Alaska Hatchery Research Group

Technical Document:[i] #12

Title: Otolith processing and quality control methods used by the ADF&G Cordova Otolith Laboratory
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Abstract

Hatchery facilities in Prince William Sound and Southeast Alaska release fry and smolt into the marine environment to enhance currently existing natural stocks. However, it is unknown if these hatchery releases have detrimental effects on the production and overall sustainability of natural stocks. The Alaska Hatchery Research Program was implemented to test some possible impacts on pink and chum salmon (Prince William Sound) and chum salmon (Southeast Alaska). In Prince William Sound, pink and chum salmon are known to stray from hatchery facilities upon their return as adults. To determine stray rates and genetic contribution of hatchery stocks to naturally occurring populations, it is essential to identify the presence of hatchery fish in historically natural streams. All Pacific salmon originating from hatchery facilities in Prince William Sound are assigned and receive an otolith thermal mark prior to release. As a result, successfully recovering and identifying these thermally marked otoliths is vital to the overall success of this project. This technical document outlines the sample preparation, quality control, storage, and data flow protocols used by the Cordova Otolith Laboratory.

Background of the Alaska Hatchery Research Program

Extensive ocean-ranching salmon aquaculture is practiced in Alaska by private non-profit corporations (PNP) to enhance common property fisheries. Most of the approximately 1.7B juvenile salmon that PNP hatcheries release annually are pink salmon in Prince William Sound (PWS) and chum salmon in Southeast Alaska (SEAK; Vercessi 2014). The scale of these hatchery programs has raised concerns among some that hatchery fish may have a detrimental impact on the productivity and sustainability of natural stocks. Others maintain that the potential for positive effects exist. To address these concerns ADF&G convened a Science Panel for the Alaska Hatchery Research Program (AHRP) whose members have broad experience in salmon

[i] This document serves as a record of communication between the Alaska Department of Fish and Game Commercial Fisheries Division and other members of the Science Panel of the Alaska Hatchery Research Program. As such, these documents serve diverse ad hoc information purposes and may contain basic, uninterpreted data. The contents of this document have not been subjected to review and should not be cited or distributed without the permission of the authors or the Commercial Fisheries Division.

enhancement, management, and natural and hatchery fish interactions. The AHRP was tasked with answering three priority questions:

I. *What is the genetic stock structure of pink and chum salmon in each region (PWS and SEAK)*?;
II. *What is the extent and annual variability in straying of hatchery pink salmon in PWS and chum salmon in PWS and SEAK*?; and
III. *What is the impact on fitness (productivity) of natural pink and chum salmon stocks due to straying of hatchery pink and chum salmon*?

Introduction

Background of Hatchery Marking Systems in Prince William Sound

To separate enhanced stocks from natural stocks, hatchery facilities throughout the North Pacific Rim are strongly encouraged to mark or tag their fish. These marks or tags may include, for example, coded wire tags (CWT), fin clips, or otolith marks. Currently, fisheries managers and research biologists use otolith mark information from Pacific salmon to facilitate management of commercial fisheries for Management Area E, consisting of Prince William Sound (PWS), Copper River, and Bering River districts. All hatchery-produced pink salmon *Oncorhynchus gorbuscha* and chum salmon *O. keta* released in PWS receive a thermal mark specific to each hatchery, species, release location, and in many cases, brood year (BY) (ADF&G 2002). Thermal mark patterns are negotiated with the Prince William Sound Aquaculture Corporation (PWSAC) and Valdez Fisheries Development Association (VFDA) through the North Pacific Anadromous Fish Commission (NPAFC) and their Working Group on Salmon Marking (WGSM). The NPAFC and State of Alaska maintain a database of thermal marks released from all countries producing Pacific salmon to minimize duplicate marks. This allows immature and juvenile salmon captured in offshore areas to be assigned to area of origin in addition to age determination applications (Urawa et al. 2001); for more information, please visit http://wgosm.npafc.org/.

