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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

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DRAFT

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 current 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 *Exxon Valdez* Oil Spill Trustee Council (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 commercial 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. Chemical marking of herring otoliths, by exposure to chemical dyes, has been successfully used in Japan and similar approaches might also be considered for PWS/. There are, however, a number of important biological differences and different regulatory considerations that would need to be addressed. 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. Although it was not explicitly discussed in the workshop, the different tagging approaches are not mutually exclusive. For example, Coded Wire Tagging or otolith marks could be used to validate genetic studies, etc.

The workshop provided a unique opportunity to assemble 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 (cwt's), used mainly on large juveniles and adult fish;
- (3) otolith (‘earbone’) fluorescent dyes and stress-induced marks applied to very young fish
- (4) natural tags, with emphasis on chemical fingerprints, mainly from otoliths
- (5) acoustic tags, suitable for large juveniles or adults
- (6) genetic tags, that require prior development of spawning broodstock
- (7) fatty acid signatures, that reflect different diets.

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 substantial local knowledge and capability exists 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 but the results will only indirectly be 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 Alizarin Complexone. However, this specific chemical may incur severe permitting problems if applied in North 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 for 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.

Acoustic tagging holds considerable promise for herring, 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 organizations, so the present time is an excellent opportunity to pursue this approach. Acoustic tagging methods, have not been conducted previously with herring, but they have been used on other small 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 the 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 methods 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 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 report. Chapter two presents a summary and brief discussion of the individual contributions. Chapter three presents a synthesis that discusses the key issues raised in 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 format so that they could be presented in the collected Table of Contents.

Limitations and potential applications of the report

This report does not present a definitive conclusion or recommendations because many biological, technical and procedural uncertainties remain. Nevertheless, the 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 Council)
- 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 in a form that is 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 many different future scenarios that could develop (Hay 2008). Common to all approaches to supplemental production 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 ADF&G, 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 rationale 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 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 of 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 can 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 electrical field– at which time they can emit a signal. The tags of special interest for PWS are acoustic tags. They are relatively large so they 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 along with the date, time location, and other auxiliary information. 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 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. 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 elsewhere in the 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 or 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 probable 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 routinely in herring literature but sometimes are interpreted incorrectly 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 larvae 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 are not intended as critical reviews of each contribution. Instead the purpose of the summary and commentary is to provide a brief review 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 1950s. Maximal abundance was probably much greater than one million tonnes, but since the 1950s 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 of 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 is less than 10 cm. There is considerable uncertainty about factors affecting the distribution or survival of age 0+ juveniles, but there is strong evidence of starvation by some during the winter in PWS.

Age 0+ juveniles seem to rear within PWS. They grow larger during the second summer of life and probably 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 in the winter is an important issue, especially if some forms of enhancement are considered. It appears this life stage that might benefit from some forms of intervention in attempts to restore herring to PWS. It follows that carefully designed juvenile tagging programs could provide substantial information to 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 within the 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 pallasii*) 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 is projected to be 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 was ~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 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 since 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 in the processing plants. Often the quality of recovery information was poor with uncertain recovery origin and date. On the other hand there were many thousands of recoveries, so considerable information was gained from 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 (CWT) have been used on herring 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 use CWTs on juveniles, perhaps age 0+ juveniles.

Commentary 4

A particular concern about CWTs 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 CWTs 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. The complete report provides many useful and detailed estimates of such costs.

The practicality of CWTs for potential enhancement work in Alaska will depend on the scale of potential future enhancement projects and the natural abundance of the herring population. An important limiting factor will be the requirement to raise herring to a size that is sufficiently large to receive a CWT. Recovering CWTs will be another limiting factor but this limitation applies to all possible tagging programs, using different approaches to tags. An important positive attribute of CWT's is that they are the only tag that can be applied to a large number of fish and recovered using automated equipment.

