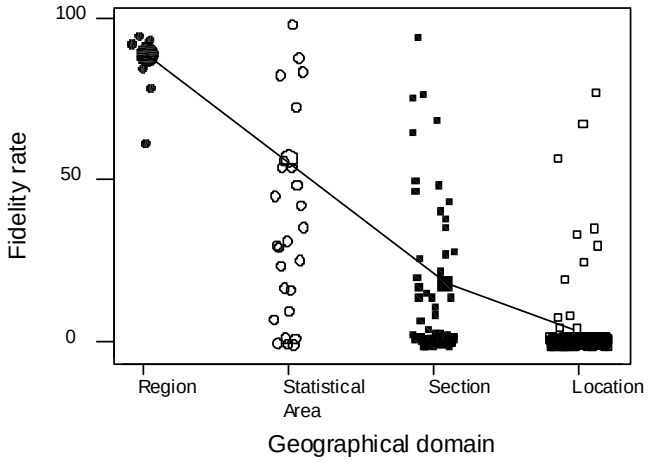

Fig. 3. Comparison of fidelity rates. The symbols show the mean fidelity for each Regions (dark circles), Statistical Areas (open circles), Sections (dark squares) and Locations (open squares). The overall mean for each geographic category is shown for each category by the largest symbol, which connects the different geographical categories.

Herring movements

Table 1 shows the release and recovery of all tags, for all times at large (1 day to 10 years) by Region. The numbers in bold show herring that were released and recaptured in the same Region. All other numbers indicate recaptures taken in different Regions. Ignoring the 'tags taken from Unknown, areas, a total of 3531 recaptured herring changed Regions.

Summary: Herring movements, fidelity and natal homing

Fidelity (F) rate varies with geographical scale. After one or more years at large, between 4-39% of herring that were released between January and April strayed to different Regions. Straying rates (S) were higher for smaller domains with about 40% among Statistical Areas, 83% among Sections and almost 99% among Locations (Figs. 3). These estimates are means, however, and a few sections had relatively high fidelity rates which indicates that some individuals were recovered in nearly the exact place of tagging and release, even after a period of years.

High fidelity does not necessarily indicate high 'homing' or 'natal homing'. From surveys made in the summer, we know some herring are widely distributed in nearshore shallow waters as well as on the continental shelf. Perhaps some herring do not migrate far, if at all, from their natal spawning areas. If so, we cannot use tag returns to distinguish between fidelity rates associated with 'homing' and those that reflect a sedentary (non-migratory) life history - and we suggest that such a distinction is not possible in some other areas, unless there were unequivocal evidence that all herring were migratory.

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Table 1. The number of recaptures of all tags shown by Region. Columns indicate Regions of recovery and rows indicate Regions of release. The recovery location of 8 311 tags was unknown (shown under column 'UNK'). Release Region 'OFFS' refers to 'offshore releases'. Region USA refers to a few recoveries from Washington State or Alaska. Tags recovered in the same area as the release are shown in bold.

	Recovery Region								
Release Region	UNK	QCI	NC	CC	JS	SOG	WCVI	USA	All
QCI	747	**2 885**	146	104	0	9	12	0	3 903
NC	679	204	**3 098**	220	18	15	11	3	4 248
CC	1 767	118	551	**8 249**	52	37	80	1	10 855
JS	536	0	4	369	**801**	142	22	0	1 874
SOG	2 922	7	12	64	282	**3 494**	287	4	7 072
WCVI	1 644	26	16	175	18	458	**12 398**	10	14 745
OFFS	16	0	0	0	0	45	9	0	70
All	8 311	3 240	3 827	9 181	1 171	4 200	12 819	18	42 767

Chapter Six - Sampling considerations

Sampling and mass marking for evaluation of herring enhancement

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Any effort to supplement natural production of herring in Prince William Sound must be subject to evaluation. It may be instructive to consider mass marking as a potential candidate by looking at examples where it has been deployed. Mass marking in the context of hatchery production refers to the ability to mark 100% of the fish that are produced and released (intentionally or not) into the environment where they may mix with un-marked fish from wild stocks. The largest application of mass marking fish is otolith marking hatchery reared Pacific salmon by member countries of the North Pacific Anadromous Fisheries Commission (NPAFC). The countries annually coordinate their respective programs to minimize mark duplication (see http://npafc.taglab.org/) and in 2007 over 1.6 billion hatchery salmon were otolith marked and released in the North Pacific (NPAFC 2007). This review will examine the foundation for the sampling program for mass marking utilized by the State of Alaska as it developed the technology of otolith marking and provide comment on how it may apply to a similar program for herring.

The State of Alaska's otolith thermal marking program begin in 1992 based on an awareness that the technology was feasible for deploying on a large scale (Volk et. al. 1990, Munk et. al. 1993). In Alaska unlike other locations, it was established primarily to identifying the contributions of hatchery salmon caught in the commercial fisheries (Hagen et al 1995). The need for this stemmed from a requirement for the hatcheries to document their contributions. They also provided information for fishery managers so they could adjust fishing patterns to meet wild stock escapement goals, or in some cases, to meet treaty obligations (Jensen 2001). Prior to otolith marking, the primary technique for identifying hatchery salmon was through the application of coded wire tags (see other Chapter 14). This method required placing a small metal wire in the fish's snout with protocol of snipping a fin to create a visual cue as to the presence of the tag. The fin clip provided a means for rapid screening of the tagged fish in the commercial catches. The difficulty with that method is that, due to the volume of the releases, 100% marking of all releases was impracticable and prohibitively costly. In addition with small size fish, applying coded wire tags was particularly difficult. As a consequence, the uncertainty surrounding the estimate of contribution was a function of the estimated proportion tagged, the estimated proportion of the run examined for fin clips as well as an estimate of tag-induced mortality and tag shedding. Collectively the variance of the estimate is derived through a compound multivariate binomial-hypergeometric distribution (Clark and Bernard 1987). While successfully deployed in many applications (Bernard and Clark 1996), but for releases of large numbers of,small fish it became problematic to produce contribution estimates with sufficient precision at reasonable costs.

The advent of a marking system that provides 100% identification greatly simplifies the basis for determining contribution estimates. The techniques and protocols for efficiently and accurately

recovering the marks from otoliths of adult salmon had been refined through practice in the early 1990's (and will be critical issue in developing a similar program with herring). In 1993 an in-season sampling program was conducted on pink salmon release two years earlier to determine if the contributions of hatchery salmon could be estimated from commercial landings by examining the catches for otolith marked fish (Hagen et al 1995). The recognition of its success in that application helped motivate the Exxon Valdez Trustee council to provide funding to Prince William Sound hatcheries and the Alaska Department of Fish and Game to adopt otolith thermal marking as replacement for the coded wire tagging program. It was considered a restoration action that could help in the recovery of pink salmon and provide an aid to the fisheries that depend on its successful management (Joyce and Evans 1999). The program quickly proved successful where the otolith derived estimates were found more accurate than those from coded wire tags (Riffe and Mathisen 2002) and soon developed as routine tool for in-season salmon management in Prince William Sound (Joyce and Evans 2001).

In its simplest deployment the ability to mark 100% of a group of fish through otolith marking (or similar approach) means the underlying distribution for the recovery of the marks can considered as a binomial (marked , unmarked) or multinomial (mark A, mark B, etc.). From that basis with a few caveats, it is fairly straightforward to draw inferences about the population with relatively high precision from small sample sizes. Much of the following discussion on sample sizes as well as consideration of caveats can be drawn from text such as Barnett (1991), Cochran (1977) and Thompson (2002). The construction of the graphs come from the using the formulas for normal approximation of the binomial which is more convenient computationally, though slightly less conservative in its estimate than other methods (Daly 1992).

To consider the sample size requirements one needs to first address the question of what level of precision is necessary when estimating the proportion ($\hat{P}$) of the marked fish in a sampled population. A second and critical question with respect to applications with herring is to define the population and the sample frame or strata to be used. Precision is typically expressed as the standard error (SE) of the estimate and there are two ways in which a target level is determined. Precision based on the absolute standard error of the estimate is typically cast as the confidence interval (CI) (e.g. $\hat{P} \pm 1.96 * SE$) while precision defined as relative standard error is referred to as coefficient of variation (CV) (S.E. / $\hat{P}$). In practice these can result in very different sampling goals. Figure 1a illustrates the number of samples required to ensure the 95% confidence interval is $\pm$ 5% the estimated proportion of hatchery fish in a population. With this goal the worse case scenario is the case of 50% hatchery fish in a population. In that situation, it is necessary to examine 400 samples to ensure that 95% of the time a similar sample size will produce an estimate between 45% and 55%. If the actual percentage is greater or less than 50%, a sample size of 400 will produce even better precision. In practice sampling goals to achieve target level of precision for a multiple mark application are not much different then the binomial situation – though the worst case scenario is for one group to be very small proportion and the remaining groups to be of equal size (Thompson 2002). In contrast to the dome-shaped confidence interval, Figure 1b shows a different shape that is based on the sample size requirements to achieve a target coefficient of variation of 10% as a function of mark proportion. Using a CV based goal, the sample size requirements become more burdensome when the population of interest is uncommon and not very rigorous when the population is abundant. In practice, CV goals are used more often when the interest is in the actual numbers and not

proportions of a group within a sample population (Cochran 1977). It is perhaps more applicable in situations in which the question is whether a marked group can be detected in the sampled population of interest.

Sampling goals based upon confidence intervals are familiar to most people, and in management applications they are well established. In most situations they provide sufficient information necessary to characterize the population sampled in the form of contribution estimates. In a mark recovery program, trying to achieve a target confidence interval can provide several advantages. For example, Figure 2 illustrates how the confidence bounds (the upper and lower confidence limits) are largely invariant of size of the population being sampled. The exception is very small populations, in which sampling without replacement can have an influence. Assuming the samples obtained are representative of the population, 500 otoliths will provide the same precision regardless of whether the population of interest is 10,000 or 10,000,000.

Another attribute of using a CI approach for setting sampling goals is illustrated in Figure 3. The graph shows how most of the precision, in terms of 95% CI range, is captured in the first 100 samples. After that it appears to be a case of diminishing returns, and there is little to be gained by processing large numbers of additional samples. This has particular advantage for programs in which the timing of decisions are critical, such as fisheries management applications. Using a multi-stage processing schedule, it is also possible to optimize the processing effort from multiple strata (e.g. weekly openings) to ensure precision goals can been meet. One way to incorporate a staged approach is via a Bayesian method where the inseason estimates inform the allocation of effort for postseason processing (Geiger 1995). Another consideration for establishing sampling criteria in a mass marking program is the incorporation of quality control process. In the coded wire tag program this involved evaluating tag induced mortality and tag shedding and monitoring tag readings. In salmon thermal marking it involved the evaluation of the 100% marking assumptions by examining fry prior to release as well as the incorporation of routine second and third readings to create agreement matrices. Applying latent class models on the agreement matrices can be used to estimate reader error and provide a means of explicitly evaluating the uncertainty in the contribution estimates in relationship to the uncertainty that stems from the sampling effort (Blick and Hagen 2002).

In consideration of establishing a herring tagging or marking program, one question that may need to be considered is what happens if the marking rate is not 100%? In those situations the sampling requirements will also go up depending on the underlying percentage of hatchery fish in the population. The formulas in the following graphs are based on theory and variance formulas established by Clark and Bernard (1987), Bernard and Clark (1996) as applied to the coded wire tags. Discussion can also be found in Schnute (1992). Figure 4 shows the sample size requirements necessary to achieve a precision goal of 95% CI $\pm$ 5% as a function of the marking rate and the percent of hatchery fish in the population. With higher levels of hatchery fish it is necessary to have a high marking rate to keep the sample sizes manageable. If the marking rate is too low then external, visible marks such as fin clips or tags become more appropriate for sampling the population. Figure 5, shows the same relationship but restricted to the case where the hatchery fish constitute 50% of the sample. When marking rates are less than 100% then an additional consideration is how to estimate the mark fraction and the incorporation of the uncertainty into the contribution estimates. The formulas can also be used to determine

the proportion of the catch that needs to sampled to achieve a given precision level as a function of the catch, the mark fraction and expected contribution.

The discussion above assumes the population of interest is well defined. In sampling commercial salmon fisheries, this is not usually a concern. Weekly openings at specific locations and accessible sites for sampling the catch, such that random samples may easily be obtain and expansion or weighting factors readily determined, make the estimates of contributions a straight forward calculation. In the case of PWS herring however, in the absence of commercial fishing it may not be so simple. In addition, by definition there would be no contribution estimate to calculate if there are no herring to be caught. If the first step in herring production is the use of small scale pilot project to evaluate success, careful consideration must be given to defining the sample population and determine the means to obtain representative samples. Salmon test fisheries are frequently sampled for otolith marks and are used as indicators of run timing. The data could also be used to draw inferences on abundance of the hatchery fish in the location, when expanded by other population assessment methods. Salmon however, more so than herring, have migration and movement patterns that are generally well defined. In addition the population structure with a stock concept associated with natal spawning areas is well established with salmon. With herring much less is known, and in considering an enhancement program and the attendant sampling effort, it will critically important to define the population in a manner such that any sampling effort conducted is representative and serves as a means to accurately estimate the success of the enhancement effort.

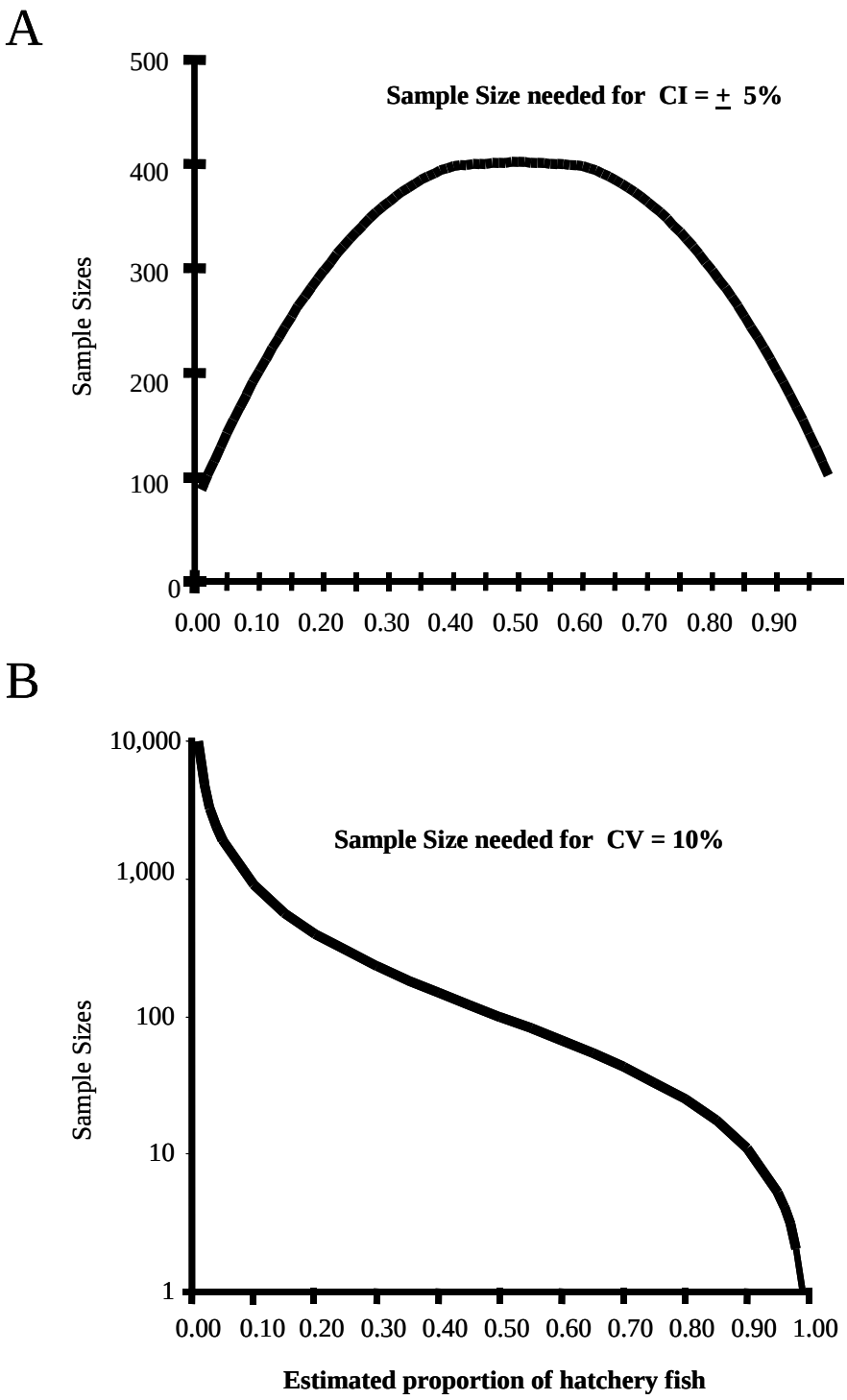

Figure 1(a). Sample sizes necessary to achieve a confidence interval of + 5 % around the estimate of the hatchery proportion in the population. (b). Sample sizes necessary to achieve a coefficient of variation of 10% on the estimate of the hatchery proportion.