Otoliths from Prince William Sound Pacific Salmon

The primary objective for the thermal mark recovery program is to provide inseason information of stock composition to ADF&G management staff for them to more effectively manage the mixed-stock commercial fisheries in PWS. However, other applications of this protocol are utilized as well, such as assessing Pacific salmon hatchery stray rates. The examination of streams for hatchery strays was prompted by findings from CWT studies of the 1990s, where increased hatchery releases resulted in higher frequency of hatchery fish in wild streams (e.g., Sharr et al. 1996). Thermal mark recovery from Pacific salmon carcasses in streams was also successfully used to identify hatchery strays in PWS (e.g., Joyce and Evans 2000, Brenner et al. 2012), prompting further study into the potential impact hatchery stocks could have on natural

populations. Other qualitative observations pertaining to the prevalence of hatchery strays include increased escapement in previously low escapement streams (Lewis et al. 2008), and increasing presence salmon species at weir sites where few were previously recorded (ADF&G, unpublished data).

This technical document outlines procedures, including quality control, used by the ADF&G Cordova Otolith Laboratory to process otolith samples collected as part of the AHRP. Goals of this section of the study are to:

1) Determine the proportions of pink and chum salmon hatchery strays in sampled streams;
2) Identify natal origin of pink salmon in sampled pedigree streams; and,
3) Determine the proportions of hatchery pink and chum salmon in the main entrance points to PWS.

Protocols and objectives of this portion of the AHRP study are a companion to sampling and otolith processing efforts in SEAK being completed by the ADF&G Mark, Tag, and Age Laboratory (MTA Lab) in Juneau, AK (Agler et al. 2015 a, b). To achieve the goals of this section of the project, samplers need to collect otoliths and other descriptive data (where applicable) from carcasses in streams located throughout PWS and from fish collected as part of an ocean test fishery (see Agler et al. 2015a for more information). Otolith mark patterns are identified and interpreted by trained otolith readers to indicate BY and natal origin in the Cordova Otolith Laboratory.

Goals of Technical Document

Goals of this technical document are as follows:

1) Describe otolith processing methods used by the Cordova Otolith Laboratory.
2) Describe the quality control measures used by laboratory staff to ensure data accuracy.

Methods for Otolith Processing and Storage

After sample trays and an inventory are delivered to the Cordova Otolith Laboratory, they are examined to ensure trays contain the correct number of otoliths and tray labels have correct descriptive information (e.g., collection date, species, and number of otoliths). Data discrepancies or missing trays issues are resolved with the contractor. Once the inventory evaluation is complete, data for each sample and tray are entered into the Prince William Sound

Thermal Mark Recovery (PWSTMR) Microsoft Access™ database[ii] through a front-end application (Frawley et al. 2015). Data entered include a unique sample identification number, unique tray identification number, harvest type, fishing district, sample date, statistical week, number sampled, sampler names, sample comments, species, gear, anadromous water stream code (all projects except ocean test fish), site name (ocean test fish only), and AHRP project type (ocean test fish, stream-stray, population structure, or pedigree).

Program otoliths are collected in either shallow 96-well trays (ocean test fish and stream-stray projects) or deep 48-well trays (population structure and pedigree projects). The 96 well trays are only used to collect otoliths whereas 48 well trays are used to collect genetic tissue samples and paired otoliths in the same tray well. Otoliths are placed into each tray type in a different order; otoliths are placed in shallow 96-well trays from left to right in rows whereas they are placed in deep 48-well trays from top to bottom in columns (Figure 1). Because Cordova Otolith Laboratory personnel have 4 months of processing commercial fisheries otoliths from shallow 96-well these trays prior to processing otoliths from deep 48-well trays, a jig is used to help ensure that otoliths come from columns and cannot fall into other wells.