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) tags 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 minimal deleterious impact on the health 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 (VIAAlpha) 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 is 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

These two reports are concerned with otoliths as structures 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 can be created by stress treatments. Short thermal shocks, for example, are known to induce distinct rings on the otoliths of many species, but it is unclear if this can be done in herring. These marks are easily applied and recovered.

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. Like otolith shape and ring structure, the natural elemental composition of the otolith can vary significantly over time and space.

A special aspect of otolith microchemistry is the potential for the addition of stable isotopes of elements that are analogues of calcium (e.g. Mg, Ba, Sr) 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 alizarin complexone 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 uncertainty regarding the effectiveness of dyes when they are applied during the egg stages. This may be an aspect worth further investigation but this is not a vital question at this time because most marking could be done at the larval or early juvenile stage. Japanese researchers marked otoliths during the egg stage with alizarin, but no otoliths are present at that stage! The likely scenario was that the chemical marker was retained by the yolk and incorporated into the otolith when the yolk reserves were metabolized for growth. But the question remains: how did a chemical with large molecules get into the egg in the first place?

One of the main uncertainties about the evaluation of otolith 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.

The logistical implications for recovery of marked otoliths warrant further consideration. As the cost of mark recovery goes up, the number of samples will probably go down: the smaller the marker, the greater the cost of recovery. Visible markers, for example, are easy to see and therefore inexpensive to recover, but elemental markers are costly because sample preparation and mark detection is time consuming and requires expensive specialized equipment.

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 be 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 about 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 laboratory conditions.

Commentary 6

Acoustic tags are relatively expensive (at several hundred dollars each) and would be limited to the large juvenile and adult 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 receivers. 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. The 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?

Ron Heintz, NOAA, Juneau
Ted Otis, ADF&G

Can fatty acid signatures (FAS) make effective markers for some types of research and monitoring activities? The answer to this question was addressed in this informative review. The review points out the uncertainty 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

There may be a role for some fatty acid analysis in certain types of short term experiments related to enhancement. For instance, over short time spans fatty acid signatures could be used to identify herring from different experimental groups that had been fed unique diets.. In a hatchery environment, where herring are reared on an artificial diet, the reared fish probably will have a different FAS 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 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 the 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. At the present time this option is more of a concept than a well-defined procedure. The technical feasibility of 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 of 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

Jeffrey R. Guyon, NOAA, Juneau,
Chris Habicht, ADF&G, Juneau
Jeffrey Olsen, USFWS, Anchorage

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 from other populations, the genetic variation may not be temporally stable. Relative to most salmonid species, herring exhibit relatively small degrees of genetic variation 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 applicable 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 vary 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, and a well constructed sampling program is critical. This chapter uses the experience gained in the developing a mass marking program for salmon to show how contributions estimates to commercial fisheries are relatively straight forward to determine when goals are to achieve a target level of precision based on confidence intervals. If assurances can be made that samples obtained are representative, then the numbers required for sampling are manageable if 100% marking rate is achieved and there is an expectation that the marked fish can be recovered from those samples. 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. 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 routes of herring are not well defined. Therefore it will be important that sampling effort is carefully constructed 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 on the issues of the numbers of marked fish that must be released, the corresponding numbers of recaptured fish in 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 spatial distributions of 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 factors of 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 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 a few seconds to positively identify a well prepared Sr marked otolith, but it can take many minutes to positively confirm that a 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

Christopher Habicht, Stock Assessment Geneticist
Alaska Department of Fish and Game, Commercial Fisheries Division,
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 potential herring enhancement activity. This chapter does not comment specifically on the potential use of genetic tags where the released fish may be genetically dissimilar to wild fish which covered in Chapter 8 that described ‘genetic’ marks).