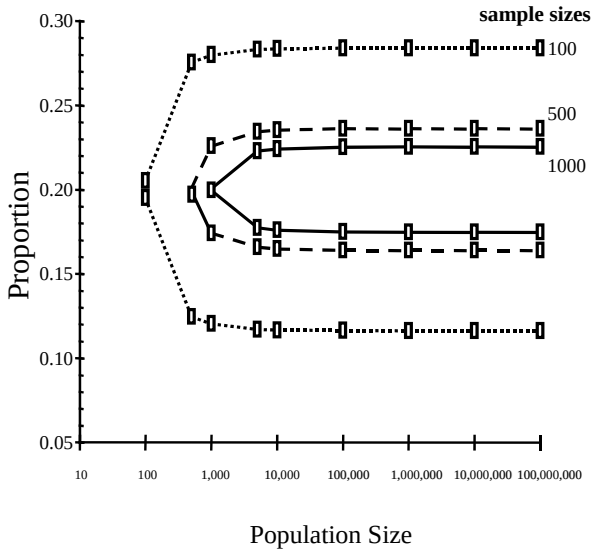

Figure 2. The 95% confidence bounds as a function of the size of the population, based on sample sizes of 100, 500, and 1000 for a sampled population containing a marked hatchery proportion of 0.20

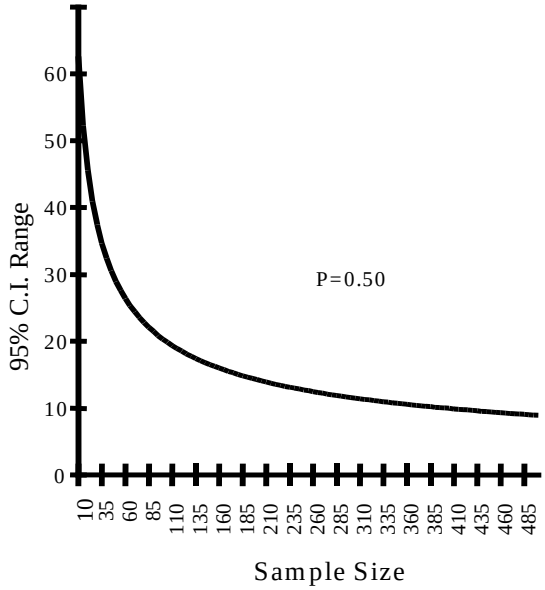

Figure 3. Changes in the 95% confidence interval range (upper bounds – lower bound) for the case of 50% hatchery fish as a function of increasing sample sizes. Graph illustrates how little there is to gain in precision by processing additional otoliths.

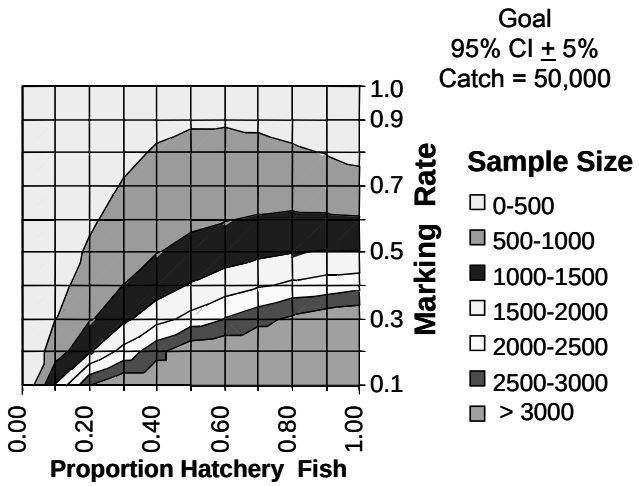

Figure 4. Sample size requirements to achieve a 95% CI that is + 5% of the point estimate as a function of the marking rate and the proportion of hatchery fish in the population. The graph illustrates how sample size requirements increase as the marking rate decreases at moderate levels of hatchery production.

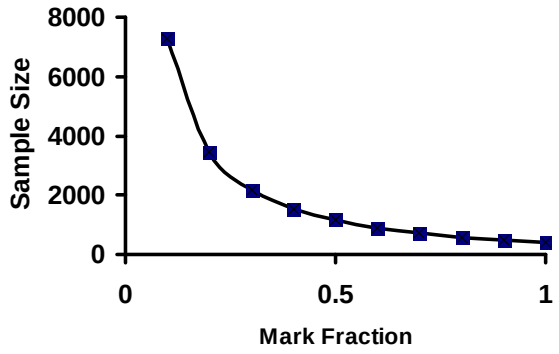

Figure 5. Sample size to achieve 95% CI with 5% precision as function of marking fraction assuming 50% hatchery fish in sample (p=50). This example is based on a catch sample of 80,000 though numbers are largely invariant of catch except at lower mark fractions

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Chapter Seven – Fatty acids

Could fatty acid signatures make effective biomarkers for large scale field experiments with Pacific herring in Prince William Sound?

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In a recent pilot study, we demonstrated that fatty acid analysis of heart tissue could be used to discriminate among Pacific herring (*Clupea pallasii*) spawning aggregates on broad (>1,000 km) and relatively fine (≤100 km) spatial scales (Otis and Heintz 2003). We're currently completing a follow-up study to evaluate the temporal stability of the stock specific chemical signatures we identified (Otis and Heintz 2005). The following white paper draws upon our recent field research, as well as the primary literature, to address the question of whether or not fatty acid signatures could be an effective natural tag to discriminate among groups of Pacific herring used in large scale field experiments in Prince William Sound.

Use of Fatty Acid Signatures as "Natural Tags"

Given the ongoing debate over the type of questions fatty acid analysis can be appropriately used to answer (e.g., Thiemann et al. 2004, Grahl-Nielsen et al. 2004), some discussion of the rationale for investigating it as a stock identification or natural tagging tool is warranted.

It is clear that many studies have documented how fatty acid compositions can change with diet (e.g., Fraser et al. 1989, Kirsch et al. 1998, Turner and Rooker 2005, Budge et al. 2006). However, the ability to trace the dietary influence of some individual fatty acids is much greater than others. Turner and Rooker (2005) documented a 35% change in the polyunsaturated fatty acids (PUFA) of juvenile red drum (*Sciaenops ocellatus*) after just 5 days of controlled feeding. Fraser et al. (1989) observed the incorporation of dietary fatty acids into the triacylglycerols (TAG) of Pacific herring larvae in a marine enclosure over the course of 43 day feeding trial. Fraser et al. (1989) found that peak 18:4n-3 levels in phytoplankton transferred through zooplankton in an enclosed marine food chain and into herring larvae in about 23 days. Haugen et al. (2006) evaluated seasonal variations in muscle growth and fatty acid composition of Atlantic halibut (*Hippoglossus hippoglossus* L.) and found that the triacylglycerol (TAG) fraction of the fatty acid profile was most affected by diet while the polar fraction was less influenced. Clearly, diet is a major factor affecting the composition of fatty acids in fish.

However, it is also clear that other studies have demonstrated that different fish stocks and strains can be differentiated using fatty acid analysis, even when they've been reared under identical conditions and fed identical diets (e.g., Joensen et al. 2000, Peng et al. 2003). Peng et al. (2003) reported that while great similarities were found in the fatty acid profiles of whole body TAG of two strains of Atlantic salmon fry, they observed marked genotypic differences in the PUFA profiles of whole body phospholipids. Pickova et al. (1997) investigated the lipid fatty acid composition of eggs from two cod stocks and concluded that the composition of

phospholipids was more related to stock than to diet. Rottiers (1993) fed landlocked and anadromous strains of Atlantic salmon identical diets and found that landlocked strains had higher lipid content. Rollin et al. (2003) also studied diet effect on anadromous and landlocked Atlantic salmon parr and concluded that "differences in specific fatty acid concentrations between fish fed the same experimental diet may be due to their individual capacities for LNA (linolenic acid) conversion to longer and more saturated n-3 PUFA". They further suggested that differences in individual capacities to process fatty acids may have a genetic basis, but also noted that other researchers have found that temperature can influence the fatty acid composition of some phospholipids (e.g., Hazel 1984). Finally, in a cautionary note to other researchers, Rollin et al. (2003) reported that the significant differences they found in fatty acid composition between salmon strains was highly dependent on the specific fatty acids considered in the analyses.

In our work, we targeted heart tissues because heart phospholipids are reported to be less subject to environmental influences than other tissues or lipid classes (Grahl-Nielsen and Ulvund 1990, Czesny et al. 2000, McKenzie 2001). Several studies have shown that dietary impacts on fatty acid composition are minimized in heart lipids. Viga and Grahl-Nielsen (1990) cultured groups of Atlantic salmon from the same stock for eight months on prescribed diets and concluded that fatty acid composition of salmon hearts was independent of diet. This conclusion is not universally supported. Owen et al. (2004) reported that the fatty acid compositions of myocardial membranes in rats fed different diets were directly related to those of their food. McKenzie (2001) also reported the tendency for heart fatty acid composition to respond to diet, but at much lower magnitude than muscle or liver. These studies suggest that examination of heart fatty acids should minimize the apparent variation imposed on populations due to diet, ration, temperature, and salinity (Henderson and Tocher 1987, Grisdale-Helland et al. 2002, Kiessling et al. 2001, Cordier et al. 2002, Jobling et al. 2002).

The concept of genetic control over the composition of heart fatty acids is bolstered by studies demonstrating relationships between cardiac function and fatty acid composition. Bell et al. (1993) reported heart lesions in Atlantic salmon fed diets with high levels of n-6 fatty acids after the fish had been stressed. Agnisola et al. (1996) reported reduced heart rate and cardiac power output in the hearts of sturgeon fed diets high in n-3 fatty acids relative to those fed diets high in n-6 fatty acids. These data demonstrate an influence of heart fatty acid composition on individual fitness, thereby providing a basis for differences among reproductively isolated aggregates. Alternatively, interactions between phospholipid composition, eicosanoid production and cardiac function have rarely been described for fish (Stenslokken et al. 2002) despite their frequently described impacts on mammalian health (Das 2001). These data may account for the conclusion that some individual fatty acids (e.g., C22:6n3) in fish heart phospholipids are not strongly influenced by diet (Thomassen and Røsjø 1989, Caballero et al. 2002, Grisdale-Helland 2002), and in fact may be under strong genetic control (Peng et al. 2003), suggesting fatty acid analysis of heart tissue may be appropriate for investigating stock structure.

Spatial, Temporal and Biological Variability

The results of our current study suggest that fatty acid analysis of heart lipids was a reliable method for discriminating putative herring stocks at multiple spatial scales (region, area, site)

corresponding to linear separations of > 750 km (region), 250-750 km (area), and sometimes even 75-250 km (sample sites), as long as samples were compared within and not across years. In most cases, our *a priori* stock identities appeared to best describe the fatty acid data structure.

The results of within-year comparisons from our current study were comparable to our pilot study (Otis and Heintz 2003). Our lower overall cross-validation success in the current study is likely due to higher intra-population variability in fatty acid compositions observed as a result of sampling all members of the population. In our pilot study, we controlled samples for age, sex, and maturity, thereby reducing inherent differences in fatty acid composition that may derive from age related diet changes and gonad maturity (Henderson and Tocher 1987, Huynh et al. 2007).

We did not observe a high degree of temporal stability in fatty acid composition for most of the stocks sampled. Cross-validation of discriminant functions and nMDS with ANOSIM revealed considerable shifts in fatty acid composition across both short (1 year) and long (4-5 year) time periods. This lack of temporal stability in fatty acid composition was observed at all spatial scales, with some exceptions. At the area/site level, only Sitka and Hoonah exhibited a high degree of temporal stability. The relatively high temporal stability we observed in Southeast Alaska is in stark contrast to the instability we observed in the northern Gulf of Alaska and Bering Sea.

The temporal shifts in fatty acid composition we observed in most herring stocks could be caused by a number of factors. Henderson and Tocher (1987) reviewed a variety of dietary and environmental factors that affect the fatty acid composition of different lipid classes. Cordier et al. (2002) reported that salinity can play a significant role in modulating the activities of enzymes acting on lipid metabolism during their natural circannual cycles. Farmed sea bass (*Dicentrarchus labrax*) fed all year on the same industrial diet showed a significant correlation between water salinity and the percentage of 22:6n-3 observed in muscle phospholipids (Cordier et al. 2002).

Our study is not the first to report temporal shifts in fatty acid composition among stocks sampled *in situ*. Kwetegyeka et al. (2006) documented temporal shifts in the fatty acid composition of Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) hearts sampled in Lake Victoria in September 2002 and June 2003. Walton and Pomeroy (2003) used blubber fatty acid profiles to detect inter-annual variations in the diets of two breeding colonies of gray seals (*Halichoerus grypus*). Despite the obvious difference in study organisms, Walton and Pomeroy's (2003) work has many parallels to this study. They too had previously demonstrated the ability to discriminate their target populations based on fatty acid profiles (Walton et al. 2000). Once that was established, they collected additional samples in subsequent years to investigate the temporal stability of each population's fatty acid profile. They found that one was highly variable while the other was temporally stable across three breeding seasons. In another distinct similarity to our own study, they also discovered trends in the distance and directionality of the fatty acid profile shifts they observed over time, as revealed by principal components analysis (PCA) plots. They hypothesized that such a result may occur if members of the population changed their diet in a similar manner.

The existence of diet effects on the fatty acid composition of heart phospholipids does not rule out genetic influences. Maintenance of myocardial membrane fatty acid compositions is essential for cardiac function and mitochondrial respiration (Hatch 2004). Three laboratory studies have reported evidence of a genetic component to fish fatty acid compositions. Joensen et al. (2000) found significant differences in the fatty acid profiles of heart tissue extracted from representatives of two cod stocks that had been reared under identical diets and environments. Peng et al. (2003) compared the fatty acid compositions of anadromous and landlocked Atlantic salmon (Salmo salar) fry, fed identical diets throughout a 44-day feeding trial, and reported significant differences in their phospholipids. In a companion study, Rollin et al. (2003) concluded that differences in the fatty acid composition of different strains of Atlantic salmon resulted from variation in the rates of desaturation and elongation of linolenic and linoleic acids. This suggests that differences in the activities of enzymes that regulate phospholipid composition might explain the stock differences identified in our pilot study on herring (Otis and Heintz 2003), as well as other species examined in field studies (Grahl-Nielsen and Ulvund 1990, Grahl-Nielsen and Mjaavatten 1992).

It is important to recognize that environments and diets were tightly controlled in each of the aforementioned laboratory studies that suggested genetic control of fatty acid composition. The variety of mechanisms by which vertebrates can control the molecular composition of their membranes (Hatch 2004) indicates that fatty acid composition is a quantitative trait subject to polygenic control. Consequently, it is reasonable to expect an interaction between genetic and environmental influences (Stearns 1992). Holding environment constant allows for identifying genetic differences. Conversely, repeated sampling of individuals allows for identification of environmental effects on fatty acid composition (e.g. Walton and Pomeroy 2003). In our study, neither environment nor genotype was held constant. Therefore, temporal shifts in the foraging environment of adult herring likely interfered with our ability to discriminate among spawning aggregates across years, assuming those aggregates were genetically distinct.

The absence of a genotype X environment (G x E) interaction in the samples collected from Southeast Alaska suggests that either the environment there is more stable than that of the rest of Alaska or that the reaction norms of these two groups are parallel (Stearns 1992). Polygenic control of the various proteins responsible for maintaining fatty acid compositions rules out parallel reaction norms as a plausible explanation. This suggests that the foraging environment in Southeast Alaska remained more stable between 2001 and 2006 than in any other part of the state.

Does this technological approach have potential applications for PWS?

Fatty acid signatures may not be the most practical method for mass-marking and recovery of millions of herring in Prince William Sound for the following reasons:

1). Fatty acid analysis is relatively expensive and time consuming. Processing large numbers of samples would likely be cost-prohibitive.

2). The fatty acid signatures of herring tissues, even those rich in the lipid classes least influenced by diet (e.g., heart phospholipids), appear to be temporally unstable, even over relatively short time spans (e.g., 1 year). Therefore, it may not be possible to "recover" (i.e., identify) fish from different experimental groups over a sufficiently long time span to complete many large scale field experiments. That being said, if experiments were of a sufficiently short time span, and the experimental groups of herring had unique fatty acid signatures at the beginning of the experiment (e.g., they'd been fed prescribed diets prior to release), fatty acid signature analysis could be an effective means for identifying individuals from different experimental groups).

3). Identification of individuals from different experimental groups using fatty acid analysis is subject to some of the same difficulties faced by geneticists conducting mixed stock analysis. Your ability to correctly place "unknown" individuals into their proper group depends on how well your baseline of fatty acid signatures includes ALL of the different groups likely contained in the pool of fish you draw you sample from. In this case, one might be able to assume that any fish not matching up with one of the baseline fatty acid signatures of the experimental groups must be a wild herring.

Are there potential or extant applications of this technology

(i.e., other species in other areas) that might have implications for PWS herring?

Fatty acid analysis is most often used to estimate the diets of marine and terrestrial mammalian predators by pulling a core sample from the subcutaneous fat of the animal and comparing its fatty acid composition to those of its potential prey items (e.g., Iverson et al. 1997, Iverson et al. 2001, Iverson et al. 2004). As discussed above, fatty acid analysis has also recently been used to discriminate among populations of marine and freshwater fish.

What logistical factors are implicit with the application of the technology?

Fatty acid analysis presents a number of logistical difficulties, particularly if a large number of samples need to be collected from the field. Samples must be rapidly frozen and maintained at -80 ºC. This is an especially difficult problem to overcome in remote locations where access to liquid nitrogen is limited. In addition, the analysis requires the efforts of skilled chemists and technicians. Consequently, samples must be processed in a laboratory dedicated to lipid extraction and fatty acid analysis. Such laboratory will necessarily need to have a system for receiving, storing and disposing of hazardous materials because hazardous solvents are typically involved in extracting lipids. Capital costs for the analysis can be quite high. In addition to glassware, balances, grinders, reagents and gases, the laboratory requires a gas chromatograph and freezers for storing materials. In addition, the method produces large amounts of data, so a data management system would be necessary.