Similar to methods described by Agler et al. (2015a) for the MTA Lab, otolith samples are prepared by first cleaning the otoliths with a mild bleach solution and then neutralizing the bleach solution. This removes blood or tissue that may be on the otolith, thereby preserving the specimen for long-term storage. Each slide is labeled with the species, sample location, sample identification number, sample date, specimen number, and a barcode. The corresponding otolith is then mounted to the slide using thermoplastic glue. After mounting, otoliths are slowly wet ground with 500-grit silicon carbide (SiC) grinding paper on a power grinder at 250 rpm until the thermal mark or wild ring pattern can be seen through a compound microscope. After mark status (marked or unmarked) and TMID of marked fish are determined, the information is entered in a Microsoft Access™ database[iii] through a front-end application, and is accessible by the AHRP (Frawley et al. 2015). If a specimen is missing, unreadable, damaged, or there are more than one pair of matched otoliths in a well, the appropriate specimen status is selected within the application (i.e., "No Read" or "Missing"), and comments are added. Once a sample is processed, a mark summary report is printed and stored in a labeled binder for future reference.

[ii] Product names used in this report are included for scientific completeness, but do not constitute a product endorsement

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Quality Control Methods

Blind Test Construction

Assessment of an otolith reader's ability to distinguish among all possible PWS hatchery and selected natural stock thermal mark patterns for a given run year is quantitatively measured with blind tests. Blind tests are conducted at the beginning of the field season, and again prior to processing AHRP samples to test for drift in readers mark identification criteria.

Prior to release, pink and chum salmon fry were collected from incubators at the Wally Noerenberg (WNH), Armin F. Koernig (AFK), Cannery Creek (CCH), and Solomon Gulch (SGH) hatchery facilities by PWSAC and VFDA staff, preserved in ethanol, and sent to the Cordova Otolith Laboratory. Otoliths are subsequently removed and mounted, sulcus side up, on a petrographic glass slide with thermoplastic glue. Mounted otoliths are placed in slide boxes labeled by origin and thermal mark. Samples of natural stock otoliths without a thermal mark are extracted from juvenile salmon captured in PWS streams and processed similarly to hatchery stock samples.

Before the commercial fishing season, 3 blind test sets of 100 otoliths each (pink, chum, and sockeye salmon) are randomly selected without replacement from samples of all possible marks anticipated for the run year. An ADF&G employee not affiliated with the Cordova Otolith Laboratory codes these prepared slides according to the randomization order to construct the blind tests. Once the blind tests are assembled, they are examined by all in-season readers prior to the start of the commercial fishing season. During the tests, otolith readers are encouraged to use notes from past years and photos from the MTA Lab voucher collection (Agler et al. 2015a; http://mtalab.adfg.alaska.gov/OTO/reports/VoucherSummary.aspx) to aid in mark identification; however, there was no discussion of the marks during the test. Because juvenile Pacific salmon otoliths are fragile, those used in the blind test are individually hand-ground to the mid-sagittal plane manually with wet 1,200-grit SiC paper and viewed under a compound light microscope at 200X or 400X magnification. Readers examine the otoliths and enter their mark interpretation into a computer template. Actual code information was not available to laboratory personnel until all tests of a set of 100 are completed. Overall ability of readers to correctly identify otoliths is determined by comparing readers' interpretations of marks to known origins. Before a reader could start processing otoliths of a given species, a score of ≥90% was required on the blind tests. However, the Fisheries Biologist III (FB III) Project Leader may deem it appropriate to start reading with a score of ≤90% if mark quality makes achievement of the goal unlikely. Generally, if the target score is not achieved, a second blind test is read after review and discussion of problem marks. If needed, retraining opportunities are available in the form of group discussion or a small workshop to ensure accuracy and further understanding of marks for future projects that season. Overall results from the 2013 and 2014 blind tests administered are outlined in Table 1. Accuracy in identifying specific marks for pink and chum salmon blind tests

are summarized in Table 2 (2013) and Table 3 (2014). For archival purposes, all blind test randomizations and otolith reader tests are digitally kept in annual files on the Cordova Otolith Laboratory's server. Additionally, all blind tests are in long-term storage for thermal mark identification practice by new otolith readers and as a reference collection for the Cordova Otolith Laboratory.

