Regional Operational Plan CF.2A.2021.02

Recovery and Analysis of Thermal Mark Hatchery Salmon Otoliths in Lower Cook Inlet, 2021

by

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February 2021

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

REGIONAL OPERATIONAL PLAN CF.2A.2021.02

RECOVERY AND ANALYSIS OF THERMAL MARK HATCHERY SALMON OTOLITHS IN LOWER COOK INLET, 2021

by

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Division of Commercial Fisheries

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SIGNATURE PAGE

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Approval

Title	Name	Signature	Date
Project Leader	Edward O. Otis		
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PURPOSE

The overall purpose of this project is to assess wild and hatchery salmon stock performance (e.g., adult returns, productivity, marine survival) in the Southern and Outer districts of the Lower Cook Inlet Management Area (LCIMA). Alaska Department of Fish and Game (ADF&G) employees in the LCIMA recover otoliths from pink *Oncorhynchus gorbuscha* and sockeye *O. nerka* salmon harvested in commercial common property (CCP) and hatchery cost-recovery (HCR) fisheries and examine the samples for hatchery marked otoliths to estimate contributions of wild and hatchery fish. These contribution estimates are used by ADF&G fisheries managers to assist in managing mixed stock commercial harvests to be consistent with sustained yield of wild and hatchery stocks (AS 16.05.730 and 5 AAC 39.220), follow appropriate principles and criteria in the Policy for the Management of Sustainable Salmon Fisheries (5 AAC 39.222), and achieve spawning escapement goals (5 AAC 39.223). In addition to fishery management uses, estimating hatchery proportions in harvests and escapements is necessary to create preseason harvest and run projections and to evaluate wild stock escapement goals. Hatchery operators use contribution estimates to assess hatchery runs and evaluate total return, marine survival, and release strategies. This regional operational plan summarizes otolith reading quality assurance measures, otolith collection and sample processing procedures, and methods employed to calculate hatchery contributions to commercial harvests in the Southern District, and escapements to select pink salmon index streams in the Southern and Outer districts in the LCIMA. This project will also facilitate collecting tissue samples, paired with otoliths, so unmarked fish can be used to develop a genetic baseline to examine pink salmon stock structure in Lower Cook Inlet.

BACKGROUND

The LCIMA comprises waters of the Cook Inlet Area, south of the latitude of Anchor Point including the western shore of Cook Inlet south to Cape Douglas, and the eastern shore of Cook Inlet along the Kenai Peninsula to Cape Fairfield. This area encompasses all coastal waters and inland drainages entering this area. The LCIMA is divided into five districts that correspond to local geography and distribution of the five species of Pacific salmon (Figure 1). Commercial salmon fishing occurs in all but the Barren Islands District.

The Cook Inlet Aquaculture Association (CIAA) reopened the Tutka Bay Lagoon (TBLH) and Port Graham (PGH) hatcheries in the Southern District of Lower Cook Inlet (LCI) for pink salmon production in 2011 and 2014, respectively. Both hatcheries now thermal mark the otoliths of 100% of the pink salmon they produce. During prior years of operation, the Tutka Bay Lagoon (1977–2004) and Port Graham (1991–2007) hatcheries annually released an average of ~43 million and ~12 million pink salmon fry, respectively (Appendices A1 and A2). Currently, each facility is permitted to incubate a maximum of 125 million pink salmon eggs. The TBLH has come close to reaching its permitted capacity (e.g., 123.5 million eggs in 2017), but production at the PGH is currently limited by water availability, an issue they plan to resolve. When both hatcheries reach full production, they will have a combined release of ~200 million pink salmon fry and total anticipated annual runs of 4–8 million adults. CIAA also operates the Trail Lakes Hatchery (TLH) near Seward, raising sockeye and coho *O. kisutch* salmon. One hundred percent of the sockeye and coho salmon raised at that facility have been thermal marked since 1991 and 2000, respectively, and both species are remotely released at several sites throughout LCI. Details regarding historical production and releases from each of the three salmon hatcheries operating in LCI can be found in Appendix Tables A1–A5.