What are the costs of the application of the technology

(i.e., cost per tag or mark, or costs of recovery or monitoring, etc.)?

Fatty acid analysis costs approximately $250 per sample. This includes the cost of grinding the sample, extracting the lipid, purifying the lipid, transesterifying the fatty acids, injecting them onto

the GC column and quantifying the results. This number does not include costs for amortizing the instrument and other capital costs, nor does it include maintenance or column costs. Add to the sample cost the cost of transporting samples from remote locations in liquid nitrogen plus the cost of shipping liquid nitrogen to the collection sites. While the sample processing costs include labor, it is important to note that the analysis should be overseen by an analytical chemist. Finally there is an unknown cost associated with maintaining the data structure that develops from the analyses.

What important issues might apply to marking PWS herring?

Important obstacles to the application of fatty acids for identifying stocks include understanding why the method appears to work in southeastern Alaska, but not in other parts of the states. In addition, algorithms for allocating mixed catch to appropriate populations would need to be developed. We envision that these algorithms would be similar to those used to allocate mixed stock sockeye salmon fisheries near the US/Canadian border. It would be important to know how long these baselines are stable. It would also be important to know how fatty acids are regulated in order to better understand the heritability of fatty acid profiles.

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Chapter Eight - Genetic Marking Strategies

Genetic Marking Strategies for Prince William Sound Herring Supplementation

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Abstract

Numbers of spawning Pacific herring declined in Prince William Sound after the *Exxon Valdez* oil spill and have not recovered to pre-spill levels. A supplementation program is now being considered to increase the numbers of herring in Prince William Sound. The genetic structure of Pacific herring in Prince William Sound is reviewed and recommendations are provided regarding using natural and transfected genetic marks to track the supplemented fish.

Definitions

For continuity, the following brief definitions regarding herring are provided:

Metapopulation: A group of subpopulations that occasionally interbreed. A metapopulation is characterized by subpopulations across a large geographical area in which subpopulations form and go extinct through time.

Population: A group of herring that randomly mate with each other, but rarely mate with individuals from other groups.

Subpopulation: A group of herring that randomly mate with each other, but at various levels, mate with individuals from other groups. Subpopulations make up meta-populations. Gene flow among subpopulations can vary over space and time.

Spawning aggregate: A group of spawning herring.

Background

Despite the lack of a commercial fishery over the last 20 years, the herring numbers in Prince William Sound (PWS) have not recovered since the *Exxon Valdez* oil spill in 1989. Five years after the oil spill, the number of spawning herring had decreased to the point that the commercial fishery was closed. This fishery has remained closed since 1994 except for 3 seasons in the late 1990's in which limited numbers of herring were harvested (Botz et al., 2006).

All stages of the herring lifecycle were affected by the oil spill. As paraphrased from the Alaska Department of Fish and Game Final Report 97165 entitled *Genetic Discrimination of Prince William Sound Herring Populations*, "adults traversed oiled habitats for spawning, eggs were deposited on oiled grass beds, larvae contained lipophilic petroleum hydrocarbons in the yolk sacs, and the juvenile herring out-migrated along the same oiled shorelines (Seeb et al., 1999)." The environment within Prince William Sound has significantly improved since the spill although oil remains in localized areas mostly outside traditional herring spawning habitat (Peterson et al., 2003; Short et al., 2007).

Despite the closure of the commercial herring fishery and improved environmental conditions, herring numbers in Prince William Sound have failed to return to historic levels. Among the potential explanations are disease, genetic damage, and changes in environmental conditions. One of the most plausible is the establishment of a "predator pit" (Hilborn and Mangel, 1997) in which a limited number of herring are preyed upon so heavily by whales and other predators that the herring would never fully recover on their own. Supplementation is being considered as a means to increase the numbers of herring in Prince William Sound. It is hypothesized that the released herring will increase the number of herring to levels outside of the "predator pit" which will allow them to then recover naturally. However, the lack of a clear understanding of the reason for the reduced numbers of herring returning to PWS, makes it difficult to predict if supplementation will temporarily or permanently increase the numbers of fish returning to spawn.

Evidence of genetic population structure in PWS herring

In 1994, the *Exxon Valdez* Oil Spill (EVOS) trustee council funded a multi-year study to examine the genetic population structure of herring in Prince William Sound (Seeb et al., 1999). Briefly, the study included two temporal samples (1995 and 1996) from four spawning locations: Rocky Bay and Port Chalmers on Montague Island, and St. Matthews Bay and Fish Bay in southeast and northeast PWS, respectively. Samples were also collected in 1995 and 1996 from a single location on the west side of Kodiak Island approximately 400 km from PWS. Five microsatellite loci and mitochondrial DNA (mtDNA) were used to characterize genetic variation within and among the eight samples. Multiple analyses were performed, grouping the samples by location and by year, to assess the relative level of spatial and temporal variation. The results of these analyses revealed two main findings. First, the samples from the five locations were distinct from one another in each year. The level of spatial variation, averaged over years, was modest but statistically significant ($p = 0.008$) as indicated by an Analysis of Molecular Variation (AMOVA). Second, samples collected from the same location, but in different years, were also distinct. The AMOVA indicated that this temporal variation, averaged over locations, was modest but larger than the spatial variation and statistically significant ($p < 0.001$). In summary, the results suggest that while significant spatial structure exists among PWS herring from different spawning locations in any given year, this structure is not reproducible from year to year.

As part of a separate study at Auke Bay Laboratories evaluating the genetic uniqueness of Southeast Alaska herring, one herring collection from Prince William Sound (Whale Bay) was included in the initial analysis (Hawkins, in preparation). This study used 22 microsatellite

markers, although 6 loci were later dropped due to difficulties in allele scoring (allele drop out, additional alleles, short repeats). As with the Seeb study, low levels of genetic diversity were realized between Whale Bay and the Southeast Alaska herring samples as F_{ST} values ranged between 0.0022 and 0.0005 (Hawkins, in preparation).

The lack of temporal stability observed in the genetic population structure of PWS herring is inconsistent with a discrete population model characterized by natal homing and local adaptation (the kind of structure typical of Pacific salmon *Oncorhynchus spp.*). However, the fact that spatial structure was observed is also inconsistent with a single panmictic (randomly mating) population. Similar results in studies of Atlantic herring (*Clupea harengus* L.) prompted McQuinn (1997) to propose a metapopulation model wherein each spawning location supports a discrete subpopulation, however, annual recruitment may come from outside the population. In this model, local population integrity is maintained because new recruits (cohorts), regardless of population-of-origin, remain with the "adopted" population by learning and repeating the population's migratory behavior (adopted-migrant hypothesis). McQuinn (1997) suggests this hypothesis best explains observed data including seemingly contradictory evidence of migration among populations and evidence that individuals from single locations tend to remain associated. Ware and Schweigert (2002) used this metapopulation model to describe the population dynamics of herring in British Columbia. More recently, Small et al. (2005) used the McQuinn model to explain inter-annual genetic variation within spatially distinct samples of herring from Puget Sound, Washington. With the exception a two highly distinct samples, the temporal and spatial patterns described by Small et al. (2005) were similar to those described by Seeb et al. (1999) on a similar geographic scale in PWS.

Although the results from Seeb et al. (1999) are consistent with the model proposed by McQuinn (1997), it must be emphasized that the existing genetic data are inadequate to strongly support a metapopulation. Further evaluation is needed so that predictions can be made regarding the effect of releasing supplemental fish into PWS.

First, the McQuinn model suggests that each cohort (year class) at a given spawning location may have a different spawning origin(s). If this is the case in PWS, then we would expect to find some evidence of significant genetic differentiation among cohorts from the same location. To do so would require a sample of individuals of known age so that the analysis may be stratified by cohort. Such an approach was applied by McPherson et al. (2004) in Atlantic herring and the results showed significant differentiation among some cohorts sampled at the same time from the same spawning location.

Second, more than one subpopulation or population may be spawning at each location. Genetically distinct "spawning waves" of Atlantic herring were revealed by Jørgensen et al. (2005) by sampling the same location multiple times in the same year. A similar result in PWS would indicate that the inter-annual variation observed by Seeb et al. (1999) was likely the result of inadvertent sampling of different spawning waves in different years.

Finally, Hedgecock (1994) used the term "chaotic patchiness" to describe similarly strong temporal variation in genetic structure of other pelagic marine fishes (e.g., California sardine, *Sardinops sagax caeruleus*). The processes that produce chaotic patchiness are not entirely

clear, but Hedgecock (1994) suggested it may result from genetic drift brought about by "sweepstakes" reproductive success in which relatively few individuals in any given spawning effort produce recruits for the next generation. One possible outcome of this type of high variability in reproductive success is an excess of homozygotes. Seeb et al. (1999) did not find an excess of homozygotes, however, only five loci were examined. A larger study, with additional loci, to address the other issues above would provide more statistical power to evaluate the influence of variability in spawning success on genetic structure of PWS herring.

Non-marking genetic methods

Because of the lack of temporally stable allele frequencies and lack of differentiation among the spawning aggregates, it was recognized that Genetic Stock Identification (GSI), which uses naturally occurring differences in allele frequencies among populations, would not likely work for Pacific herring in PWS. In other words, the lack of a discreet population structure makes GSI unsuitable for use in identifying supplemented individuals at this scale with herring.

Parentage inference (Anderson and Garza 2006), where multi-locus genotypes of the parents are assayed and offspring from these mating are identified using their genotypes, might have potential to work in PWS, but these methods have not been proven in systems with the vast numbers of potential mating pairs such as those found in PWS herring. This method might require large numbers of loci in order to distinguish wild-produced fish from supplementation-produced fish.

Genetic marking methods

There are two ways to use a genetic tab to identify supplemented fish; to insert a novel gene or to alter the frequency of a naturally occurring gene. Both methods have significant limitations. First, it is possible to add a new unique mark to the supplemented fish through transgenic techniques. The advantage of this approach is that only the supplemented fish (or their progeny) will contain the mark. The mark is usually a unique DNA sequence that can be easily assayed molecularly (if supplemented fish appear identical to wild fish) or visually (if the phenotype of the supplemented fish can be changed). Obviously, there would be significant resistance to visually changing the appearance of the herring and it is only mentioned here because it is technically feasible. A transgenic fish with a simple molecular mark would be less objectionable, although it is impossible to rule out any negative effects from the integration of the DNA marker into the endogenous genome. Procedures for adding a molecular mark have been developed for other fish species (Alvarez et al., 2007) although herring are especially prone to effects from stress and new culture protocols would have to be established. DNA marks would be introduced into the herring genome through transgenic techniques in which exogenous DNA (the marker) is injected into developing embryos at the single-cell stage. Cells are highly active at this developmental stage and DNA fragments can be incorporated into dividing DNA.

For developing transgenic fish, the injection apparatus and the necessary microscopes are standard laboratory equipment and are not cost-prohibitive although the development of a culture facility and the screening of injected fish for germ-line incorporation could be a significant

expense. To ensure adequate genetic diversity, transgenic fish would have to be created from herring taken from multiple spawning aggregations and enough transgenic fish would have to be generated to overcome a genetic bottleneck from limited broodstock. As such, the development of a group of transgenic fish would take at least 6 years, one year for injections, four for the maturation of the transgenic fish, and one year to test the offspring. Since the DNA integration site will be different for each injected fish, it is important to recognize that all the transgenic fish will be unique although they would all have the same marker. Developing an isogenic transgenic line would take additional generations, however doing so would likely result in a genetic bottleneck. Given our limited knowledge of the herring genome, it is impossible to know how the insertion of the marker will affect the biological capacity of the transgenic herring. Due to these concerns, the American Fisheries Society has a policy stating that the "uncontrolled release of transgenic fishes is undesirable" (American Fisheries Society Policy Statement 21) and this method is not recommended for further consideration.

A second method for genetically marking fish is to use controlled breeding to alter the frequency of an existing marker in the supplementation fish relative to the wild fish. There are many types of genetic markers, but the most commonly used are microsatellites and single nucleotide polymorphisms (SNPs). SNPs are single DNA base changes within the genome that lend themselves to high-throughput technology. They are less susceptible to mutational events than microsatellites (Moxon and Wills, 1999) and usually have only two alleles. While SNPs are common within the genome, no SNPs have yet been reported in the literature for herring. The use of these markers would require additional expenditures to identify, develop, and examine novel SNPs for selective neutrality. As for microsatellite markers, they are highly polymorphic (many alleles exist for each locus) making it easy to select a marker or groups of markers that are relatively unique. Many recently published genetic studies for herring have used microsatellite markers (Beacham et al., 2001; Beacham et al., 2002; Bekkevold et al., 2007; Hotta et al., 1999; Jorgensen et al., 2005; McPherson et al., 2004; O'Connell et al., 1998b; Seeb et al., 1999; Shaw et al., 1999; Small et al., 2005) and at least 22 microsatellite loci have been identified to date (McPherson et al., 2001; Miller et al., 2001; O'Connell et al., 1998a; Olsen et al., 2002). Most microsatellite markers are thought to be neutral (not under selection) although some are in coding regions and have been linked to various neurological diseases in humans (Macdonald et al., 1993). A recent study in Atlantic herring showed that only 2 of 12 tested herring microstatellite markers were not neutral loci (potentially under selection) (Watts et al., 2008). To prevent the selection of particular traits, it would be important to select a genetically neutral mark for tracking the success of herring supplementation. Although there are statistical methods to identifying a mark that does not appear to be linked to a selected trait, there is no guarantee that the mark will not be associated with a gene under selection when new environmental conditions are encountered.

Altering the allele frequency at a neutral locus among supplementation fish may provide a genetic mark to assess the effectiveness of supplementation to recruitment (Gharrett et al., 2001). To do this, individuals would be chosen for use as broodstock based on the possession of a relatively rare allele. The rarer the allele is in the wild, the more statistical power will be available to detect the effect of supplementation. However, the rarer the allele is in the wild, the more fish that would need to be screened during the broodstock selection. In addition, a

minimum number of fish would be required to be used as broodstock in order to avoid loss of genetic diversity through the Ryman-Laikre effect (Waples and Do, 1994).

One of the key assumptions for this method to work is that the relationship (migration) among herring spawning aggregates is understood. If no migration occurs into or out of the spawning aggregates, then the effect of supplementation can be calculated by examining the change in the allele frequency of the manipulated allele and the number of fish within the year-class. Migration among spawning aggregates would have an affect on this relationship. In addition, temporal instability of allele frequency as noted previously (Seeb et al., 1999) could also make it difficult or impossible to interpret supplementation results. This temporal instability of allele frequencies will require much larger sample sizes during the assessment stage and a larger divergence in allele frequency change to provide adequate statistical power to determine the efficacy of the supplementation effort.

Options for genetic marking

Option #1 – Develop the mark using only males.

This option is based on the assumptions that (1) male and female herring cannot be individually marked with a physical mark and then held for mating and (2) that herring sperm will maintain its viability during the 1 to 3 day genotyping process (this has not been tested). For this process, one relatively rare allele (maybe present in about 1-10% of the spawning aggregate) from one neutral locus (not under selection) without null allele issues will be selected and used as the supplementation marker. Null alleles are anticipated alleles that are missing from an analysis potentially due to amplification difficulties. A number of neutral microsatellite markers without null allele issues have been identified for some populations of herring (Watts et al., 2008; Olsen et al., 2002). Additional work would be necessary to identify new SNP markers, although these markers are easier to score in quantity.

Collect milt and a fin clip from spawning male herring and store the milt until finished with genotyping. Male herring can be released or killed after the milt is taken. The DNA would be isolated from the milt or the fin clip and genotyped using a marker selected as described above. Fish that are homozygous or heterozygous for the chosen marker would be selected for breeding. Genotyping a group of samples (say 1,000) for a single marker should take 1-2 days. Sperm from the selected males would be used to fertilize eggs from randomly sampled females to create the supplementation fish. At least 25% of the supplemented fish will contain the genetic mark.

Option #2 – Develop the mark using males and females.

This option assumes that male and female herring can be held in a hatchery setting for mating. For this process, one relatively rare allele (maybe present in about 20% of the spawning aggregate) would be selected and used as the supplementation marker. As with option #1, this locus should not be under selection and have no null allele issues.

Tissue samples would be collected from the isolated or marked herring in the hatchery and the DNA would be isolated. Fish that are homozygous for the marker will be selected by

genotyping. This will require the isolation of individual fish while the genotyping tests are being completed which may be difficult for sensitive fish like herring. Genotyping of a group of fish (say 1,000-5,000) for a single marker should take 2 days. If we anticipate that 4% of the spawning aggregate would be homozygous for an allele with p=0.20, 25,000 herring would have to be screened to find 1,000 that are homozygous for the marker. The selected fish would be bred in the hatchery to create the supplementation fish. Since homozygous male and female fish will be selected, 100% of the supplemented fish would contain the genetic mark.

For both options –

The number of fish used for supplementation must be large enough (at least 200 males and 200 females) to prevent the Ryman-Laikre (1991) effect. This probably will not be an issue since this number of spawning adults will at least be necessary to create a quantity of supplemented fish large enough so that the efficacy of the project can be evaluated. For example, if the fecundity of herring is estimated at 20,000 eggs/female and 200 females are spawned, then there will be 4,000,000 eggs. If mortality prior to recruitment is assumed to be 50%, this leaves 2 million herring for supplementation which may or may not be sufficient to detect when mixed with the wild stocks.