Post-Season Quality Control: Second Reads

After reading all otoliths for a specific project, a subset of the processed otoliths are read a second time ("second-read") by a different otolith reader to examine consistency in mark identification between readers. Because of limited funding and time (all Cordova Otolith Laboratory's staff are seasonal employees, including the supervisor), only 10–30% of a species and project type are usually read a second time. If time and funding permit, more samples undergo the second read process. Samples to be second-read are selected based on mark frequency and original reader. Once complete, samples are given to the Fisheries Biologist I (FB I) Laboratory Supervisor to reconcile discrepancies between readers. If there are major discrepancies between first and second readers, then more samples from the first reader are examined. Additionally, retraining opportunities can be offered if time permits (e.g., a group study of tricky marks or an additional blind test) to increase consistency among readers for the remaining projects.

Although some marks had low reader agreement, particularly for chum salmon, reader agreement of marked and unmarked otoliths was greater than 97% for pink and chum salmon from the AHRP (Tables 4–7). Additionally, overall reader agreement for all marks for a given species was 94% or higher.

Discussion

Chum Salmon

Historically, chum salmon thermal marks are difficult to read. They may not take some thermal marks well, and difficulty in reading is not limited to a certain facility or a certain mark. A thermal mark can appear very clearly on one specimen, but be very difficult to interpret on the next specimen. Additionally, chum salmon otoliths display a large amount of incremental growth that can obscure thermal marks. Because of this variability in thermal mark among fish, the voucher process identifies, quantifies, and catalogs any thermal mark variants that show up in the voucher samples. Variations are measured and photographed for use by readers to aid in identifying similar marks. Additionally, the voucher process alerts hatchery facilities of any errant marks so adjustments to marking protocols can be made for future brood years. However, even with the voucher catalog available for otolith readers, chum salmon marks can still be extremely difficult to identify.

For 2013 and 2014 chum salmon runs, there were several variants of assigned thermal mark patterns which resulted in marks that were similar to (or the same as) other marks released during those years. In particular, BY 2008 chum salmon from Port Chalmers and WNH had variants that appeared the same as assigned marks for other release sites for the same brood year. For example, the 3,2nH mark (released at AFK) had a 5,2nH variant, which was also an assigned mark for BY 2008 chum salmon released at WNH. Because these two marks were from the same brood year, readers could not use an estimate of age to narrow down mark possibilities. Additionally, the mark 4,1H also looked like a 3,2nH at times, again adding additional uncertainty to mark identification. As a result, readers found it impossible to determine the exact mark for these chum salmon otoliths.

In 2013 and 2014, none of the otolith readers were able to achieve the target score of ≥90% correct identifications on chum salmon blind tests, mostly due to the poor marks and variant marks documented in the voucher otoliths. Readers mostly had problems with BY 2008 marks, as well as specific marks from BY 2010 (AFK 1,2,3H) and BY 2011 (AFK: 1,2,2H). The latter two marks were confused because of mark spacing; most readers felt they were so similar that they could not distinguish between the two marks. The number of otoliths with each mark is not the same in a blind test; some marks are randomly selected more often in the tests than others, potentially skewing the percent accuracy statistic reported. Despite the difficulty in identifying individual marks, readers were able to identify the unmarked otoliths with a relative high rate of success (80–100%, with between 3 and 20 unmarked otoliths for a single test). The ability of a reader to identify unmarked otoliths can be impeded if otoliths are improperly ground during sample prep or if the assigned mark was very similar to natural mark patterns seen in PWS. Because no reader achieved the target score on the chum salmon blind tests in 2013 (one or two tests per reader) or 2014 (all readers took three tests), the FB III Project Manager decided to waive the other blind tests and allow all readers to read chum salmon, provided more second reads were completed on chum salmon at the end of the season (Table 1).