A different but partially related policy issue would be the potential for disease transfer, associated with fish transfers or release of cultured fish. This issues was not addressed in 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 or larvae, would induce such a mark, but the possibility cannot be ruled out. For instance, recent work with the elemental composition and morphological attributes of otoliths from hatchery raised chinook demonstrated that the simple act of rearing fish in a hatchery could be enough to create a unique marker that could be used to differentiate otoliths recovered from captive reared fish from their wild counterparts. 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 dyes (Rows 2-4 and Columns 1-2 in Table 1)

It is uncertain if chemical dyes or elemental isotopes 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 probable that the mark would be very tiny, and correspondingly difficult to detecting recaptured fish at 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 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 improbable 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 difficulty 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 and 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 argue that it is within the ovary, when genetic marks could be applied within an ovary. (Others may argue, perhaps correctly, that it is 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 acid signatures could be applied and recovered at any 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’) indicates 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. 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 dyes otoliths		Elemental markers otoliths		external tag		internal 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)	m3*	yes	yes	yes	yes	yes	yes	yes	yes	yes		yes	yes	yes
6 2+ Pre-recruit(y+2)	m3*	yes		yes	yes	yes	yes	yes	yes	yes		yes	yes	yes
7 Adult(y+3+)	m3*	yes		yes	yes	yes	yes	yes	yes	yes		yes	yes	yes
8 Broodstock	m3*	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.
 M3* In theory, larger fish could be marked but they would require long holding periods.

Comparisons among approaches

Table 2 provides a more detailed summary of each tagging or 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 methodology that has promise is the array of chemical dyes and elemental marks (Table 3). At the present time, however, it is not clear which specific chemical dye would be best. The simplest approaches, which involve a chemical dye mark applied to the otolith at an early life history stage, seem to be the most promising and least expensive. Such approaches have been successfully applied in Japan using the alizarin complexone. During discussions of this approach in the workshop it was clear that the advantages and disadvantages of each specific dye or chemical vary. Some are 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.

Brief discussion subsequent to the workshop has focused on the specific question of how and when chemical dyes can be taken up by otoliths. Remarkably, it seems that some dyes can be effective when applied at the egg stage, even before otoliths are formed! The explanation for the later incorporation of dye into the otolith is that the dye is taken up and stored in the yolk prior to being deposited in the otolith of an embryonic herring.

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. The life history stages are the egg, larvae, age 0+juvenile (0+ juv), age 1+ juvenile (1+ juv), recruit (or a fish that is entering sexual maturity ofr the first time, normally at age 3 or 4) and an adult (sexually mature fish).

Workshop Presenter	Type of tag or mark	<u>Life history stage - application</u>					
		egg	larvae	0+ Juv	1+ Juv	recruit	adult
Doug Hay	external Floy tags, internal belly tags			m	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	m			
Andrew Munro	chemical analysis of otoliths	m	y	m			
Olsen/Guyon	genetic marking	y					y
Otis-Heinz	fatty acids as marks	m	m	m	m	m	m

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. The life history stages are the egg, yolk-sac larvae, feeding larvae, age 0+juvenile (0+ juv), age 1+ juvenile (1+ juv), recruit (or a fish that is entering sexual maturity for the first time, normally at age 3 or 4) and an adult (sexually mature fish).

Workshop Presenter	Type of tag or mark	Life history stage - application						
		egg	yolk-sac larvae	feeding larvae	0+ juv	1+juv	recruit	adult
1. Stress marks – developing unique dark rings on otoliths								
	thermal marks		m	y	y			
2. visible markers - fluorescent chemicals								
	Alizarin Complexone	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
	microstructural		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. There could also be negative public perceptions for some types of chemical otolith markers, especially strontium.

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. There also would be requirements for disposal of chemical and elemental markers.

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 holding and rearing of young herring juveniles. The type, location and scale 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.

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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).