Assessing the success of supplementation could occur when the fish return to spawn by comparing the frequency of the marker among the age supplemented age group. If the allele frequency of the selected marker increases, the supplemented fish likely participated in producing the next generation. If the allele frequency remains unchanged, the supplementation program was not likely successful.

It is important to recognize the limitations of the power of these analyses. If you select for a marker with a low natural allele frequency (say 1%) and supplement with a set of fish that are homozygous for the marker (p=100%), the genetic analysis is statistically most powerful since the differences are the greatest. The higher the natural allele frequency and the lower the frequency within the supplemented fish, the worse the analysis. Steps done to expedite the selection (say the isolation of heterozygous fish or the selection of a high allele frequency) would be offset by the increased numbers of fish that would have to be analyzed to measure the success of the supplementation program.

To illustrate these tradeoffs, we will use two scenarios of Option #1. If we select a marker with an allele frequency of 1%, approximately 200 fish with the chosen allele should be discovered from every 10,000 samples assayed. Sperm from the selected males would be used to fertilize eggs to create the supplementation fish. Randomly sampled females would be used for the eggs. This process would yield a mark frequency of 26% in the supplemented fish and 1% in the wild fish. If the returning supplemented fish composed 1% of the total return, then the overall mark frequency of the return would be the weighted average 0.01*26% + 0.99*1% = 1.25%. This is an overall increase of only 0.25%, but a significant proportional increase for this allele.

Smaller numbers of fish could be assayed for broodstock selection if the baseline frequencies are higher, but the differences in allele frequencies between the supplemented fish and the wild fish would be smaller, resulting in lower statistical power during the assessment stage. For example,

if the allele frequency in the wild is 10%, approximately 200 marked fish should be discovered from every 1,153 fish screened. This process would yield an allele frequency of 31% in the supplemented fish and 10% in the wild fish. Again, if the returning supplemented fish composed 1% of the total return, then the overall mark frequency of the return would be the weighted average 0.01*31% + 0.99*10% = 10.21%. This is an increase of only 0.21% and a much smaller proportional increase than the previous example.

There is a tradeoff in costs between the effort expended on marking fish and the effort expended on detecting the fish when they return to spawn. To demonstrate this, assume that the allele frequency in the wild fish is known without error and that it is temporally stable. As described above in the population structure section, we know these assumptions may not be true for herring (Seeb et al. 1999), therefore this example will underestimate the number of fish required for screening during the assessment stage, but should provide valid relative numbers under the two marking scenarios. If the supplemental fish account for 1% of the returning fish, then 19,000 fish would need to be screened, under the first marking scenario above, just to be 95% confident that the supplementation fish are present (this sample size is required to conclude, 95% of the time, that the overall returning mark frequency is greater than the original wild mark frequency). To provide the same level of detection, 225,000 fish would need to be screened under the second scenario. The reason for these large numbers is due to the fact that the allele used as a marker also appears naturally in the spawning herring and the difference between the overall mark frequency and the original wild mark frequency is small and hard to detect.

Conclusion

In conclusion, the genetic population structure of Pacific herring in PWS does not lend itself well to traditional genetic stock identification and the use of a genetic marker for evaluating the efficacy of the supplementation program has a number of concerns which have been identified. The advantage of using a genetic mark is that the mark will be retained in future generations in offspring of the supplemented fish, although allelic temporal instability could make it difficult to evaluate the efficacy of the supplementation program. Regardless of the type of marker used, it is recognized that any supplementation program will likely have genetic issues that will need to be addressed in the future.

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Chapter Nine – Overview of Pacific Herring Otolith Marking

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Otoliths are ideal structures for marking because their incremental growth is extremely sensitive to biological and environmental change, which means their structure and composition can be easily manipulated to create a unique signatures that can later be used to distinguish them from their conspecifics. Because otoliths are biologically inert, these marks become a permanent part of its structure. Such marks can be used to distinguish hatchery-raised fish from their wild counterparts, evaluate enhancement programs, identify stocks, estimate population size, and determine movement patterns.

An ideal otolith mark is permanent and expressed in 100% of the fish exposed to the procedure. Its application should be straightforward and simple, and it should produce a mark that is clear and unambiguous relative to the natural background "noise" typically present in an otolith's structure. Ideally, the marking technology should allow for the creation of multiple mark patterns so that one could distinguish between different release groups within a year as well as between years, and the marking procedure should not be harmful to either the fish or possible consumers.

Otolith marks whose application and subsequent recovery are not restricted by age can provide an opportunity for the creation of diverse marking patterns. This "marking window" is dependent on three factors: 1) the timing of otolith formation, 2) hatchery retention time, and 3) the type of mark being applied. At this point, it remains unclear as to when the otolith begins to form in Pacific herring. Data from marking herring in Japan indicate that at least the otolith core, or "primordia", is present in late-stage eggs (Hay 2007). Visible daily ring accretion apparently begins in post-hatch yolk-bearing larvae (Fox et al. 2003), which emerge approximately 3 weeks after fertilization. This would likely be the earliest point at which marking can occur. If herring are released prior to winter, then the marking window will be approximately 6 months long, whereas if they are released mid-winter, the window will be about 10 months in duration. This window, however, varies with mark type. Stress-induced marks, which are essentially modified daily growth rings, must be applied when daily rings are visible. This typically occurs early in development, which narrows the marking window considerably. Chemical marks, in contrast, do not rely as heavily on a visual expression of daily growth and tend to be applicable over a greater time frame, provided adequate adjustments to application protocols are made.

In addition to application efforts, one must consider the recovery issues. Recovery effort and its associated costs vary with mark type. Stress-induced marks, for example, require very little preparation for detection: the otolith is mounted to a glass slide with thermoplastic cement, ground down to the core, and examined under a regular microscope. Otoliths with fluorescent chemical marks are prepared in a similar manner, except they usually must be examined under a UV light source. The preparation and recovery of elemental markers is considerably more labor intensive and time consuming: each otolith must be ground to the primordia, polished until it is mirror smooth, cleaned and rinsed, mounted such that there are several otoliths per slide, and examined with specialized and sensitive analytical equipment designed to detect low levels of

minor and trace elements (e.g. electron microprobes (EM) and laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)). As this recovery effort increases, the sample turnover rate decreases and the per-sample cost increases. As cost increases, often the overall sample size must typically decrease. By way of an example, it costs the ADFG's Mark, Tag, and Age Laboratory approximately $1 per otolith to recover a thermal mark, whereas it costs $6.50 to simply prepare an otolith for examination under an electron microscope to detect a strontium mark – actual mark detection costs an additional $10. Costs related to elemental analyses are higher still, with sample preparation and analyses ranging from $80 to $130 per otolith. Clearly, greater recovery costs are going to put severe limitations on sample sizes. If the ultimate goal is the real-time data recovery from a large number of samples, mark recovery must be quick, simple and cost-effective.

Stress-Induced Otolith Markers

To create a stress-induced mark, developing larvae are exposed to stressors that alter metabolic processes to produce unique daily rings within the otolith's matrix. There are two common methods of stress-marking:

Thermal Marking:

This method is currently applied to the majority of hatchery-raised salmonids released in Alaska (approx. 1.5 billion in 2007) and involves exposing embryos to a three or four degree (Celsius) shift in temperature that temporarily disrupts otolith growth to create a dark ring in the otolith microstructure. By imposing a set number of temperature changes, a unique pattern of dark rings can be produced in all exposed fish (Munk et al. 1993). These unique mark patterns can provide information regarding brood year and hatchery of origin. Recovery of marked individuals from the fishery is then used to identify cultured fish from their wild counterparts. This method has not been applied to herring, although Folkvord et al. (2004) suggest it is possible because they observed that temperature affected daily ring structure in Atlantic herring.

Dry Marking:

A periodic change in water level during incubation generates the stress to create distinct daily rings that are similar in appearance to thermal marks. The developing eggs are exposed to air, but kept humid, for 24 hours, then submerged for 24 hours to develop a single ring. This method, however, will likely not work on herring because there is probably not enough daily accretion on the otolith during the egg stage to produce a visible mark.

Stress-induced marks are good because they mark 100% of the exposed fish and are easy and cheap to apply (assuming gas prices continue to drop). The resulting mark is visible under a regular microscope with little preparation so mark recovery is quick and inexpensive. This quick turnaround makes real-time data generation possible. The method can be used to create multiple mark patterns that are not harmful to consumers. There are some problems, however: only the otolith is marked, the stress may affect survival rates, marks can be obscured by naturally occurring ring patterns, and the marking window is limited. Stress marks should be applied early

in development when daily ring structure in an otolith is most pronounced – this not only reduces the marking window, but limits the availability of different mark patterns.

"Bone-Seeking" Fluorescent Chemical Markers

In this technique, broad spectrum chemicals are introduced by injection, immersion or ingestion and get incorporated into any calcified structure that grows incrementally (e.g. otoliths, fin rays, vertebrae, etc) to produce a unique ring in the calcified matrix.

Alizarin Complexone (ALC):

This dye is attracted to calcified tissue to create a fluorescent red ring in any bony structure that has incremental growth. The ring, however, is only visible under UV light and is not always distinct. The Japanese have successfully applied this mark via immersion to Pacific herring larvae, and its recovery was used to verify homing behavior (Hay 2007). They also reported successful application to late-stage eggs, which is surprising because the otolith essentially exists as a small primordia. It was also believed that the egg capsule and/or chorion would prevent entry of large, complex molecules.

Oxytetracycline (OTC):

OTC is a widely used broad-spectrum antibiotic that binds to any calcified tissue to create a ring that appears fluorescent yellow under UV light. Immersion in this dye, however, does not always mark 100% of exposed fish and mark retention may decrease with age. Although the mark is widely used in age validation studies, I could find no record of it having been applied to Pacific herring.

Calcein (e.g. Flourescein):

Immersion in this dye produces a yellow-green fluorescent mark when viewed under UV light. It marks scales and fin rays as well as otoliths and bones. Although the chemical has been available for several years, its application to fishery science is relatively new and research regarding its application is ongoing. Experiments conducted by the MTA Lab and NOAA's Auke Bay Lab regarding its application to salmon indicate the mark is stable and its expression does not fade with age. The mark, however, may fade with exposure to the environment, so rings laid down in scales and fin rays may fade with cumulative exposure. There are no previous applications to Pacific herring.

Strontium Chloride:

Immersion of larvae in a solution of 3,000 ppm strontium chloride for 24 hours produces a single bright white fluorescent ring in the otolith matrix. The ring, however, can only be detected when a highly polished specimen is examined using an electron microscope equipped with a

backscatter electron detector. Although there have been no applications to Pacific herring, the mark is successfully applied each year to sockeye salmon produced at Gulkana hatchery.

One of the biggest draws for chemical marks is their ease of application (simple immersion in the appropriate solution) and recovery (UV-equipped light microscope, excepting strontium marks) Like stress-induced marks, samples can be read quickly and cheaply, making real-time data generation possible. Dyes can potentially be applied at any stage, so the marking window is wide open. The fact that multiple mark patterns are possible, and the dye marks a variety of hard parts only adds to their appeal. There are, however, several drawbacks. Immersion may not mark all fish equally, and the chemicals themselves can be costly. Their application requires extensive governmental approval and they suffer from negative public perception. There are issues associated with chemical storage, handling, and disposal, as well as unknown affects on the health of the fish and their consumers.

Non-Visible Applied Elemental Markers

In this marking method, minor or trace elements are added to the water and are absorbed by the fish, which alters the Ca / Element ratio in the otolith to produce an "elemental" mark. These markers are typically isotopic forms of elements that are analogues of calcium (Br, Sr, and Mg), although other chemicals, such as rare Earth elements like lanthanides, work as well. Exposure to different concentrations and/or multiple isotopes can be used to produce unique mark patterns. To date, applied elemental markers have not been used with herring but have been applied to other species. Munro et al. (2008) successfully marked hatchery-reared golden perch with ^{137}Ba and ^{86}Sr, and Ennevor and Beames (1993) used lanthanides to mark otoliths and vertebrae in Coho fry and smolts.

Elemental marks have the advantage of marking 100% of the exposed fish in an easy, cost-effective manner, although the chemicals can be costly. Since the chemical can be absorbed by the fish at any stage of development, there are numerous possibilities with regards to mark patterns. And as a bonus, these patterns are potentially present in any calcified structure within the fish. The drawbacks are similar to those associated with the visible fluorescent markers: their application requires governmental approval, there are issues with handling, storage, and disposal, and they can suffer from negative public perception. Unlike the aforementioned mark technologies, an elemental mark recovery requires extensive sample preparation and the use of specialized equipment (LA-ICP-MS). Consequently, recovery costs can potentially be high, which can affect recovery effort (sample size) and the ability to manage marked fisheries in real time.

Natural Marker Options

This approach uses naturally occurring structures and chemical features formed in an otolith in response to environmental or genetic factors to discriminate among groups, reconstruct life history, etc. There following techniques are often used together for stock discrimination:

Shape Analysis:

This technique uses spatial differences in otolith shape to distinguish among groups. Such comparisons, however, are often confounded by age-related changes in otolith morphology, variability within age groups between years, and large scale environmental disturbances (cyclones, tsunamis, earthquakes). This method has been used to distinguish between migrant and resident Atlantic herring populations in Irish and Celtic Seas with an accuracy of 95% (Burke et al. 2008).

Microstructural (Ring) Analysis:

This method compares otolith increment patterns (e.g. daily and annual ring counts) and incremental distances among groups to distinguish between stocks. Such comparisons are essentially equivalent to comparisons of age and growth, and as such are confounded by the same factors affecting otolith shape. Regardless, the technique has proven useful at discriminating between hatchery and wild Chinook salmon in the Sacramento River valley with a high degree of accuracy (91%: Barnett-Johnson et al. 2007). It has also been applied successfully to herring in the Atlantic, where comparisons of daily ring counts helped distinguish between autumn-spawned slow growing fish and faster growing winter-spawned groups with and accuracy of 91% (Brophy & Danilowicz 2002, Clausen et al. 2007). In addition to otoliths, scales have been used to differentiate among stocks. Microstructural analysis of fish scales were used to differentiate between wild and reared Atlantic salmon (Lund and Hansel 1991), and should be considered for possible applications with regards to herring stock identification.

Elemental Fingerprints:

This is approach is founded on the premise that the elemental fingerprint associated with every location, water mass, and climatic event experienced by a fish is recorded in chronological order within the growth increments of it's otolith. Comparisons of elemental signatures within and among groups can therefore be used to reconstruct environmental histories, assess movement patterns & natal origins, and identify stock structure. However, elemental profiles can vary significantly over time and space. Consequently, to be useful for stock discrimination, spatial variation must exceed temporal variation, and differences in elemental concentrations should be consistent over the time period in which the assignments are made. Elements previously found useful for stock discriminations include regular and isotopic forms of Mg, Ba, and Sr. In a pilot study examining elemental composition of otoliths from juvenile herring collected from 5 nursery bays in Prince William Sound, Dr. Nate Bickford (EVOS Project 060782) with the Fisheries Otolith Group at the University of Alaska, Fairbanks found that comparisons of Ba/Ca and Sr/Ca ratios among the locations indicated three distinct nursery groups existed within the Sound. Additional results from a larger-scale EVOS funded study by T. Otis (ADFG) and R. Heintz (NOAA) are pending. Similar elemental comparisons by Gao et al. (2001) involving stable isotopes of carbon and oxygen were used successfully to identify herring stock structure in Puget Sound, Washington. Although no publications could be found that used rare earth elements for stock identification, the possibility should be given some consideration since herring larvae spend much of their early development in the near-shore environment.

Is it reasonable to believe rearing fish in a captive environment can alter otolith morphology and/or composition enough to create a mark that is can be used to differentiate them from their wild counterparts? Given the uniqueness of the hatchery environment and the sensitivity of otoliths to environmental change, it is. Unique signatures in hatchery-raised fish can derive from ambient hatchery-induced stress, incubation temperatures, water chemistry, leaching from pipes, diet, and dietary supplements. Barnett-Johnson et al. (2008) used $^{87}Sr/^{86}Sr$ ratios to identify natal origins of hatchery raised and naturally spawning Chinook salmon in the Sacramento River Valley. Spawning locales and hatchery-of-origin were assigned correctly 82% of the time. This improved to 94% and 98%, respectively, when comparisons included microstructural markers. The elemental signatures were traced geochemistry and biofeed. Given these results, this approach is worth exploring.

There are numerous advantages to using natural markers: 100% of the fish are marked, there are no costs associated with mark application, cultured fish are not subjected to additional stress, and the technology is applicable to both hatchery-reared and free ranging populations. Elemental and morphological analyses are not only useful for stock discrimination, but can be used to ascertain natal origins, movement history, and site fidelity. Natural markers, however, are not the perfect solution. Shape, microstructural, and elemental analyses are all subject to debilitating levels of variability. In addition, the sample preparation associated with elemental fingerprinting is labor intensive and the recovery of elemental marks requires the use of expensive equipment (LA-ICP-MS). These factors make elemental fingerprinting expensive, which limits sample size and can make real-time mark recovery problematic. Shape and microstructural analyses, however, do not suffer as much from such limitations.

Transgenerational Marking:

The final method for consideration is transgenerational marking. This method involves injecting the abdomen of gravid females with an elemental marker that subsequently becomes incorporated into their offspring. Thorrold et al. (2006) injected gravid clownfish and serranid females with $^{137}BaCl_2$, which was later recovered in the core of their offspring. Marked larvae were produced over multiple clutches, up to 90 days after a single injection. Such markers are likely restricted to elemental tags because larger, more complex molecules will likely get filtered out by the mother's system, the egg capsule, and chorion. This method is appealing because it can be applied easily to wild fish as well as hatchery reared individuals, but it will be difficult to accurately quantify marked releases.