Pink Salmon

There were no major issues with our reader's ability to identify pink salmon thermal marks. In 2013, there was some difficulty in determining the presence of the accessory mark (-H3) in both WNH and AFK specimens. The accessory mark, though consistently placed, is extremely easy to grind away during processing. Some readers, especially those in their first season, tended to have a slightly heavy hand when grinding, causing these accessory marks to be extremely faint or completely ground away. Another issue some readers had was to interpret a very prominent, naturally occurring otolith feature into a mark, causing false identification of either the 4H3 or 8H3 marks.

In 2014, there were no marks with post-hatch accessory rings, and the only issue some readers had was distinguishing the 7H SGH glitch and the 8H WNH marks. Historically, SGH marks

were easy to distinguish from WNH marks because of the spacing between rings and thickness of the rings themselves. In 2014, the BY2012 SGH mark looked very similar to the WNH mark, causing readers some difficulty in identifying these marks.

Despite these issues, reader agreement during second reads was high (94–95%), and agreement in identifying unmarked otoliths ranged from 96–99%.

Resolving Mark Identification Issues

Because some mark issues became apparent through blind tests and second reads, several strategies are used to improve the accuracy and precision of readers. First, readers are asked to try to resolve a questionable mark by studying the online voucher database as well as consulting their observational notes. Additionally, readers discuss mark questions during sample reading and ask other readers about their mark identification criteria for certain specimens and thermal mark patterns. This type of open communication can help readers improve their understanding and pattern recognition skills, increase productivity, and improve job satisfaction.

Second, a review workshop was conducted after the commercial fishing season in 2013 and 2014 that focused on increasing accuracy of problem marks. Photographs of chum (predominantly) and pink salmon marks from in-season samples were projected for readers to view and discuss. Each reader provided their criteria for identification of the mark based on their experience. Mark criteria used by readers were discussed and mark images compared to voucher images until a group consensus about mark identification was achieved. These review sessions provided an environment for readers to discuss problem marks and offer assistance or tips to other readers. Sometimes this workshop would take a couple hours; however, this exercise increased consistency with mark identification while building reader's confidence and *esprit de corps* (extremely important when collectively reading 50,000+ otoliths in a 9-month season!).

Finally, we would second read more otoliths from readers who did not score well on blind tests of a particular species or if preliminary second reads indicated more disagreement than anticipated. In either case, more experienced readers would second read more samples by that particular reader.

These strategies, when combined with the preseason blind tests and standards and procedures already in place, allows the Cordova Otolith Lab to have a consistently high rate of reader agreement. Although additional improvements are possible, these protocols allow the lab to produce quality data and maintain high standards for our fisheries biologists, hatchery managers, and collaborators to ensure that Alaskan resources are properly managed and utilized throughout the season.

Summary

- Otolith processing and quality assurance protocols are similar to those used in the ADF&G MTA Lab in Juneau, AK (Agler et al. 2015 a, b). There are very few differences between the two laboratories' protocols.
- Blind tests provide a quantitative measure of reader ability to identify thermally marked otoliths as well as individual marks. Blind tests are read at the start of the commercial fishing season in May, and sometimes at other parts of the year.
- Second reads of processed samples allow supervisors to measure the consistency among readers. Depending on time, funding, and frequency of reader discrepancies, 10–30% of samples for a given project type and species are read a second time by a different reader.
- Readers in the Cordova Otolith Laboratory reached a 94% or higher reader agreement for the pink and chum salmon specimens processed as part of the AHRP projects in 2013 and 2014. Ability to distinguish between marked and unmarked fish was 97% or higher for both years.

Questions for AHRP Science Panel

1) Are the methods presented here adequate for assessing accuracy of detecting the presence of a hatchery (thermal) mark?
2) Are the methods presented here adequate for assessing the accuracy of identifying hatchery-specific marks?

AHRP Review and Comments

This technical document has been reviewed.

This document covers some of the well established procedures for thermal mark recovery at the ADF&G Cordova Lab. There were very few comments on this document.