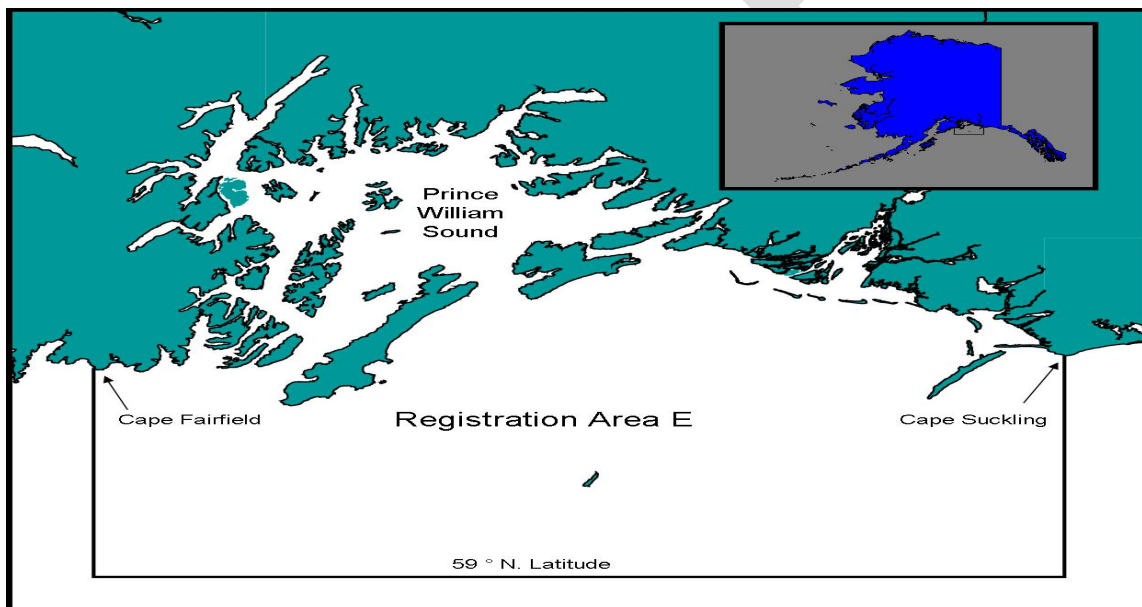


Figure 1. Pacific herring Registration Area E (5 AAC 27.300 Description of Prince William Sound Area).

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 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 and 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 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 will not 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).

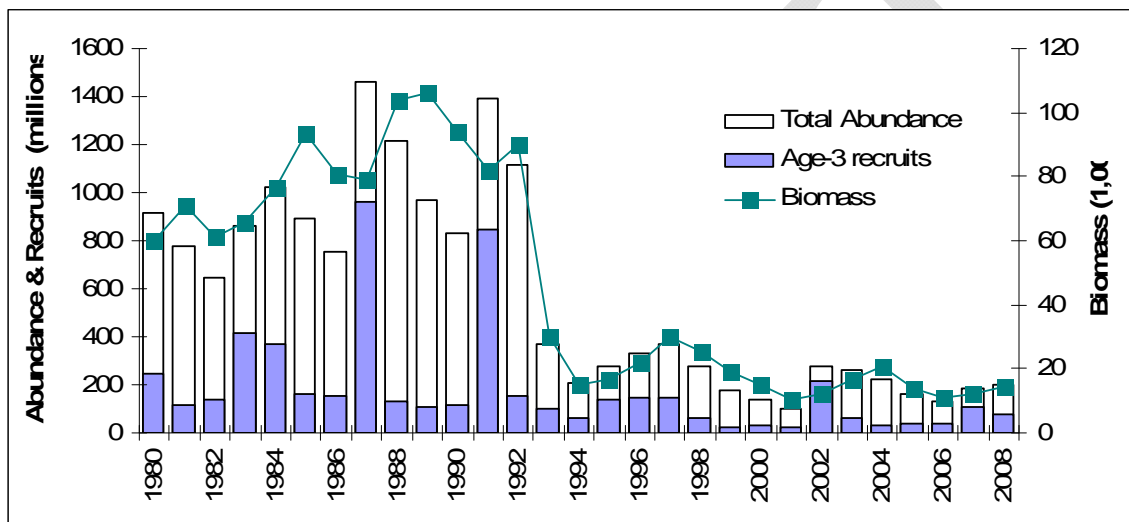


Figure 2. Total abundance, age-3 recruitment, and estimated prefishery run biomass from the 2008 version of the ASA model for Prince William Sound herring.