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Chapter Ten – chemical marking in otoliths

Chemical marking in otoliths potential applications for the restoration and enhancement of herring in Prince William Sound, Alaska

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Stocking of fish is a standard practice to aid in the recovery and enhancement of depleted populations. However, evaluating the effectiveness of such stocking programs is often challenging because of the difficulty in discriminating stocked fish from wild fish, especially for species that are stocked when they are small and fragile; in these cases, traditional physical tags might not be feasible to use. An alternative approach is to mark the otoliths (or other calcified structures) with chemicals. The following provides a brief overview of a variety of methodologies for chemically marking fish otoliths and evaluates their potential application for Pacific herring (*Clupea pallasi*).

Natural chemical signatures

The otoliths of fish are formed from the deposition of calcium carbonate laid down in layers over a fish's life. As otoliths grow, trace elements present in the water, and to a lesser extent in the diet, are accreted into the otolith structure. As there is no turnover of the deposited material, the otolith forms a permanent record (an otolith chemical signature) of the chemical environment to which a fish has been exposed throughout its life (Campana 1999). By measuring the relative amounts of various trace elements present in different regions of the otolith (e.g., the larval/juvenile growth region in the core), it is possible to identify the recruitment sources of individual fish (e.g., Campana et al. 2000, Thorrold et al. 1998), including whether a fish was reared in a hatchery or in the wild (e.g., Weber et al. 2002).

The principle of this technique is that discrimination between the otolith or scale chemical signatures of hatchery and wild fish is possible due to inherent differences in the rearing conditions between hatcheries and the wild. For example, the water chemistry of hatchery rearing ponds may differ from the natural environment due to the source of water used (e.g., well water versus water sourced from a river) or the use of inorganic fertilisers to stimulate algal and zooplankton production. Alternatively, the diet of hatchery versus wild fish may contribute to natural differences in chemical composition. In one study, hatchery salmon were fed a marine based diet whereas wild salmon fed on a freshwater based diet, resulting in differences in otolith sulphur isotope ratios between the two groups of fish (Weber et al. 2002). The otolith or scale chemical signatures of hatchery and wild fish can be measured using a variety of techniques including electron microprobes, atomic absorption spectroscopy (AAS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (Campana 1999).

Advantages

The main advantage of this methodology is that there is no requirement for a marking procedure, as discrimination is based on inherent differences between hatchery conditions and the natural environment that result in identifiable chemical signatures. Consequently, this approach avoids the potential stress and injury of handling and tagging. Also, because every fish contains a mark, any captured fish represents a recapture and can be used. Furthermore, natural otolith signatures can be used to better understand stock and population structure (e.g., Campana et al. 1994, Edmonds et al. 1995, Gillanders 2002b), to explore connectivity among populations (e.g., Gillanders 2002a), and to trace movement and origin of fish (e.g., Limburg et al. 2001, Thorrold et al. 1998).

Disadvantages

The natural signature technique relies upon there being distinct and consistent signatures among the groups of interest (e.g. hatcheries). Because factors influencing the chemical compositions of otoliths can vary from year to year, consistently distinct signatures might not always be possible. As a result, temporal variation in the signatures can sometimes confound any spatial differences that might be present. Therefore, it may be necessary to collect and analyse known samples on a periodic basis in order to assess the temporal stability of the signatures and to build an otolith signature "library" that can then be referenced. Other disadvantages of this method are that accurate analysis of trace elements can be problematic and that it requires specialized staff in order to obtain and interpret meaningful results.

Implications/considerations for herring

Perhaps the most important aspect of using natural otolith chemistry signatures is establishing the question(s) that are to be addressed. For example, is it sufficient to be able to simply discriminate between hatchery and wild fish, or is the goal to be able to discriminate among wild fish from different spawning areas? The amount of sampling required will depend on the goals, and scale of inference. Furthermore, it is also important to establish a baseline of spatial/temporal differences among the groups of interest (e.g. hatcheries, spawning/larval rearing areas) in order to determine what questions are feasible to answer before a full-scale project is initiated. Finally, some knowledge of the local geology, water chemistry and temperature/salinity regimes would be useful in determining what elements and/or isotopes might be the most useful for discrimination for the Prince William Sound region. This will, in part, dictate what instrumentation is needed as well as sample preparation methods.

Marking otoliths with elements/enriched stable isotopes

Otoliths incorporate many different elements and isotopes, some at relatively low concentrations (see Campana 1999). Numerous studies have investigated the potential for mass-marking hatchery fish otoliths with these trace and minor elements or isotopes (e.g., Behrens Yamada and Mulligan 1982, Ennevor and Beames 1993, Schroder et al. 1995). Strontium has been the element of choice for many studies because it is metabolically inert and replaces calcium in the

aragonite matrix of the otolith, thus producing a permanent mark in the otolith. One issue with marking fish with strontium, however, is that the concentration in water is naturally variable (0.06-8.1 ppm; Drever 1982). Therefore, one needs to be certain that the concentration of strontium used, and thus the mark produced, cannot be mistaken for a natural mark.

Marking with isotopes is an alternative to elemental marking that has received little attention. A few studies have investigated the used of radioactive isotopes (see Thorrold et al. 2002), but there are a number of potential risks that prevent their use from being seriously considered. Artificially enriched stable isotopes, on the other hand, do not pose environmental and health risks and show great potential for marking hatchery fish. These isotopes are non-radiogenic and their natural abundances are stable, meaning that marks produced with the enriched isotopes will produce marks that cannot be confused with natural signatures (Munro et al. 2008). Fish can be marked by either immersing them in isotopically enriched water as fingerlings (e.g., Munro et al. 2008) or as larvae (e.g., S. Woodcock, unpublished data). Alternatively, embryos can be transgenerationally marked by injecting the maternal parent with the enriched isotopes (e.g., Almany et al. 2007, Munro et al. In review, Thorrold et al. 2006). Multiple isotopes can also be used to produce numerous unique signatures that can be used as batch marks (e.g., Munro et al. 2008).

Advantages

One of the main advantages of marking fish otoliths with elements or isotopes is that it is easy to batch mark large numbers of fish with limited handling. Marking at different life history stages is also possible, and the marking procedure can be incorporated into existing hatchery procedures with little or no modification. Fish marked at the larval stage can be held at higher densities and for longer periods without water changes, thus making it a more cost-effective method than marking at the fingerling stage. Transgenerational marking (i.e. broodstock injection) is also a simple and cost effective method for administering the marking agent as the fish are born pre-marked and only the maternal parent needs to be handled. This method can also be used in the field to mark wild fish (e.g., Almany et al. 2007). If marking with elements that are naturally low in abundance (e.g. rare earth elements), unmistakable hatchery marks can be produced. However, it is easier to produce an unequivocal mark using enriched stable isotopes because the natural isotopic ratios are known and relatively invariant with respect the magnitude of shift that is possible in the otolith. Furthermore, combinations of elements or isotopes (e.g., Munro et al. 2008) can be used to create unique batch-marks that can indicate information such as hatchery of origin or year of stocking. Finally, though enriched stable isotopes can cost anywhere from $1/mg to >$20/mg, depending upon the element and the natural abundance of the isotope in question, only a small amount is needed to effectively shift the isotopic ratio in the otolith (Munro et al. 2008, Munro et al. *In review).*

Disadvantages

Issues with mark recovery are the major disadvantage of marking with elements or isotopes. Fish need to be sacrificed and otoliths prepared (sectioned and polished) in order to retrieve the marks. In addition to the costs associated with marking the otoliths, there is the cost of retrieving the mark, which varies depending upon the instrument being used to measure the elements or

isotopes of interest and the preparation required for a particular instrument. Other issues include determining which elements and/or isotopes are feasible to use and developing the appropriate marking protocols. Given the correct protocols, however, 100% mark success can be achieved with no impact on growth or survival of the fish. Gaining approval for using elements or isotopes to mark fish also needs to be addressed, as well as disposal of the wastewater. To date, strontium chloride has been approved for use for marking fish. However, as enriched stable isotopes are naturally occurring isotopes that are non-radioactive, their use should not be a major issue, other than possible public misperceptions.

Implications/considerations for herring

Marking herring otoliths with elements or isotopes is a potentially viable method. However, considerable time and effort will need to be invested in order to get this method to the stage that it can be used as the primary means for mass marking herring. The most appropriate elements and/or isotopes need to be determined and approved for use. Also, marking protocols will need to be developed. Some of the questions that will need to be answered are: Which life history stage will be the most appropriate to mark? What concentrations of marking agent need to be used? And, how are the marks going to be retrieved (i.e. which instrument will be used to detect the marks)?

Fluorescent marking via osmotic induction

Various fluorescent chemical stains have been used to externally mark fish as well as their otoliths. Calcein is becoming increasingly popular as a fluorescent marker (e.g., Bashey 2004, Leips et al. 2001, Negus and Tureson 2004), but it is expensive (~$17-$25/g). Low-cost alternatives, such as Alizarin Red S, have also been used to externally mark fish (e.g., Bashey 2004). Typically with fluorescent marking, immersion times range from several hours to over 1 day; however, Mohler (2003) described an "osmotic induction" method to quickly mark fish with Calcein. In the osmotic induction method, fish are first immersed in a bath of hyper-saline water and then transferred to a high concentration solution of the fluorescent dye. This method enables mass marking of fish both internally and externally.

Advantages

Fluorescent marking via osmotic induction is a quick and efficient method for externally marking fish as well as producing a permanent mark in the otoliths. It is possible to mark a large number of fish in a single application without having to handle individual fish (batches of > 20,000 fish are possible). Marking takes only a few minutes (typically < 20 min total) as compared to traditional fluorescent marking methods. With the correct immersion times and concentrations, 100% of the fish can be marked without affecting growth or increasing mortality (Crook et al. *In press)*. Although Calcein is relatively expensive, it is possible to reuse the dye bath several times; cheaper fluorescent compounds that are suitable for osmotic induction are also available (Crook et al. 2007). While some of these alternative chemicals have not been approved for use for marking fish, Calcein does have approval. Since fluorescent marking via osmotic induction produces an external mark, it is not necessary to sacrifice the fish in order to

detect the mark. In addition, portable field detectors are available for both Calcein (Crook et al. In review, Mohler 2003) and Alizarin Red S (Bashey 2004).

Disadvantages

The main disadvantage of using fluorescent compounds to mark fish is that there are a limited number of marks (colors) and they cannot, therefore, be used for individual or batch marks. Mark retention is variable and depends upon the chemical and concentration used, the species of fish and the size at time of marking; environmental conditions also affect mark retention (Bashey 2004, Honeyfield et al. 2008, Negus and Tureson 2004). Furthermore, the use of fluorescent chemicals requires proper approval and permitting. Calcein has been approved for marking fish, but it is one of the more expensive fluorescent compounds. Lower cost alternatives, such as Alizarin Red S, have not been approved for marking fish and obtaining approval for using them could take considerable time. Although, Mohler and Bradley (2008) describe a process for removing Calcein from wastewater, further consideration of storage and disposal of chemicals is essential.

Implications/considerations for herring

To date, fluorescent marking via osmotic induction has been limited to freshwater fish and the freshwater stage of anadromous fish; therefore, it is not certain that osmotic induction would work for a marine/estuarine species. Considerable testing and refinement of the osmotic induction method, similar to the experiments by Crook et al. (in press), would need to be carried out for herring in order to determine the appropriate concentrations and immersion times to produce quality marks in 100% of the fish without affecting growth or survival. The main drawback for implementation of the osmotic induction method is the issue regarding the permitting and approval for different chemicals as well as the disposal of the chemical waste. Despite this, osmotic induction is a quick and efficient method to mass-mark large quantities of fish. Furthermore, because the mark is external and portable detectors are available, hatchery fish can rapidly be identified in the field.

Costs

The cost of marking and retrieving marks for the methods described above are variable and difficult to weigh against each other, but see Munro et al. (2008) and Munro et al. (In review) for comparisons. Costs for marking will depend on which chemicals or isotopes are used and how many are used (if trying to produce unique batch-marks). Natural otolith signatures have no marking cost associated with them; however, sample of known origin fish do need to be analyzed to determine if there are differences among the groups of interest and repeated sampling may be required if there is temporal variation in the signatures that could confound any spatial differences. Fluorescent chemicals vary in cost and range between about $3/g (Alizarin Red S) and $25/g (Calcein). Based on the concentrations used in Crook et al. (In press), it is estimated that it would cost about $198/1000 fish to mark with Calcein and $1.06/1,000 fish with Alizarin Red S. Enriched stable isotopes cost considerably more ($1 to >$20/mg), but only small amounts are needed to produce marks. Munro et al. (2008) estimated that it costs about $9.80/1,000 fish to mark fingerling golden perch (*Macquaria ambigua*) with 15 µg/L of enriched

^{137}Ba over 4 days. Marking at other life history stages alters the cost. Transgenerational marking is the most variable because of natural variability in spawning success and survival to stocking size (Munro et al. In review), while marking at the larval stage is the most cost efficient, even when taking in account for mortality to stocking size (S. Woodcock, unpublished data).

Retrieval of marks also needs to be considered. The cost of retrieving natural otolith signatures and artificial elemental/isotopic marks are similar, but dependent upon a number of factors including the type of instrument used to analyze the otoliths and the rate the users are charged. Typical costs vary from ~$400/day to > $1,000/day for laser-ablation inductively coupled plasma mass spectrometers. Munro et al. (2008), estimated it costs about $8/otolith to analyse fish marked with enriched stable isotopes. The cost of reading marks in otoliths marked with fluorescent compounds is less expensive than with elemental/isotopic marks. Typically, fluorescent microscopes with the appropriate filters are used to detect marks in the laboratory and cost approximately $15,000 (Munro et al. 2008), but there is also the cost associated with preparing the samples. External marking with fluorescent compounds via osmotic induction eliminates the need to extract and prepare otoliths and the availability of portable field detectors means that marked fish can easily be identified in the field without needing to take samples back to the laboratory for analysis.

Summary

There are a variety of methods for chemically marking otoliths that have the potential to be successfully used to mark hatchery herring. The methods fall into three main categories: natural otolith signatures, artificial element/isotope marks, and fluorescent chemicals. Natural otolith signatures require no marking of the fish, but rely upon detectable differences among the groups of interest and temporal variation in signatures can potentially confound spatial differences among groups. Elemental/isotopic marking of otoliths is a cost effective means of mass-marking otoliths and it is possible to create multiple unique marks that can be used as batch-marks to identify different hatcheries or stocking events. Herring specific marking methods would need to be developed and approval for use of different elements or enriched stable isotopes would need to be obtained. Mark retrieval in fish marked with fluorescent chemicals is the least expensive and can be done in the field without needing to sacrifice the fish. As with elements and isotopes, some fluorescent chemical still need approval for use, but Calcein has been approved for marking fish. Herring specific marking protocols would still need to be developed and disposal of waste chemicals would need to be addressed. Each of the above methods have their advantages and disadvantages, and the most appropriate method to use will depend greatly upon the question(s) that are being asked. Also, each of the marking methods are not mutually exclusive – combining methods, such as batch marking with isotopes at the larval stage and fluorescent marking prior to stocking could prove to be more useful than each method on their own.

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Chapter Eleven – genetic issues

Alaska Department of Fish and Game Fish Transport – genetic issues and strategies

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Introduction

The number of Pacific herring *Clupea pallasi* returning to Prince William Sound (PWS) has fallen to such low levels that the commercial fishery has remained closed since 1994 except for 3 seasons in the late 1990's in which limited numbers of herring were harvested (Botz et al., 2006) The cause of the decline is not well understood and may be due to anthropogenic (i.e. Exxon Valdez oil spill) or natural (i.e. metapopulation cycles) causes or a combination of the two. A restoration/supplementation program has been forwarded as a means to increase the numbers of herring to supplement the commercial catch. To evaluate the efficacy of such action, some type of marking methodology needs to be identified. The **Exxon Valdez Oil Spill Herring Marking Meeting** was designed to evaluate the different methods available. Part of the evaluation of marking methods includes the permitting issues associated with release of herring into Prince William Sound.

An Alaska Department of Fish and Game (ADFG) Fish Transport Permit (FTP) is required for any transport of fish or eggs within or into the State (Statutory Reference: AS 16.05.050; 16.05.251; 16.40.100; 16.40.160; Regulatory Reference: 5 AAC 41.001). Therefore, an FTP is required to take wild fish or eggs into a culture facility and to release eggs or fish into the wild. The FTP is reviewed by Commercial Fisheries Division staff (Fish Health Services Pathologist, Regional Resource Development Biologist, Regional Supervisor, Principal Geneticist, and Director) and by the Sport Fish Division's Regional Supervisor. The permit is then signed by the Commissioner of the Department of Fish and Game.

The following is a review of the genetic concerns associated with the issuance of an FTP for research, restoration, or enhancement of Pacific herring in Prince William Sound where release of herring into the wild is requested. These concerns will need to be addressed in a successful FTP application. There will likely also be concerns associated with the stocking of Pacific herring into PWS from the other FTP reviewers, but these will not be addressed here.

Genetic Review

Wild stocks have provided all the fishing opportunity for Pacific herring throughout the State of Alaska. Although the number of Pacific herring spawning in PWS has declined in recent years, wild stocks still provide for robust fisheries in Southeast and Western Alaska. The Division of Commercial Fisheries has a Genetic Policy that was written with Pacific salmon in mind

(http://www.genetics.cf.adfg.state.ak.us/policy/genepol.pdf), but its tenets also apply well to Pacific herring. This policy places primary emphasis on the protection of wild stocks. As such, the genetics review of FTP application centers on ensuring that the actions proposed do not harm wild stocks. If the actions proposed have any potential to harm wild stocks, the genetic review determines if the likely benefits from the proposed actions are likely to outweigh the potential harm.