One reviewer felt that the use of "stray rate" in the abstract was confusing. The science panel generally prefers to refer to the "proportion of hatchery origin fish" on the spawning grounds. That reviewer also thought that the term "genetic contribution of hatchery stocks to naturally occurring populations" might be misleading considering that the hatchery fish presumably have the same genes as wild fish.

This document is acceptable to the AHRG.

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Tables

Table 1: Reader accuracy (percentage correct) in determining mark status and identifying facility of origin as determined by blind tests, 2013–2014. Otoliths that were unreadable or overground were not included in the final scores.

	2013 Pink Blind Test #1	2013 Pink Blind Test #2	2013 Chum Blind Test #1 [a]	2013 Chum Blind Test #2 [a]	2014 Pink Blind Test #1	2014 Pink Blind Test #2	2014 Pink Blind Test #3 [b]	2014 Chum Blind Test #1	2014 Chum Blind Test #2 [c]	2014 Chum Blind Test #3 [b]
Reader 1	97%	–	88%	–	96%	100%	98%	84%	91%	91%
Reader 2	92% [d]	96%	–	60%	84%	93%	92%	44%	73%	68%
Reader 3	89%	–	64%	–	87%	94%	92%	75%	83%	87%
Reader 4	91%	–	68%	77%	94% [e]	91%	88%	70%	85%	79%

[a] Although readers did not achieve the target of ≥90% because of poor mark quality, the FB III project leader allowed all readers to process chum salmon otoliths. Reader 1 read most of the chum salmon otoliths during the season, as she has the most experience and scored the highest on this test. After the commercial season and before processing AHRP otoliths, a workshop was conducted with all readers to refine mark identification technique for chum salmon marks. No third blind test was available for further testing.

[b] A third blind test in 2014 was completed after the commercial season and prior to processing the AHRP study specimens.

[c] Although readers did not achieve the target of ≥90% because of poor mark quality, the FB III project leader allowed all readers to chum salmon. Reader 1 read most of the chum salmon otoliths during the season and the AHRP project, as she has the most experience and achieved the target score on the blind test.

[d] No wild otoliths were identified during this test, but were reported by the reader as overground. The FB III project leader and FB I lab supervisor deemed it necessary to retest to ensure competency in mark identification.

[e] This reader left a large number of otoliths blank. Even though the score was above 90%, only 80 otoliths were read. The FB III project leader and FB I deemed it necessary to retest to ensure competency in mark identification.

Table 2: Reader accuracy (percentage correct) in determining mark identification from blind tests, 2013. Otoliths that were unreadable (typically ~5% total) were not included in the final scores.

Species	Hatch Mark	Reader 1, Blind Test #1	Reader 2, Blind Test #1	Reader 3, Blind Test #1	Reader 4, Blind Test #1	Reader 1, Blind Test #2	Reader 2, Blind Test #2	Reader 3, Blind Test #2	Reader 4, Blind Test #2
Pink Salmon	3,3H	100%	100%	86%	87%	–	94%	–	–
	4H	86%	92%	86%	86%	–	100%	–	–
	4H3	92%	90%	58%	92%	–	94%	–	–
	6H	100%	100%	63%	88%	–	100%	–	–
	8H	100%	72%	95%	84%	–	94%	–	–
	8H3	100%	100%	95%	100%	–	100%	–	–
	wild pink salmon	100%	NA [a]	100%	100%	–	88%	–	–
Chum Salmon	1,2,1,2H	100%	–	83%	80%	–	33%	–	80%
	1,2,3H	100%	–	0%	67%	–	33%	–	67%
	1,2n,3H	100%	–	86%	71%	–	60%	–	100%
	1,3,3H	63%	–	67%	60%	–	40%	–	77%
	1,3n,2H	100%	–	50%	50%	–	–	–	–
	1,4,1H	100%	–	100%	100%	–	–	–	–
	1,5H	78%	–	75%	67%	–	63%	–	50%
	3,2nH	93%	–	64%	71%	–	50%	–	40%
	3,4nH	100%	–	90%	67%	–	93%	–	100%
	4,1H	71%	–	29%	86%	–	83%	–	33%
	5,1H	100%	–	25%	60%	–	100%	–	50%
	5,2nH	71%	–	50%	57%	–	40%	–	80%
	wild chum salmon	100%	–	100%	80%	–	100%	–	100%

[a] Blind test #1 wild pink salmon were all marked as unreadable and thus not factored into the final blind test score.