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).

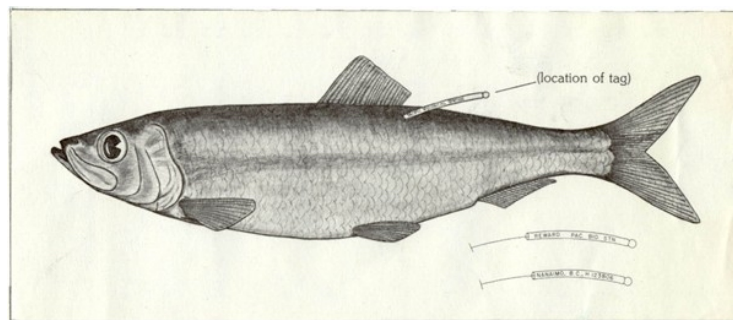


Fig. 2 Drawing of a herring with an attached 'anchor tag'. The drawings at the lower right show tags before insertion.

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 km²) 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 km² has a fidelity rate of about 80-90%. At the other extremes, there are almost no Sections, with areas < 100 km² that have detectable fidelity above 0. There are, however, a number of sections with fidelity estimates between 10%-80% that are approximately 200-500 km² sea-surface area.

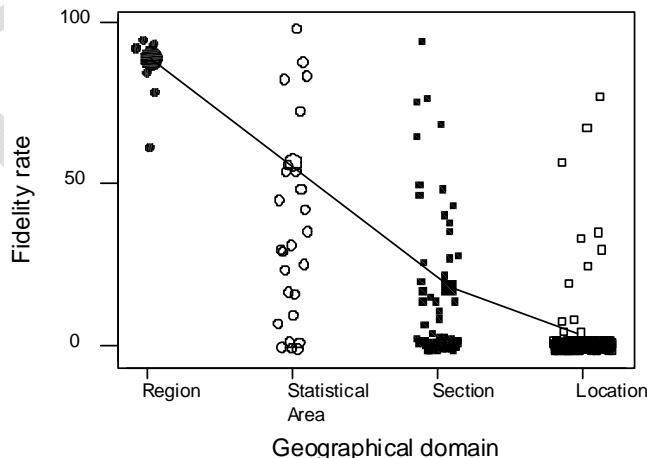


Fig. 3. Comparison of fidelity rates. The symbols show the mean fidelity for each Regions (dark circles), Statistical Areas (open circles), Sections (dark squares) and Locations (open squares). The overall mean for each geographic category is shown for each category by the largest symbol, which connects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

Release Region	Recovery Region								All
	UNK	QCI	NC	CC	JS	SOG	WCVI	USA	
—									
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 salmon example

The State of Alaska's otolith thermal marking program began 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 identify 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 (CWT-see 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 CWTs 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), 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 CWT 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 CWTs (Riffe and Mathisen 2002) and soon developed as routine tool for in-season salmon management in Prince William Sound (Joyce and Evans 2001).

Sampling goals for making contribution estimates

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 be 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) (SE / \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 worst 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 than 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.