Pacific herring appear to best fit into a metapopulation model of population structure which will be discussed in detail under the Genetic Marking session of this workshop (Guyon et al.). An understanding of the conceptual basis for this model (and model variations) is critical for evaluating the potential for genetic risk. While the concept is useful, there are many unanswered questions that have not be adequately addressed related to the genetic and population structure of Pacific herring throughout the range of the species, and specifically to herring spawning within Prince William Sound. Among these are the following: 1) How much adaptive genetic variation is there and how is it structured?; 2) How important is the genetic variation in buffering both anthropogenic and natural perturbations?; 3) How well does the adopted-migrant model fit Prince William Sound herring?; and 4) What are the relationships among Prince William Sound herring and herring that spawn in other areas?

Genetic Concerns Associated with Release

There are a number of genetic risks that should be considered with any project where fish are taken from the wild, bred, and progeny released back into the wild. Given the complexity of the fish genome, it is impossible to know every outcome although certain generalizations can be made to help minimize the potential for unanticipated adverse effects. For a review of these types of risks in the Pacific salmon literature see Araki et al. (2008). These concerns are listed below:

Loss of genetic diversity

One risk associated with supplementation programs is the potential loss of genetic diversity through the Ryman-Laikre Effect (Ryman and Laikre 1991). This effect occurs when supplemented fish are the progeny of a relatively small number of parents, but, due to higher survival under culture (through reduction from predation and reduced environmental stress), they represent a disproportionately large portion of the total population. Genetic diversity helps buffer populations from changing environmental conditions and it can be lost if the environment cannot support the abundance of fish after supplementation. For example, if the environment can only support 10 million fish and a supplementation plan adds 90 million of a particular stock, the population will eventually revert to 10 million fish. If selection were equal for fish from both sources, the supplemented fish would now represent 90% of the remaining population, in effect, diluting the overall genetic diversity. The Ryman-Laikre effect can be ameliorated by using a large brood stock (effective population size) as the source of the supplemented fish. In addition, assessment of the genetic diversity of the broodstock will be necessary to determine the effective population size of the cohort, because census and effective population sizes can diverge greatly.

Loss of natural breeding stock

When applying a genetic mark to a supplemented group of fish, risk is associated with the removal of a large portion of the potential breeding stock from the natural population in order to identify sufficient individuals with the marker for broodstock. Depending on the genetic marker, it is anticipated that it would require screening 25,000 herring to identify 1,000 that are homozygous for a diploid marker with an allele frequency of 20%. This number can increase if less common marker alleles are used or could decrease if heterozygous fish are used in the brood stock. Due to the handling mortality and disease susceptibility during the handling of live herring, the individuals screened for supplementation broodstock may be more likely to die or release into the wild may not be permitted to avoid transmission of pathogens to the wild population.

Reduction in fitness in the released fish

The reduction of genetic fitness of released fish could occur due to a number of reasons including: 1) the domestication selection for traits well adapted to culture conditions, but poorly adapted to wild conditions, 2) the relaxation of selection during the fertilization, incubation, and rearing of progeny allowing for alleles that would be deleterious in the wild to survive, or 3) inbreeding depression as a result of mating among relatives. A reduction of genetic fitness can occur during a single cycle through the culture environment and can have effects on subsequent generations even if they spawn in the wild (see Akari et al. 2008 for evidence in Pacific salmon). If the supplementation project lasts for multiple generations and supplemental fish cannot be individually identified, this loss of fitness may be compounded due to the use of supplemental-produced herring as broodstock. For this reason that the Genetics Policy for the Department of Fish and Game states that "Gametes may be removed, placed in a hatchery, and subsequently returned to the donor system at the appropriate life history state (eyed egg, fry or fingerling). However, no more than one generation of separation from the donor system to stocking of the progeny will be allowed." This stipulation will make it more difficult to extend the supplementation/rehabilitation program for more than one generation.

Unknown deleterious genetic effects:

Even if genetic defects are not noted in the hatchery setting, deleterious genetic effects could appear under different conditions experienced in the wild. Effects may be invisible under some environmental conditions, but critical under others. For example, the loss of genetic variation may result in high survivals if oceanic conditions are good, but may not provide the variation needed to allow the population to survive natural or man-made perturbations. Since it is impossible to test all possible conditions to evaluate the fitness of a stock, it is impossible to determine the seriousness of these risks.

Straying of the supplemented fish outside Prince William Sound:

A final genetic risk is disruption of local gene complexes through increased or novel straying among populations. Although the genetic population structure among herring populations appears to be shallow, there is evidence that adult herring generally spawn where they have previously spawned. This life-history trait provides the opportunity for different populations to adapt to particular habitats. Increased straying among populations may result in decreases of fitness due to the loss of adaptation. In addition, straying may increase genetic homogenization among populations which may reduce the ability of populations to react differently to man-made or natural perturbations to the environment. Therefore, it will be critical to understand the mechanism used by herring in returning to the spawning grounds and in adopting migrants and ensure that supplemented fish follow similar patterns. This involves identifying where, when, and at what stage the progeny from the supplementation should be released to ensure similar behavior and a monitoring program to assess differences between wild and cultured behavior.

Assessment of Genetic Concerns

Many of the above concerns are difficult to eliminate. However, if the applicant can propose mitigations for them that reduce the concerns to an acceptable level, a successful applicant would be expected to show that the benefits of implementing the FTP outweigh the potential risks to the wild population. Among the questions that need to be addressed here include, but are not limited to:

1) How will the experimental releases answer scientific questions?
2) Is the supplementation or rehabilitation process likely to attain the objective of increasing the number of herring that spawn in Prince William Sound – what is the evidence?
3) How will the efficacy of the action be measured?
    a. Marking to identify cultured fish (the workshop is a good start)
    b. Survey design to achieve adequate statistical power to detect the effect of stocking
    c. Account for life history of Pacific herring – homing may not be a reasonable assumption.

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(I still need to reference many of these in the text above)

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Chapter Twelve – Acoustic tags

Feasibility and considerations of using acoustic tags to examine movement of Pacific herring in Prince William Sound

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Background

Pacific herring (*Clupea pallasi*) has been immensely important to the people and marine animals of Prince William Sound (PWS) in south-central Alaska for millennia. After the *Exxon Valdez* oil spill, the Pacific herring population in PWS collapsed and there has been no fishery since 1998. To date, there has been no satisfactory answer to explain the lack of recovery. Currently, there are large life history information gaps, including seasonal movement patterns of adult herring. After spawning in small bays, it is assumed that adult herring either remain near Green Island in Montague Strait, or leave PWS for the open waters of the Gulf of Alaska (Fig. 1; Brown et al., 2002). As fishing is primarily prosecuted in the spring on spawning aggregations in small bays in PWS, traditional fishery research methods have not provided information about the movement of adult Pacific herring throughout the remainder of the year. Several questions exist regarding movement of Pacific herring, such as timing of seasonal migrations, length of time spent in spawning bays, and spawning site fidelity. Resolution of these issues may provide insight into the lack of recovery of the PWS herring stock by identifying vulnerable times in Pacific herring life history. Acoustic tags are a fisheries research tool that enables one to gather time-space movement information from marine organisms. These tags emit acoustic pulses that encode an ID number that is recorded when a marked fish is present within range of an acoustic receiver. Miniaturized tags are able to be implanted in fish as small as 12 cm (Welch 2007). These tags have been used to track small marine organisms including salmonid smolts, rockfish, and squid over long distances and multiple years with high efficiency as they move along the continental shelf of the North Pacific Ocean (www.postcoml.org). These miniaturized acoustic tags may provide a new tool for unlocking the secrets of the life history of adult Pacific herring. However, herring are a notoriously fragile fish species that are highly susceptible to handling stress which may result in death. Therefore, it is prudent to examine the feasibility of implanting acoustic tags in Pacific herring under controlled laboratory conditions.

Feasibility study

On 13 October 2008, we began a feasibility study for acoustic tag implantation in Pacific herring. Collaborators from the United States Geological Survey Marrowstone Marine Field Station provided 150 Pacific herring for abdominal implantation of acoustic tags. The captive fish had a mean age of approximately 2.5 years, and mean fork length of 18 cm. We followed a surgical protocol that was developed for salmonids, and has been used successfully with very high survival rates in over 7500 salmon smolts since 2004 (Welch et al. 2007; Chittenden et al. 2008). The salmon smolts generally have ranged in size from 12 cm FL to 18 cm FL at the time

of tagging (i.e., similarly sized to the Pacific herring provided by the Marrowstone Lab), and the tag types used are the same as those used in this experiment. To distinguish between the effects of surgery and the effects of two tag sizes, our Pacific herring experiment included two treatment and two control groups (Table 1).

For the tag treatment groups, a dummy (non-signal emitting) acoustic tag was placed into the body cavity. Tags were made of epoxy and two sizes were used: Vemco V7-1L Tag (7 mm diam x 18 mm length, 0.7 g, round in cross section, rounded ends) and Vemco V9-6L tag (9 mm diam x 21 mm length,1.6 g, round in cross section, rounded ends). The V9-6L tag model is the standard tag that has been used in most salmonid studies while the V7-1L tag is a next-generation smaller tag in testing. The V7 is suitable for smaller fish, but has less transmission power and a shorter transmission range. The surgical incision control group underwent the same handling procedures as the tag treatment groups, an incision was made and closed with sutures, but no tag was inserted. Finally, the no-surgery control group was anesthetized and then allowed to recover without surgery.

Treatment	Sample Size	Mortalities	Extrusions
Vemco V7-1L tag (7 mm diam. x 18 mm length)	50	2	1
Vemco V9-6L tag (9 mm diam. x 24 mm length)	50	2	2
Control (Surgical incision, no tag)	25	0	0
Control (Anesthesia, no surgery)	25	0	0

Table 1. Experimental and control groups in the Pacific herring acoustic transmitter implantation experiment at Marrowstone Marine Field Station as of 17 December 2008.

Prior to surgery, the fish were removed from the main holding tank one at a time and anesthetized in 60 ppm buffered MS-222. Once sedated, the fish were placed on the surgery table ventral side up and an incision just large enough to allow passage of the dummy tag along the ventral midline anterior to the pelvic fins (11-12 mm) was made. The tag was lightly pushed through the incision and forward until it lay within the abdominal cavity (Fig. 2). The incision was closed with two or three simple interrupted sutures. The entire surgical procedure took approximately two minutes per fish. After surgery, the fish were returned to an oxygenated 5-gallon recovery tank, and then moved to the main tank once they were swimming upright and stable in the water column.

As of mid-December 2008, about nine weeks into the experiment, the mortality rate for Pacific herring implanted with acoustic tags was 4% and the tag extrusion rate was 3%. There have been four Pacific herring mortalities (two V9 tags and two V7 tags) and three extruded tags (1 V9 and two V7s) (Table 1). Mortalities occurred 9, 10, 15 and 25 days post-surgery while extruded tags were found on the bottom of the holding tank 39, 50 and 51 days post-surgery. There was hemorrhaging around the incisions of all dead fish while one had a tear up the body wall from the incision and one looked as if the incision never closed and the sutures had loosened. Of the remaining fish, there have been no mortalities since 7 November and all appear healthy. The experiment will continue through March, at which point all of the fish will be sacrificed and re-weighed, re-measured and necropsied to examine the internal effects of the tag.

Nonetheless, Pacific herring appear to be amenable to acoustic tag implantation, therefore this method appears to be suitable for monitoring the movement of Pacific herring in Prince William Sound.

Detecting acoustically tagged Pacific herring

To detect acoustically tagged Pacific herring, acoustic receivers must be deployed in Prince William Sound. To date, there is one array of acoustic receivers in PWS. During autumn 2008, the Prince William Sound Science Center installed a listening line of acoustic receivers across the entire entrance of Port Gravina (Figure. 1). This hydrophone array consists of ten VEMCO VR3 underwater receivers spaced approximately 800 m apart, the optimal spacing for detecting V9-6L tags (MA Bishop, PWSSC, pers. comm.). Each receiver is attached to a 50 kg mooring with the unit positioned approximately 1–2 m above the seafloor. Four smaller arrays of VR2W underwater receivers were deployed near the VR3 array. VR3 and VR2W receivers contain identical receiver hardware and therefore, both record the presence of acoustically tagged animals, but the VR3 offers several enhancements including remote communication capability, increased computing power and memory capacity, a two channel receiver and field upgradeable software.

In addition to the existing listening arrays, two international fish tracking projects will soon expand and place listening arrays in or near Prince William Sound. POST (Pacific Ocean Shelf Tracking) is a member project of the Census of Marine Life and the flagship for the Ocean Tracking Network (OTN), a CAN$168M fish tracking network. The POST project currently (http://www.postcoml.org) operates the largest permanent acoustic telemetry array in the world, consisting of more than 300 receivers deployed in multiple cross-shelf listening lines between California and Alaska. The OTN soon will begin deploying a global array of hydrophone receiver listening lines. POST plans on installing two listening lines across the Gulf of Alaska continental shelf just to the east and west of Prince William Sound (http://www.postcoml.org) while the OTN hopes to deploy listening lines across the entrances to PWS (http://oceantrackingnetwork.org/; Figure 1).

POST and OTN have committed to developing new and innovative tracking technology and testing them in Prince William Sound. One recent development is “business card” (BC) tags (http://www.vemco.com/), which combine a miniaturized receiver and a transmitter in a single unit. A BC tag is carried by a large predator such as a salmon shark (*Lamna ditropis*) and will record interactions when the predator comes within range of another acoustically tagged organism, such as a Pacific herring. In Prince William Sound, the large BC tags hopefully will be attached to salmon sharks by 2010, making them “roving receivers” capable of continuous data collection from acoustically tagged organisms. By attaching geolocating tags to these BC-tagged salmon sharks, it is also possible to determine where these interactions occurred.

POST/OTN has offered to store and distribute data retrieved from hydrophone receivers placed in PWS, provided investigators use tags and receivers manufactured by VEMCO of Canada. By acting as a data “clearinghouse,” investigators will be able to retrieve fish movement data from multiple arrays, even if the fish migrate to unexpected locations. These tag data will be incorporated into the POST/OTN data management systems and distribution will follow the

guidelines on data sharing and terms of use that have been defined by POST/OTN (http://www.postcoml.org/), which promote making data public and open-access as soon as possible, while respecting the needs of individual researchers to publish their results.

Considerations for the application of acoustic tags

Considering the low mortality rate of Pacific herring in the acoustic tag implantation feasibility study and the impending deployment of several hydrophone receiver listening arrays in Prince William Sound, acoustic tagging of these fish is a promising method of monitoring movement of adult herring in PWS. Given the cost of tags, receivers and annual gear maintenance (subsequently discussed), as well as the time required for surgically implanting tags, deploying hydrophone receivers, retrieving data and maintaining listening arrays, acoustic tagging is not a practical method for *mass*-marking Pacific herring in PWS. However, by tagging a representative sample of the population, one may address and increase understanding of important biological questions related to PWS herring such as timing of migration to and from spawning grounds, migration routes, spawning site fidelity, and areas of seasonal residency.

Acoustic tagging technology used to investigate other fish species may also be used for investigating herring in PWS, assuming that the investigators use VEMCO products and participate in the POST and OTN data management systems. Listening arrays that are designed for other species of fish in PWS will detect tagged herring as well. For example, the only currently existing listening array in PWS (the Port Gravina array; Fig. 1) was installed for monitoring rockfish and lingcod movement (MA Bishop, PWSSC, pers. comm.). Because the investigators are providing their data to the POST management system, the receivers may also be used for detecting acoustically tagged herring in the same area. There is also a study proposal submitted to POST to acoustic tag octopus and rockfish, in which the investigator will install a fine scale listening array around Green Island (Figure 1). The outlook is promising for investigations using acoustic tags for other fish species as at least one funding agency (North Pacific Research Board) has identified monitoring movement of lingcod, rockfish and sablefish in PWS as a funding priority. Hopefully, funds will be available for acoustic tagging study of other fish and invertebrate species as the spatial and temporal resolution of movement data for Pacific herring will be directly related to the number of hydrophone receiver listening arrays in Prince William Sound.

The three main considerations when planning and implementing an acoustic tagging program are deploying hydrophone receivers, implanting tags, and retrieving data, all of which require advance planning. To anchor hydrophone receivers on the ocean bottom, one must obtain state and/or federal permits, which may require up to 12 months of lead time. Equipment, including tags, hydrophone receivers and gear to anchor, tether and mark the receivers should be purchased at least three months in advance of deployment as considerable discounts are given when orders are placed at least 90 days in advance of delivery. Vessel charters, which may require a year of lead-time, are needed for deployment of receivers and tags and for data retrieval (up to three times per year). Considering that most of the implementation of an acoustic tagging program is

fieldwork, a well designed program does not require year-round work, but rather a few field outings planned around periods of historically high fish abundance and amenable weather.

When establishing a new acoustic tagging program, consultation with POST by both scientific and technical staff is highly recommended because POST has vast experience in designing and implementing acoustic tagging studies. Consulting with an experienced investigator (from POST or otherwise) will prevent many mistakes and streamline the planning and implementation processes. For investigators establishing a new acoustic tagging program, it is recommended to have at least one person with scientific knowledge which is important when designing a monitoring experiment, and at least one person with technical knowledge which is important when assembling and deploying gear in the ocean. In any case, always have an experienced fish surgeon with a proven track record implant the acoustic tags.