Table 3: Reader accuracy (percentage correct) in determining mark identification from blind tests, 2014. Otoliths that were unreadable (typically >4% total) were not included in the final scores.

Species	Hatch Mark	Reader 1, Blind Test #1	Reader 2, Blind Test #1	Reader 3, Blind Test #1	Reader 4, Blind Test #1	Reader 1, Blind Test #2	Reader 2, Blind Test #2	Reader 3, Blind Test #2	Reader 4, Blind Test #2	Reader 1, Blind Test #3[a]	Reader 2, Blind Test #3[a]	Reader 3, Blind Test #3[a]	Reader 4, Blind Test #3[a]
Pink Salmon	3,3H	92%	100%	100%	100%	100%	88%	92%	92%	95%	77%	100%	95%
	4H	100%	50%	71%	100%	100%	100%	100%	95%	95%	89%	82%	90%
	6H	100%	89%	86%	77%	100%	59%	100%	94%	100%	93%	92%	93%
	8H	90%	71%	76%	100%	100%	93%	85%	81%	100%	100%	90%	67%
	wild pink salmon	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Chum Salmon	1,2,1,2H	80%	20%	60%	60%	100%	50%	100%	100%	100%	43%	100%	100%
	1,2,2H	92%	77%	77%	100%	80%	80%	80%	100%	85%	67%	75%	69%
	1,2,3H	70%	0%	50%	0%	100%	29%	14%	71%	91%	18%	91%	91%
	1,2n,3H	83%	100%	100%	83%	100%	100%	100%	100%	100%	75%	100%	100%
	1,3,3H	100%	89%	100%	100%	100%	100%	100%	100%	92%	83%	92%	100%
	1,5H	43%	0%	43%	29%	88%	50%	71%	71%	75%	75%	75%	75%
	3,2n,1H	100%	0%	75%	100%	89%	75%	88%	100%	100%	100%	100%	100%
	3,2nH	86%	33%	83%	67%	100%	71%	100%	100%	100%	100%	100%	40%
	3,4nH	100%	43%	100%	100%	100%	85%	100%	100%	100%	80%	90%	70%
	4,1H	86%	0%	40%	25%	100%	100%	100%	100%	67%	33%	50%	33%
	5,2nH	57%	57%	43%	40%	50%	40%	20%	20%	86%	43%	71%	43%
	5n,2H	92%	25%	92%	83%	87%	67%	93%	80%	89%	78%	89%	89%
	wild	100%	100%	100%	100%	83%	100%	83%	100%	100%	100%	100%	80%

[a] The third blind test in 2014 was completed after the commercial season and prior to processing the AHRP study specimens.

Table 4. Reader agreement (percentage) for Prince William Sound Alaska Hatchery Research Program projects completed for pink salmon during the 2013 season. Overall reader agreement among all marks across all projects was 94%.

	Ocean Test Fishery	Stream-Stray	Stream-Pedigree (including Stock Structure)	Overall Agreement
AFK11A	60%	73%	62%	63%
AFK11B	82%	50%	82%	75%
CCH11	83%	79%	86%	82%
SGH11	93%	92%	82%	92%
WNH11A	75%	77%	72%	76%
WNH11B	71%	36%	96%	49%
Wild	96%	99%	99%	99%

Table 5. Reader agreement (percentage) for Prince William Sound Alaska Hatchery Research Program projects completed for chum salmon during the 2013 season Overall reader agreement among all marks across all projects was 96%.