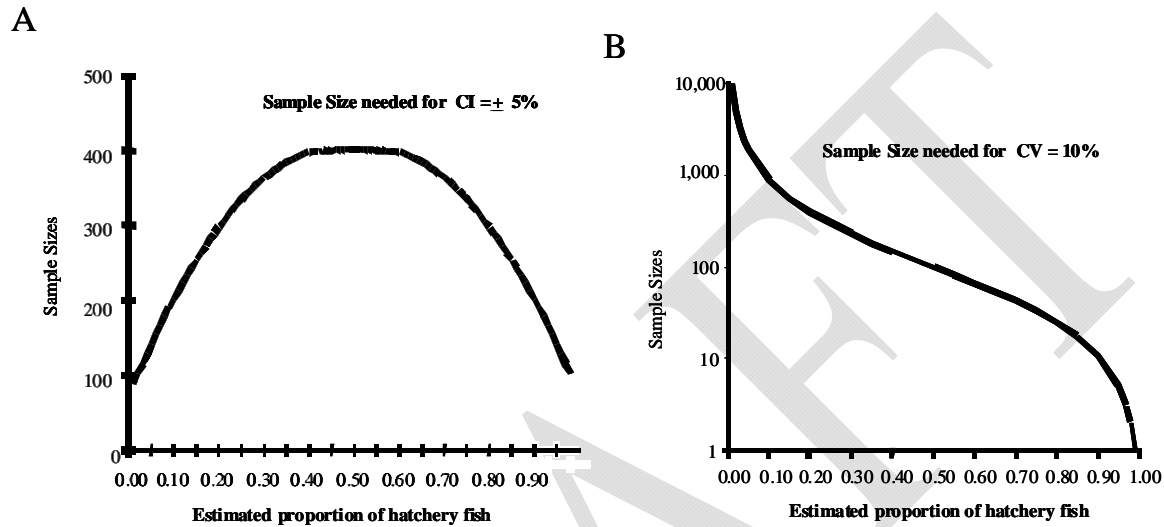


Figure 1(a). Sample sizes necessary to achieve a confidence interval of + 5 % around the estimate of the hatchery proportion in the population. **(b).** Sample sizes necessary to achieve a coefficient of variation of 10% on the estimate of the hatchery proportion.

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.

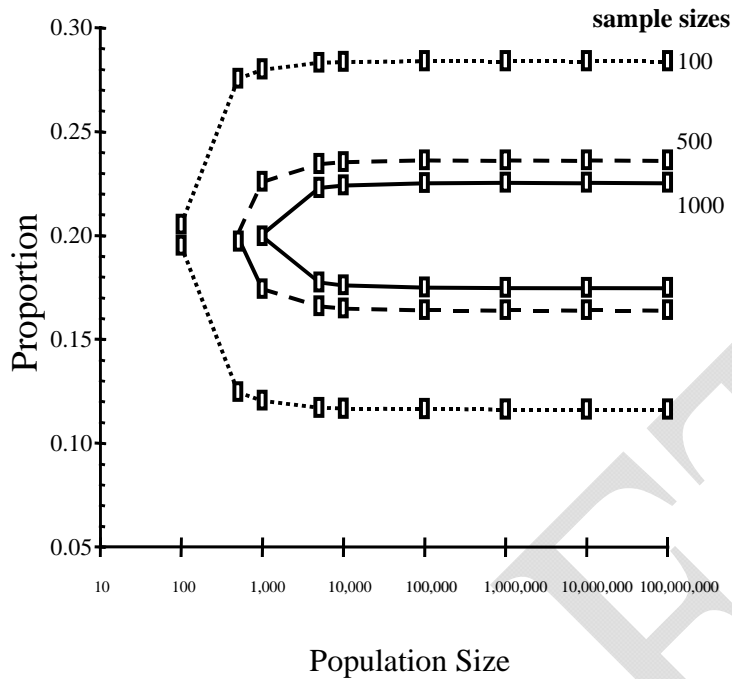


Figure 2. The 95% confidence bounds as a function of the size of the population, based on sample sizes of 100, 500, and 1000 for a sampled population containing a marked hatchery proportion of 0.20

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 be met. 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).