The costs associated with an acoustic tagging project are largely dictated by whether the installation and maintenance of listening arrays fall on the investigator or whether existing listening arrays are used. In either case, one must purchase acoustic tags (V7 and V9 tags are CAN $350 each). Should an investigator opt to install and maintain his/her own listening array(s), the cost of hydrophone receivers varies widely. VR2W receivers are affordable (CAN $1410 each), but are work intensive as they require physical recovery of the unit for data retrieval. VR3 receivers are much more expensive (CAN $7680 each), but data retrieval is much simpler as the unit can be queried by a shipboard modem (CAN $9540 each), thus they do not require physical recovery of the unit. Deployment of each receiver costs approximately CAN $4000 each when considering shiptime, protection collars to avoid trawl damage and anchor and tether systems. After the purchase and installation of a listening array, recurring costs include data retrieval and maintenance of the array (estimated $2300/year/receiver) and replacement of lost and expired receivers (estimated 10%/yr). In sum, it is estimated for experiments using V9 tags that listening lines cost CAN $13,750/km to install and CAN $4,250 to maintain (assuming 800 m spacing between receivers), while the costs are doubled for experiments using V7 tags (assuming 400 m spacing between receivers because of weaker transmission power in V7 tags). It should be noted that these estimates are absolute upper limits of costs for acoustic tagging experiments. VEMCO offers advance order and bulk order discounts, ships of opportunity may be used for array installation and/or maintenance and POST/OTN listening lines will have no user fees, all passing considerable savings to the investigator.

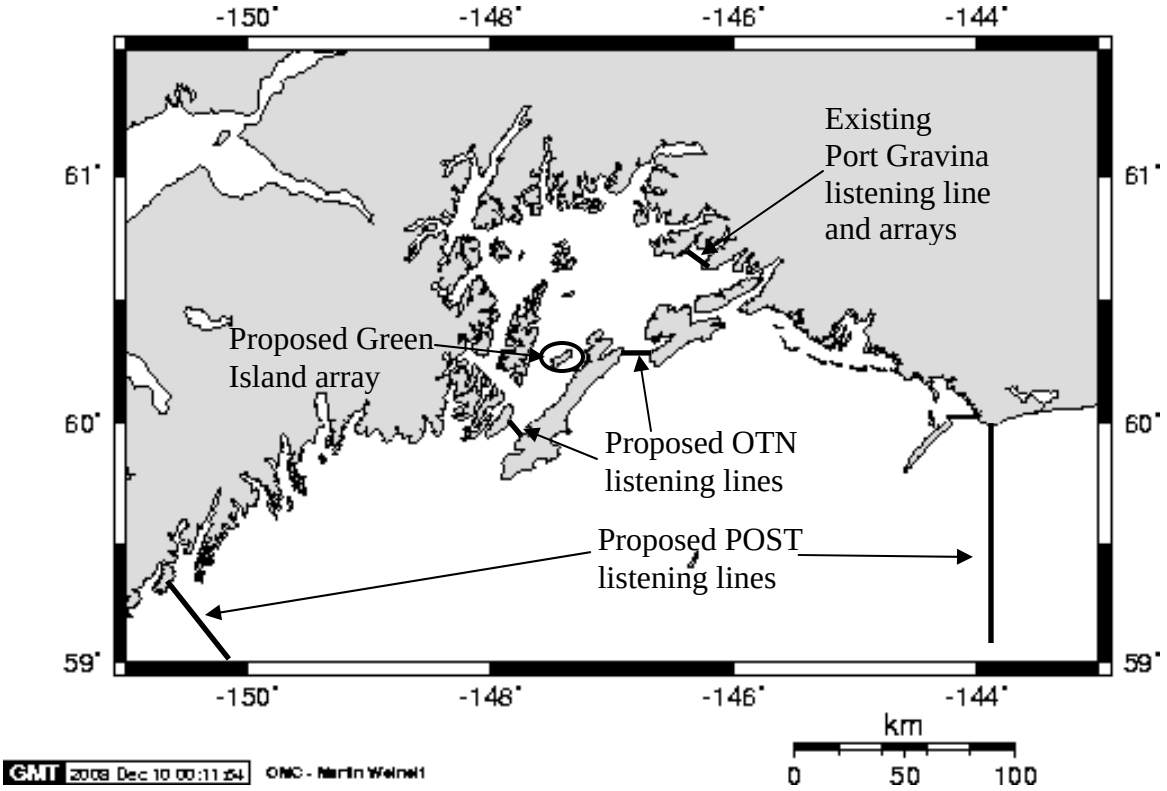

Figure 1. Existing and proposed hydrophone receiver listening arrays in and near Prince William Sound.

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Chapter 13 - Instrumentation

Instrumentation and Recovery of Marks on Fish Hard Parts (Particularly Otoliths)

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The instrumentation I discuss here is focused on the measurement of elemental, not isotopic marks. The problems associated with the measurements of isotopic signals are similar. I also refer mainly to otoliths which are almost entirely metabolically inert after deposition, thus allowing them to preserve a chemical signature better than a structure that is subject to reworking or remodeling. (Campana et al. 1997).

Induced vs. Natural Marks

Thermal marking is well established for salmon, where an obvious optical mark is induced into the otolith of young fish. The point here is that the mark has to be unambiguously distinguishable from any optical bands that would naturally occur. The technique has been developed to the point where the marks can be used to distinguish multiple marks from multiple times and hatcheries. Again, the point is that these induced marks that are (ideally) read on a presence or absence basis.

Chemical marking (particularly with strontium) is being used on a routine basis to distinguish Gulkana Hatchery Sockeye salmon from wild Copper River Fish. Fry are immersed in water with elevated levels of strontium (caused by the addition of SrCl, 3000ppm for 24 hours and a layer of Sr -enriched aragonite is deposited on the otolith. Qualitatively this mark contains upwards of several weigh percent Sr (natural levels rarely exceed 0.1 wt %), and the mark is readily distinguishable using backscattered electrons in a scanning electron microscope. Ideally the mark is read on a present/absent basis. AIL has processed samples from other researchers where the Sr immersion is reported to be in lower concentration and of shorter duration (1500ppm and six or 12 hours), and the mark is visible, but less obvious. The immediate conclusion might be that the varying quality of mark could be used to differentiate batches of fish, but this has not been tried in a practical setting.

There are several disadvantages to applied marks that stem from the fact that the fish must be manipulated in some fashion. There are manpower costs associated with the application of the mark, and perhaps more importantly, there is the possibility of disrupting the fish itself, with disruption ranging anywhere from mortality to subtle, but perhaps important, changes in fish behavior. None the less, applied marks can be read unambiguously if the samples are prepared properly.

Natural marks have the major advantage of “just being there, waiting to be read.” Because they develop naturally, their source, whatever it may be, presumably has had no effect on the fishes

behavior or survivability other than what would naturally be expected. But natural marks have major disadvantages that stem in part from our lack of knowledge as to what causes them (witness the typical litany in the introduction of almost any otolith chemistry paper: "... trace element uptake into the otolith reflects the physical and chemical environment, albeit with significant physiological regulation ..." Campana et al. 1997), and where marine fish are concerned, from the fact that if indeed otolith chemistry is affected by water chemistry, then the differences are likely to be subtle simply because marine water is relatively homogeneous.

For natural marks then, the analysis must be much more **precise ,** and ideally accurate, than the simple presence / absence required for detecting an induced mark. As with induced marks, sample preparation is important, and even in the tightly controlled inter-laboratory-inter-instrument studies such as Campana et al, 1997, both the precision and accuracy of analyses are rarely better than about 10%. This implies that differences between elemental signatures, whatever they might be, should be at least on the order of 10% in order for them to be considered as reliable indicators, particularly if the analyses are to be performed in or compared between different laboratories using different techniques. This may not be the case when comparing patterns (i.e. "an increase in '*unobtainium*' concentration was seen at the margin relative to the core") but it certainly is the case when comparing absolute values. Ratio values seem to fall somewhere in between.

Spatial constraints on Sampling

A further complication may result if the desired mark occurs in only a specific spot on the otolith. Simply put, it takes a certain number of atoms for those atoms to be detected, and a greater number of atoms for them to be quantified. To be sure, instruments vary it what those numbers may be, but there is some certain minimum number, and often a minimum concentration that must be present if that particular element is to be detected. ICP-MS is often quoted as having detection limits in the low part per trillion range, but this is only for samples in liquid form. Laser-ablation ICP-MS is more typically in the part per million range. Electron microprobe (EPMA) is typically in the several hundred ppm range.

One might conclude then that LA-ICP-MS is clearly the method of choice because of its superior detection capabilities. This would be true **if** the spatial resolution of LA-ICP-MS were similar to that of EPMA. However, LA-ICP-MS typically ablates samples from trenches 5-20 microns wide and tens of microns deep (Jones and Chen 2003), while EMPA typically analyzes a volume 8-10 microns across and only 2-3 microns deep (Goldstein et al.). Furthermore, the quantification of EPMA data is much better understood than is the quantification of LA-ICP-MS data, both from the theoretical standpoint and also from availability of suitable comparative standards, although the availability of standards is improving rapidly. Finally, there are some elements (such as Ba) that are present in quantities accessible to LA-ICP-MS, but not to EPMA, while other elements that have shown utility in stock separation (K, Severin et al. 1995) are not practical to analyze via LA-ICP-MS. In short, much to the delight of the instrument manufacturers, there is no one single perfect instrument for otolith compositional analysis, but each has its strengths and weaknesses.

Analysis Time

As a lab junkie I tend to have a problem when asked "how long does it take to analyze a sample" because without knowing exactly what is wanted from the analysis, the question is so vague as to be meaningless. It only takes a couple seconds to positively identify a well prepared Sr marked otolith, but it can take many minutes to convince myself that a poorly prepared specimen does not have a mark. It may only take a couple minutes to get the raw data for a quantitative elemental transect across a specimen using LA-ICP-MS, but this is in addition to the hour or so of instrument warmup, 10-15 minutes of sample stabilization and calibration, and so forth. We have found that simple presence/absence determination of Sr marks averages about 8-10 samples per hour, assuming that the samples are mounted such that we can load around fifty samples at a time into the microprobe (the loading procedure takes 5-10 minutes). This includes recording the results into a spreadsheet and also getting a notebook (not publication) quality image of each sample. For quantitative results the rates drop dramatically to the range of one to two samples per hour, depending on the number of individual analyses required, as transects across an otolith can be quite long. For LA-ICP-MS analysis, the actual analytical time on the sample is relatively short, on the order of several minutes, but the overhead imposed by instrument stabilization suggests that analyzing a single slide in much less than an hour is doing quite well. If multiple samples are included on a single slide, they can be analyzed together, which dramatically cuts the overall time, but our experience matches will with those of others (Fowler et al. 2005) that a day will produce good data for 10-12 otoliths.

Sample preparation

I have mentioned sample preparation several times. For otoliths, which are anything but simple in their structure, good preparation is the key to a good analysis. The researcher must carefully define the portion of the otolith that contains the signal of interest, recognizing the limitations of the technology that is to be used for the analysis. EPMA, for example, demands that the sample surface be as flat as possible for good quantification, ideally finished to a flatness much less than a micron. Surface finish is not as critical for LA-ICP-MS, but variability in sample ablation due to surface imperfection can induce noise into the signal. Surface topography can also add artifact to a backscattered electron signal. The section must be prepared so that the analyst can locate their analyses as precisely as necessary. It is also critical to remember that otoliths do not grow at a constant rate, and that an analysis of a ten micron area might cover **a** segment that was deposited in only a few days in an area near the core, but cover a period of months if that area is near the margin. Finally, some analytical techniques analyze material many microns deep into the sample, and subsurface growth patterns and their effects should be considered.

Sample preparation includes more than just the preparation of the individual otolith. The otoliths must be mounted onto something before analysis. In general, including several otoliths on a single preparation will minimize analytical time, if for no other reason that it takes a certain amount of time to insert and remove a preparation from the instrument. For some analytical techniques it is critical that standards be included in the instrument along with the sample, and this must be taken into account as well. If it is critical (as it usually is), that a specific surface of the otolith be exposed, then it is often easier to combine several submounts into one preparation before analysis.

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Chapter 14 – Internal tags

Tagging Herring with NMT's Internal Tags

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Introduction

Northwest Marine Technology (NMT) specializes in implant tags for live fish, crustaceans, reptiles and amphibians, and other aquatic animals in a manner that minimizes biological impact while providing clear and unbiased data. NMT manufactures three types of internal tags, and their associated injection and detection equipment. The tags are Decimal Coded Wire Tags (CWT), Visible Implant Elastomer tags (VIE) and Visible Implant Alpha tags (VI Alpha). NMT's marking and tagging systems all involve implanting tags into tissue beneath the skin so that, following healing of the initial wound, the tags become encased in healthy tissue. This requires that the tags are (1) small, (2) bio-compatible, and (3) nothing remains penetrating the skin. These three characteristics are the primary differences between NMT tags and others which tend to be larger and have an external code-bearing component that is anchored internally through a permanently raw lesion. Problems with the latter typically include unquantifiable levels of tag shedding, reduced growth and poor survival.

Coded Wire Tags (CWT)

The Coded Wire Tag (CWT) was developed over 40 years ago (Jefferts et al. 1963) for large-scale studies on migratory salmonids and this is still their dominant application today. Each year over 40 million Coded Wire Tags are put into Pacific salmon with around 300,000 tags recovered (Johnson 1990), but the system is also well suited to smaller-scale projects with wild salmonids and a huge range of other fish and shellfish species. Hundreds of species representing 40 families have been tagged with Coded Wire Tags. A list of species and families that have been marked successfully with CWT is constantly being updated online (www.nmt.us). In general, tag retention is very high across species, particularly when there is careful attention to tagging procedures.

The CWT is a small length of stainless steel wire 0.25 mm in diameter and typically about 1.1 mm in length, though half, length-and-a-half, and double length tags are also used in some circumstances. The tag is coded with a series of etched decimal numbers, which allow identification of the spool or batch of wire from which it was cut, depending on the format. Tags can be used to identify large batches of fish, small batches, or even individuals. The tag is cut, magnetized and implanted into suitable tissue with an injector.

Coded Wire Tags are detected in live or dead fish using magnetometers. However, the tag must be recovered from the fish for code identification. Most often this is done by dissecting the tag

from a dead fish after capture by an angler or commercial fisherman. The code is then read under a low power microscope. There are possibilities for data recovery from live fish.

The overwhelming advantages of the CWT over most other tagging methods with significant coding capacity are that they have virtually no adverse impact on the fish to which they are applied, and they provide unlimited code capacity. The tag is biologically inert, and is injected beneath the skin or deeper within the tissues of the fish, without a permanent wound or lesion. It has been demonstrated to have minimal impact upon subsequent survival, growth and behavior of the fish (Vander Haegen et al. 2005). In contrast, conventional external tags, attached via penetration of the skin, can cause a wound that is very slow to heal or may never heal. The two main limitations of the system are the requirements to individually handle and tag each fish, and to recover the tag to read the code.

Advantages of Coded Wire Tags	Limitations of Coded Wire Tags
Very high retention rates are achievable, over considerable time periods and size increases.	Individual fish must be handled for tagging.
Minimal impact on fish survival, growth and behavior.	Tag must usually be removed from fish for deciphering.
Virtually unlimited coding capacity; codes are never reissued.	Capital equipment is expensive.
Considerable scope for automatic scanning of large catches and samples.	Tags will not be reported by anglers/fishermen unless the fish carry a secondary visible mark.
Tags are completely stable over time, and not affected by external environment.	
Well-established technique with extensive literature on successful applications in hundreds of species of fish, amphibians, crustaceans and other animals	
Can be used in very small fish.	
Tags are inexpensive.	

Coded Wire Tag Injectors

There are two main types of CWT injectors in widespread use. The Mark IV Automated Tag Injector is designed for large-scale projects involving tens or hundreds of thousands, or even millions, of animals. It automatically cuts, magnetizes and injects the tag and can be used with head molds or with a needle support tube for tagging in a range of body locations. Although often used in hatcheries or in research facilities, the Mark IV is suitable for field use in any situation where it and the required batteries can be carried.

The Handheld Multishot Tag Injector (Multishot) is a highly portable device designed for mobile use or for projects where smaller numbers of fish are involved. As a general guide we would expect the Multishot to be used for projects involving hundreds or thousands of fish; for those involving many tens of thousands, the Mark IV is a more realistic proposition.

NMT's AutoFish System is a self-contained mobile unit for handling very large numbers of juvenile salmonids. The system incorporates modified Mark IV injectors and accomplishes adipose fin clipping and/or coded wire tagging without the fish being anesthetized or touched by

hand. It can process over 60,000 fish in 8 hours, but AutoFish is available only for salmonids and is unlikely to be adapted for herring.

Coded Wire Tag Detectors

There are three types of CWT detectors available for deployment in different circumstances. They work by detecting the magnetic field of the injected tag, and require the tag or the detector to be moving relative to the other. The detectors can detect and help locate the tag but they do not read the code; the tag has to be recovered and viewed under a low power microscope to read the code.

The V-Detector is powered by an internal battery and is based on the original detector developed almost 40 years ago. It is robust and sensitive as long as it is placed on a firm, static base. The tagged specimen is moved relative to the sensitive faces of the detector and presence of a tag is indicated by a sound and light signal. The main limitation to the V-Detector is that it is sensitive to vibration, so is not really suitable for using in a small motor boat for example. V-Detectors are used mainly in hatcheries and laboratories.

The Handheld Wand Detector (Wand) operates by being moved over the suspected tag location with the specimen held still. The range is limited to about 3 cm with a standard length tag. The Wand is an ideal field tool as it is light and easily carried, is powered by a light internal battery, and can be used in moving boats or in the presence of vibration.