	Ocean Test Fishery	Stream-Stray	Overall Agreement
PORTCHALMERS07	100%	NA	100%
PORTCHALMERS08	86%	75%	83%
PORTCHALMERS09	94%	100%	96%
PORTCHALMERS10	NA	NA	NA
WNH07	NA	NA	NA
WNH08	60%	45%	57%
WNH09	79%	87%	81%
WNH10	NA	NA	NA
WNH-AFK08	84%	60%	82%
WNH-AFK09	100%	NA	100%
WNH-AFK10	NA	NA	NA
Wild	91%	100%	99%

Note: NA=Not applicable.

Table 6. Reader agreement (percentage) for Prince William Sound Alaska Hatchery Research Program projects completed for pink salmon during the 2014 season. Overall reader agreement among all marks across all projects was 95%.

	Ocean Test Fishery	Stream-Stray	Stream-Pedigree (including Stock Structure)	Overall Agreement
CCH12	100%	94%	94%	94%
AFK12B	97%	81%	74%	77%
SGH12	99%	100%	81%	96%
WNH12B	94%	94%	98%	97%
Wild	99%	98%	96%	97%

Table 7. Reader agreement (percentage) for Prince William Sound Alaska Hatchery Research Program projects completed for chum salmon during the 2014 season. Overall reader agreement among all marks across all projects was 96%.

	Ocean Test Fishery	Stream-Stray	Overall Agreement
PORTCHALMERS08	100%	0%	5%
PORTCHALMERS09	100%	0%	63%
PORTCHALMERS10	64%	0%	47%
PORTCHALMERS11	100%	0%	67%
WNH08	60%	NA	60%
WNH09	92%	45%	71%
WNH10	100%	0%	96%
WNH11	50%	0%	25%
WNH-AFK08	100%	0%	58%
WNH-AFK09	100%	0%	50%
WNH-AFK10	75%	0%	50%
WNH-AFK11	100%	0%	7%
Wild	99%	99%	100%

Note: NA=Not applicable.

Figures

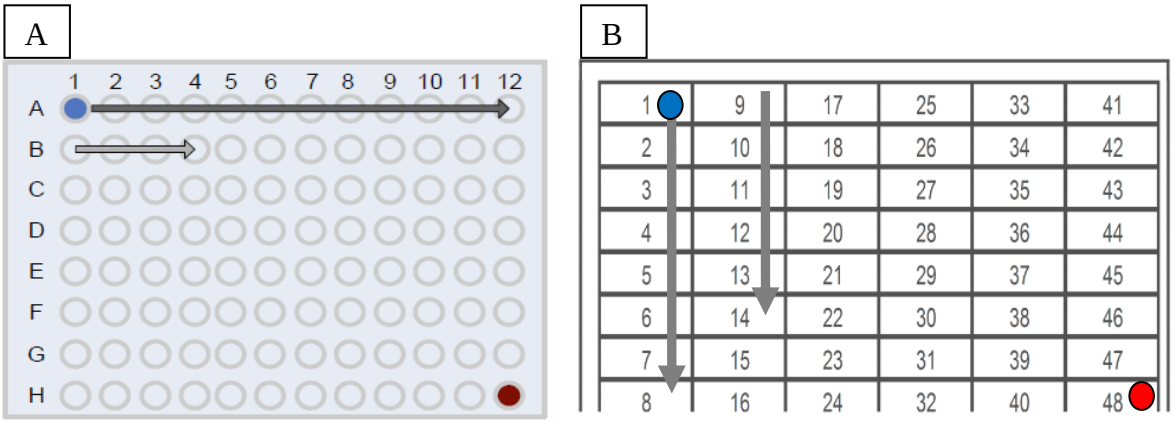

Figure 1. Schematics of A) otolith only (stream-stray and ocean test fishery otoliths) shallow 96-well trays and B) stock structure and pedigree deep 48-well trays. Arrows show the order otoliths are loaded during sampling and the order in which they are processed. Blue circles are the first cell, red circles are the last cell in the respective trays. (Tray templates courtesy of PWSSC, 2013).