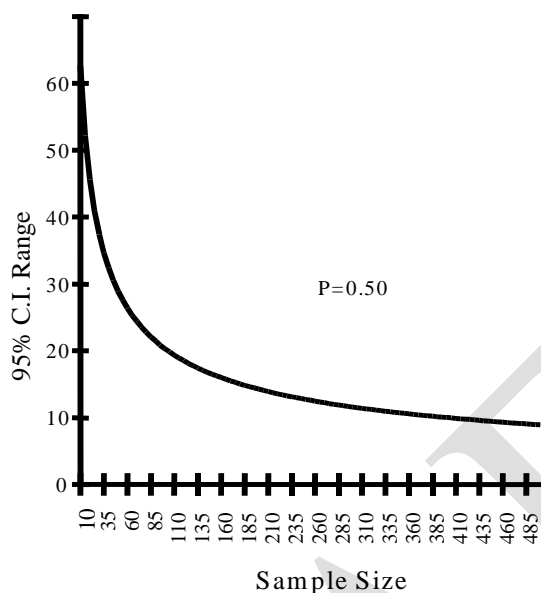


Figure 3. Changes in the 95% confidence interval range (upper bounds – lower bound) for the case of 50% hatchery fish as a function of increasing sample sizes. Graph illustrates how little there is to gain in precision by processing additional otoliths.

In 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 be sampled to achieve a given precision level as a function of the catch, the mark fraction and expected contribution.

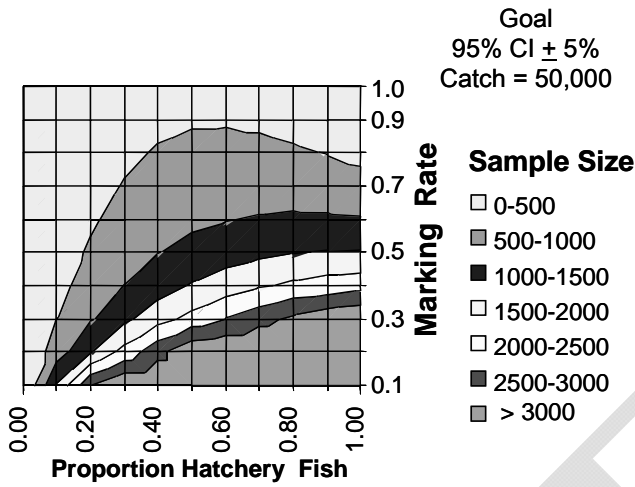


Figure 4. Sample size requirements to achieve a 95% CI that is + 5% of the point estimate as a function of the marking rate and the proportion of hatchery fish in the population. The graph illustrates how sample size requirements increase as the marking rate decreases at moderate levels of hatchery production.

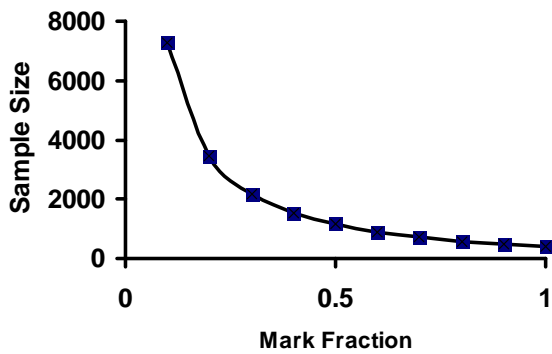


Figure 5. Sample size to achieve 95% CI with 5% precision as function of marking fraction assuming 50% hatchery fish in sample ($p=50$). This example is based on a catch sample of 80,000 though numbers are largely invariant of catch except at lower mark fractions

Lessons for herring

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 obtained 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, as discussed in chapter 11, much less is known. In considering an enhancement program and the attendant sampling effort, it will be critically important to anticipate how the marked fish may intermix with the wild fish such that the sampling effort conducted is representative and serves as a means to accurately estimate the success of the enhancement effort.

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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 °C. 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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DRAFT

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 of 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 tag 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 microsatellite 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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DRAFT

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. Fluorescein):

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. The 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 an 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 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 its 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 $^{87}\text{Sr}/^{86}\text{Sr}$ 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 unique elemental signatures in the hatchery-reared Chinook 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 $^{137}\text{BaCl}_2$, 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 pallasii*).

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 use 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., Albany 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., Albany 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 to 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

¹³⁷Ba 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 pallasii* 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 been 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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DRAFT

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 pallasii*) 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.