Tunnel Detectors detect tags in fish passed through them, either by gravity or on a conveyor belt. Four sizes are currently produced from a 4 inch tunnel to a 13 inch tunnel. These detectors are typically used at locations where large volumes of fish must be scanned for tags, such as at hatcheries or fish processing plants. Automated detection systems have been used for scanning large volumes of fish.

Tag recovery programs are specific to the particular situation but a number of common features will be apparent. Where a significant proportion of the sample of fish to be scanned is likely to be tagged, a straightforward check of all fish in the sample is an ideal option. Where tagged specimens are likely to represent only a small part of the sample to be checked some difficulties arise. Obtaining an adequate number of returns (tagged fish) is likely to involve scanning very large numbers of fish, which is not only a time-consuming operation but can lead to operator fatigue and careless use of the detectors. Missing the occasional tagged fish when they represent a large proportion of the catch may introduce only a minor bias in the results, but missing the one tagged fish in a sample of a thousand for example represents a serious matter. In these cases, automated systems for sorting tagged and untagged fish are critical.

Visible Implant Elastomer (VIE)

The VIE system provides internal colored tags that are visible externally. The system uses a bio-compatible, two-part, elastomer material. After mixing, the elastomer is a liquid that is injected into tissue with a hypodermic syringe; most species of fish, and many other animals, have suitable areas of transparent or translucent tissue. Within hours or days this material cures into a

pliable solid. The elastomer holds the pigment in a well defined mark, without damaging surrounding tissue. By the use of different marking sites, and perhaps two or more marks on each individual, development of numerous group or individual codes is possible. Some of the colored pigments used are fluorescent, and use of appropriate lighting can significantly enhance detection of tags. The material is biocompatible and carries no known human health hazards.

Advantages of VIE tags	Limitations of VIE tags
May be applied to very small fish and other animals	Tags may become difficult to detect in ambient light if growth is considerable and pigmented tissue is laid down over the tag, though it can usually be detected using the VI light
Minimal impact on fish survival, growth and behavior	Limited coding capacity (but use of several colors, several body locations, and possibly more than one tag allows a greater coding capacity to be developed)
High retention rates	Tags may not be noticed and reported by casual observers
Low capital and material costs make it viable for small-scale projects	
Detection can be further enhanced with appropriate illumination	
Tags detected visually in ambient light	
Fast to apply	
Well-established technique with extensive literature on successful applications in hundreds of species of fish, amphibians, crustaceans and other animals	

Table 1. Advantages and limitations of VIE tags

Hundreds of species of fish, crustaceans, amphibians and reptiles have been tagged with VIE, including herring. A list of species and families that have been marked successfully with VIE is constantly being updated online (www.nmt.us). In general, tag retention is very high across species, particularly when there is careful attention to tagging procedures.

Visible Implant Alpha (VIAlpha)

The VI Alpha tag is a small fluorescent tag with an alphanumeric code designed to identify individual specimens. VI Alpha tags are implanted internally but remain externally visible for easy recovery. The tags are implanted with syringe-like injectors, and are available in several colors and in two sizes: standard - 1.0 x 2.5 mm and large 1.5 x 3.5 mm. Because the tags are made from a biocompatible medical grade elastomer, they do not irritate the tissue at the implant site and seem to have little negative effect on the host animal when properly used.

Although many fish have transparent tissue (adipose eyelids, fin membranes, clear boney tissue, etc.), tag retention varies by species. Size of the tagged specimens is also important. Shedding

rates from adipose eyelids of salmonids less than 150 mm total length have been excessive while retention in larger fish often exceeds 90%

Advantages of VI Alpha tags	Limitations of VI Alpha tags
High retention rates in suitable tissue/species	Not all species have suitable tissue.
Tags detected visually and readable in live specimens without removal	Unsuitable for very small fish
Visibility and readability is enhanced using the VI Light	Tag readability may become occluded by pigmentation.
Provide individual identification	
Low capital costs	
Minimal impact on survival, growth and behavior	

Table 2. Advantages and limitations of VIE tags

Does this technological approach have potential applications for PWS?

Coded Wire Tags are suitable for addressing or increasing understanding of important biological questions concerned with Prince William Sound herring, and for tagging specific groups in conjunction with other tagging technologies. Depending on the scale of the project, they could be considered for mass marking and have the advantage of being able to be electronically detected for automated sorting at recovery.

NMT's Visible Implant Elastomer and VI Alpha tags are unlikely to be suitable for this project. VI Alpha tags are typically used in smaller projects (hundreds to a few thousand fish) where individual identification is required. The fish must be large enough to accommodate the tags. Visible Implant Elastomer tags are retained well in herring but application rates will be too slow for the number of fish being tagged, and there is no scope for automated recovery. The remainder of this paper will discuss only Coded Wire Tag technology.

Are there potential or extant applications of this technology

Chapter 1 **(i.e., other species in** other areas) that might have implications for PWS herring?

Coded Wire Tags are used extensively in managing Pacific Salmon and other species around the world. They have been used to answer the types of questions listed in the table below. The applicability of other studies to PWS herring will depend on the specific questions being asked, the logistics of tagging (e.g. at what stage will the fish need to be identified, how long do you have to do the tagging, how many fish will be tagged, where will they be tagged, etc.), and the logistics of tag recovery (e.g. how will fish be collected to search for tags, where will they be recovered, how many will be recovered, what data will be recorded, who will collect the data,

etc.). Before implementing any Coded Wire Tagging program, the entire process needs to be planned.

Management (Basin-wide implications, on-going marking)	▪ How many fish survived to adults (estimate smolt to adult survival)? ▪ Where do the fish go? Should they be there? ▪ When are they there? ▪ How many and where are fish caught (fisheries contribution, harvest rate)? ▪ In a particular area where fish are caught, where did they come from? ▪ Who caught the fish and how many (fishery resource allocation)? ▪ Are enough of the right fish returning to reproduce the next generation? ▪ What is going to happen next year? Can we make changes to affect it? ▪ Over time, are these fish runs increasing or decreasing (run size estimation? ▪ What is the stock distribution among fisheries and spawning areas?
Hatchery Evaluation (Site-specific implications, on-going marking)	▪ How many fish survive to adulthood? ▪ Where do they go? ▪ Who catches them? ▪ Where do they spawn? ▪ Are these fish fulfilling the reasons for which they were produced? ▪ Is the hatchery program effective at producing the quality (age, size, weight) and number of fish needed? ▪ Are these fish increasing or decreasing in numbers over time? ▪ Are population characteristics changing (age, size of adults, male/female ratio, number of jacks, etc.) ▪ What other fish are returning that don't belong there?
Experimental Marking (Fixed length studies)	▪ Are these fish of wild or hatchery origin? ▪ Are fish being released at the right time? ▪ Are fish being released at the right place? ▪ Are fish getting the right diet? ▪ Are there better ways to control disease? ▪ Can we change things at the hatchery that: o Result in more adult fish? o Affect where the fish go? ▪ Are there better ways to mark fish? ▪ Is the right strain of fish being used?
Habitat Evaluation	▪ Does the habitat produce quality smolts and the number of adults needed? ▪ Over time, do habitat improvements result in more adult fish returning? ▪ What other fish are showing up in the habitat that do not belong there? ▪ To what extent do fish move between habitats?
Wild Fish Tagging	▪ Is it OK to use hatchery fish to evaluate a wild stock (specific locations)? ▪ Do wild fish behave differently than hatchery fish? ▪ Do wild fish survive differently than hatchery fish? ▪ Natural stock spawning composition ▪ Stock distribution (among fisheries, spawning areas) ▪ Run size estimation ▪ Smolt to Adult return rate

Table 3. Biological questions related to coded wire tagging: management, hatchery evaluation, experimental marking, habitat evaluation and wild-fish tagging.

As well as using CWT on all these other species, CWT have been used with herring, so there is good experience with tagging techniques, and some of the logistics of tagging and tag recovery. Some of these projects are summarized below.

Applications of cwt tags in herring

Atlantic herring stock sizes – an application of cwt tags in herring

Morrison (1990) describes a pilot study that tested the logistics of using CWT to estimate Atlantic herring (*Clupea harengus harengus*) stock sizes in the North Sea. In this study, he tagged mature fish on board after capture in a seine net during June and July, 1983. The fish were released immediately after tagging. Because most of the commercial herring catches in this region were transferred to processing ships, there was little access to those fish for recovering CWT. Instead, chartered fishing trips were used to recapture fish and an automatic tag recovery system was designed for scanning the catch for tags. Advantages and challenges of the system were discussed.

A population estimate of blueback herring in a large reservoir

Isely and Tomasso (1998) reported on a mark-recapture population estimate of blueback herring (*Alosa aestivalis*) in a large reservoir on the Georgia – South Carolina border. In April and May, 1996, over 100,000 fish (mean length 140 mm) were tagged in the snout with sequential CWT. The sequential tags were used to identify when and where in the reservoir the fish were originally captured. Between May and August 1996, 155 tags were recovered from the 144,227 fish examined. As it was apparent that the fish tagged in different parts of the reservoir had not fully mixed, a stratified population estimate procedure was adopted. This gave an estimate of the total population of adult fish in the reservoir of about 89 million. This study illustrated the concepts of confidence limits being dependent largely upon the number of tags recovered, and the value and importance of stratifying tagging and sampling where complete mixing cannot be assumed.

Spawning ground use and migration of Pacific herring

In 1999, biologists at Fisheries and Oceans Canada in British Columbia began a study that used CWT to investigate trends in interannual spawning ground use and migration intensity (Flostrand and Schweigert 2002; Flostrand and Schweigert 2003; Flostrand and Schweigert 2004; Flostrand and Schweigert 2005; Flostrand and Schweigert 2007a; Flostrand and Schweigert 2007b; Flostrand et al. 2007; Schweigert and Flostrand 2000; Schweigert et al. 2001). An initial study was conducted to examine tag retention and survival of tagged fish retained in net pens; survival of tagged fish and controls was similar, and tag retention close to 100%.

Tagging began in 1999 and lasted until 2004. About 450,000 herring were tagged and released between 1999 and 2004. Fish for tagging were dip-netted from a seine, and a pipe was used to return tagged fish to the sea. Tagging rates of up to 1175 per hour were achieved. Tags were recovered from 2000 through 2006. Catches were scanned (up to 40 tonnes per hour) at processing plants using R9500 Tunnel Detectors with conveyor belts; when a signal triggered the detector a batch of fish was diverted from the belt for closer examination. About one quarter of

the total catch reported in the province in 2000-2001 was scanned for tags. This represented from 78 to 93% of the tonnage handled by the plants where detection equipment was installed. A total of 1108 tags were recovered; 535 in the year of tagging, 464 the following year, 131 in the second year after tagging plus 15 of uncertain duration. One year at large recoveries ranged from 0.06 to 0.26 % of fish tagged (or 0.32 to 1.13% when adjusted for tonnage searched). Equivalent figures for two years at large recoveries were 0.2% (0.69 to 0.80 when adjusted for tonnage searched). Of particular interest were four tagged fish captured in regions other than that in which they were tagged, all in the year following tagging.

A major logistic constraint is the short fishing season (typically just a matter of days) and thus the need to scan landings simultaneously at several processing plants. The authors also discuss the desirability of a greater level of stratification of tagging and sampling to provide better estimates of stock intermixing, survival rates and stock estimates. The project was ended due to a lack of funding.

Atlantic herring stock characterization (Maine)

To address research objectives proposed by the New England Fishery Management Council and the Atlantic States Marine Fisheries Commission a pilot tagging project was implemented in 2001 and 2002 to assess stock discreteness, exploitation rates and reevaluate catch allocations (Kanwit 2002). CWT were selected as the best option for marking herring, because they are less invasive than other tags, result in high retention rates and automated tag detection can be integrated into bulk processing facilities.

Researchers captured fish in midwater trawls and in purse seines and tagged fish onboard using MKIV Automated Tag Injectors in a variety of seasonal and environmental conditions. Two automated detection units were incorporated into processing lines at a single processing facility. Testing showed that tag recovery within the plants ranged from 80-100%. In spite of the success of the tagging portion, this project ended after 2002 because implementing the tag recovery part of the project failed. The CWT detectors were both severely damaged by misuse at the plant. Liners were not installed inside the tunnels to protect them from the conveyor belts (as had been done in the DFO project), and one conveyor belt cut into the tunnel, which filled with fish carcasses and water, and destroyed the electronics. The second detector was hit with a fork lift. Losing the detectors represented a significant setback to the project both financially and functionally, and a lack of personnel to monitor the recovery coupled with waning support from processors, effectively ended the project.

What logistical factors are implicit with the application of the technology?

The logistics of implementing a CWT program vary considerably depending on the questions being asked as these will dictate the scale of the tagging program, the timing of the tagging and tag recovery, and the geographic area over which tagging and tag recovery. Obviously, larger more complicated programs will require more planning and more equipment than a small study. Implementation can often be phased in, particularly when there is a lag between tagging and tag recovery or with very large tagging programs. However, it is critical for the success of the program

that the entire process of tagging through tag recovery be well planned before any tagging begins. Implementation can range from having to "start from scratch" to expanding existing programs to incorporate new tagging programs. For example, ADFG already has some expertise and equipment for Coded Wire Tagging, and a laboratory set up for dissecting and reading tags. It is possible that this "infrastructure" could be expanded to accommodate parts of a program for tagging herring. Existing catch samplers may be able to search for tagged herring, or monitor automated systems. Tag recovery is simpler if the catch to be scanned for tags is landed at centralized locations, and more difficult if landings are dispersed.

What are the approximate time scales for setting up and implementing a program and does the program require year-round work or is there a seasonal component?

The time to set up and implement a program depends on the scale of the program, both in terms of the number of fish being tagged and scanned for tags, and the geographic area over which the program will be implemented. A small program in which tagging will take place in a single or a few locations can be implemented quite quickly. Very large programs are often phased in over some years, giving time to develop the logistics and personnel expertise required. The recovery phase of the program may be incorporated into existing surveys, in which case it can be implemented quite quickly, following training. Using automated recovery systems at processing plants (if any are operating) or onboard research vessels will require some time for construction as well as for testing and calibration when large numbers of the fish to be scanned are present.

Most tagging programs tend to be seasonal – there are times and life stages that are easier to handle and tag. Typically, there is a period of tagging that may last from a week or two up to a few months, followed by a longer period of tag recovery. Recovery activities also tend to be seasonal coinciding with times when the tagged fish are accessible, and with long-lived species may last much longer than the tagging component.

What capital investments are needed and what are they
(i.e., land-based, laboratory, or vessel support)?

- Injection equipment – this may be land or vessel based. Recommend using Mark IV Automated Tag Injectors with either a Quality Control Device or V-Detector
- Coded Wire Tag detectors and any automated recovery systems to be used with them.
- Tag reading laboratory (may be as small as a single desk with a tag detector, low-powered microscope and tag reading jigs, or may require several tag reading stations, depending on the scope of the program). Could also contract other labs to do this.

What personnel requirements and skill sets
(academic, technical and experience) are needed?

A Coded Wire Tagging program typically involves the following types of duties:

- **Project planning and coordination**
    - Clearly define study objectives
    - Select appropriate sites for tagging and tag recovery
    - Coordinate onsite logistics for tagging and tag recovery
    - Train and supervise personnel
    - Order and track tag wire
- **Tagging**
    - Onsite tagging supervisor able to oversee details of tagging operations, track tag wire and associated data
    - Quality control checks during tagging to maximize tag retention
    - Measure tag retention rates after tagging to estimate tag loss rates
- **Tag recovery**
    - Quality control checks during recovery to estimate detection rates.
    - Establish chain of custody for recovered tags.
    - Record tag recovery data.
- **Tag reading**
    - Careful attention to detail and continue chain of custody for recovered tags.
    - Double read tags to ensure accuracy
    - Enter tag recovery data
    - Archive recovered tags with associated data.
- **Data compilation, sharing, analysis and implementation of results**

The number of different people that are actually involved depends on the scale of the project. At least one person would be at the "project leader" level, and be able to clearly define the objectives, coordinate logistics for the entire project, be responsible for training other personnel in each aspect and analyze data. In very large programs (e.g. the Pacific salmon CWT program), hundreds of different personnel are involved with every part of the program. :

. What are the costs of the application of the technology

(i.e., cost per tag or mark, or costs of recovery or monitoring, etc.)?

Costs depend on the scale of tagging program, and whether some of the activities can be integrated with existing programs. For example, ADFG already has some tag injectors, and a laboratory equipped to recover and read tags. Equipment is available for rental or purchase, and can sometimes be borrowed from other programs. Customized installations may be required.

Sample equipment prices (all prices are in US dollars, are subject to change, and do not include any applicable taxes or shipping). A full price list is available at www.nmt.us.

Mark IV Tag Injector	$21,700 (can be rented for $2,110 per month)
Coded Wire Tags	$88/1000 (based on a quantity of 100,000 to 999,000)
R9500 Tunnel Detector	$17,500 (can be rented for $1,750 per month)
Handheld Wand Detector	$5,000 (can be rented for $415 per month)
V-Detector	$5,000 (can be rented for $415 per month)

What important issues (or obstacles or questions) might apply to marking or mass marking of PWS herring?

What data do you need to collect?
Do the herring need to be tagged to collect that data?
Is there an appropriate tagging technology that can be applied at the life stage you are interested in?
If yes, will that tag give you the data you need?
Will it give you part of the data you need?
How many fish need to be tagged to get the precision you need in your data?
How are you going to recover the tags?
Can you afford it?

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