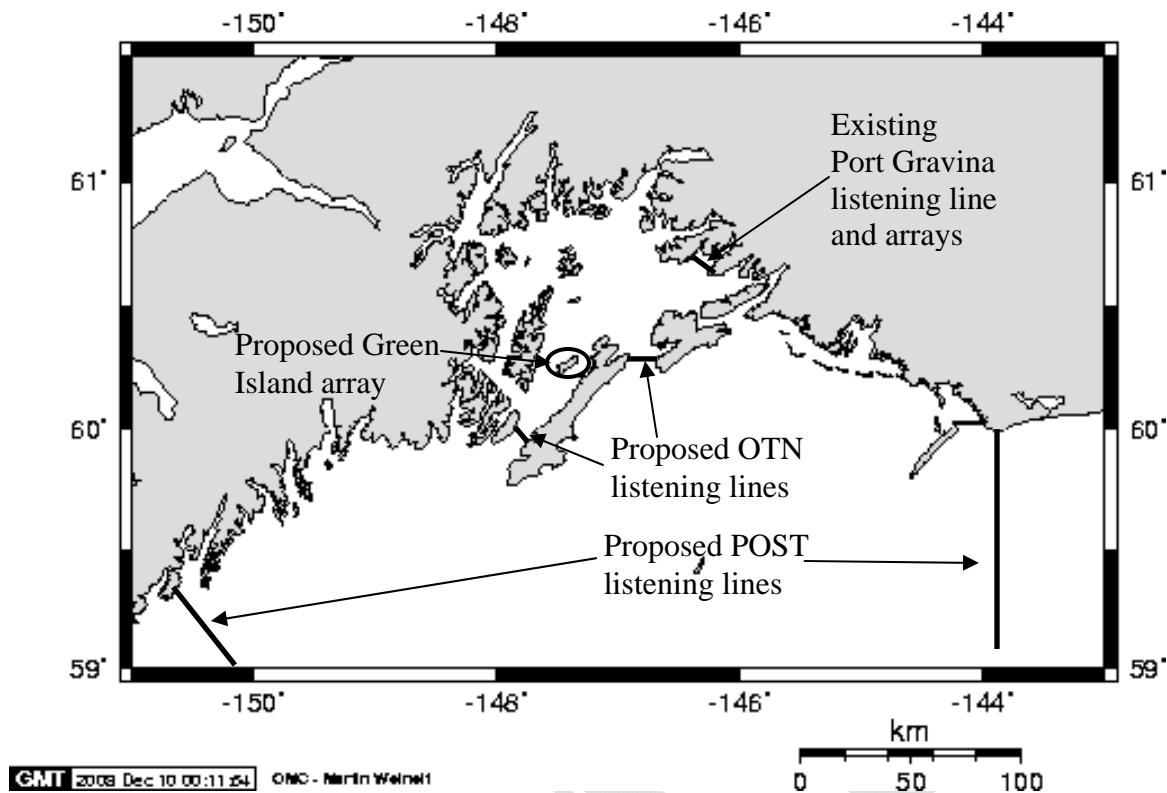


Figure 1. Existing and proposed hydrophone receiver listening arrays in and near Prince William Sound.

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Chapter Thirteen - 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 SrCl₂, 3000ppm for 24 hours and a layer of Sr-enriched aragonite is deposited on the otolith. Qualitatively this mark contains upwards of several weight 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 in 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 EPMA 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 well 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 Fourteen – 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 (VIAAlpha)

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

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.

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<p>Management (Basin-wide implications, on-going marking)</p>	<ul style="list-style-type: none"> ▪ 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?
<p>Hatchery Evaluation (Site-specific implications, on-going marking)</p>	<ul style="list-style-type: none"> ▪ 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?
<p>Experimental Marking (Fixed length studies)</p>	<ul style="list-style-type: none"> ▪ 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: <ul style="list-style-type: none"> ○ Result in more adult fish? ○ Affect where the fish go? ▪ Are there better ways to mark fish? ▪ Is the right strain of fish being used?
<p>Habitat Evaluation</p>	<ul style="list-style-type: none"> ▪ 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?
<p>Wild Fish Tagging</p>	<ul style="list-style-type: none"> ▪ 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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