ALASKA HATCHERY PROGRAM DEVELOPMENT

Through a series of actions in the 1970's the Alaska Legislature enabled development of salmon hatcheries with the explicit intent to rehabilitate the state's depressed salmon fisheries. In 1971, the Legislature created the Fisheries Rehabilitation, Enhancement and Development (FRED) Division within ADF&G to "develop and continually maintain a comprehensive, coordinated, and long-range plan for the orderly present and long-range rehabilitation…of all aspects of the state's fishery" (CIRPT 2007). In 1974, the Legislature passed the Private Non-Profit (PNP) Hatchery Act, the preamble for which states:

> *"It is the intent of this act to authorize the private ownership of salmon hatcheries by qualified non-profit corporations for the purpose of contributing by artificial means to the rehabilitation of the state's depleted and depressed salmon fishery. The program shall be operated without adversely affecting natural stocks of fish in the state and under a policy of management which allows reasonable segregation of returning hatchery reared salmon from naturally occurring stocks."*

In the mid-1970s, the Alaska Legislature took further action to: 1) authorize the designation of salmon enhancement regions across the state, 2) establish regional aquaculture associations (RAAs), and 3) develop regional planning teams (RPTs) to oversee enhancement activities in their region. This led to formation of the Cook Inlet Aquaculture Association (CIAA) in 1976 and the Cook Inlet Regional Planning Team (CIRPT) in 1977. The CIRPT provided a detailed description of their long-term plans in a document titled: *Cook Inlet Regional Salmon Enhancement Planning, Phase I Plan: 1981–2000* (CIRPT 1981). This plan was updated in 2007 to cover *Phase II: 2006–2025* (CIRPT 2007). Currently, CIAA is among five RAAs in the State of Alaska that maintain private hatcheries with the capacity to produce salmon for harvest in common property fisheries. After acquiring the assets and permitted egg capacities of the Port Graham Hatchery Corporation (PGHC) in early 2015, CIAA is now the third largest RAA in Alaska in terms of overall permitted egg capacity.

As specified in the establishing legislation (PNP Hatchery Act of 1974), Alaska's hatchery program must be operated in a manner that protects wild stocks. Organizationally, that protection is put into practice through: 1) a rigorous hatchery permitting process that includes genetic, pathology, and fishery management reviews; 2) policies that require hatcheries to be located away from significant wild stocks; 3) use of local brood sources; 4) legal mandates that require wild stocks to be given priority in fishery management; 5) requirements for tagging/marking of hatchery fish; and 6) as necessary, requirements for special studies on hatchery/wild stock interactions (McGee 2004). Specific policies, plans, and regulations that help guide hatchery operations in Alaska include: the *PNP Hatchery Act of 1974* and current Alaska Statutes under *Title 16, Chapter 10, Article 9: Salmon Hatcheries*, the State of Alaska *Genetic Policy* (Davis et al. 1985) and supporting background document (Davis and Burkett 1989), *Regulation Changes, Policies, and Guidelines for Fish and Shellfish Health and Disease Control* (Meyers 2010), the *Sustainable Salmon Fisheries Policy* (5 AAC 39.222), the CIRPT, and the *Cook Inlet Regional Salmon Enhancement Planning Phase II Plan: 2006–2025* (CIRPT 2007).

Additional details on LCI hatcheries, including historical releases, can be found in Appendix A of this document, CIRPT (2007), Stopha and Musslewhite (2012), Stopha (2012a), Stopha (2012b) and Hollowell et al. (2019).

THERMAL MARKS

Prior to the advent of thermal marking technology, coded wire tags (CWTs) were used to mark juvenile salmon to estimate contribution. However, high cost and other problems associated with CWT technology led biologists to consider alternatives for marking larger portions of populations with relatively inexpensive non-intrusive methods. By marking all hatchery production, recovery sample sizes can be much smaller without compromising the accuracy and precision of contribution estimates. Non-intrusive marks that are not lost and do not affect survival or behavior eliminate important sources of error in mark-recapture population and hatchery proportion estimates. Mosegard (1987), Volk (1990), and Munk et al. (1993) found that Chinook, coho, sockeye, chum, pink, and Atlantic salmon *Salmo salar* otoliths can be marked through carefully controlled changes in incubation water temperature. The technology was subsequently incorporated into a mixed stock fisheries assessment program by Hagan et al. (1995) and technical issues and costs for PWS were first discussed by Geiger et al. (1994). Thermal otolith marking techniques have many advantages over the CWTs. The cost of applying marks is relatively low, tag loss and differential mortality are non-existent, and the required adult sampling effort is lower.

OTOLITH PILOT STUDY

LCI staff began recovering and analyzing otoliths from sockeye salmon harvested in the commercial set gillnet fishery (SGN) in 2013 and from pink salmon escapement in 2014, which was the first return year for pink salmon thermal marked at the TBLH. Commercial harvest sampling was expanded in 2015 to include purse seine (PS) harvest of both pink and sockeye salmon. Lack of directed funding limited the scope and scale of sampling efforts from 2014 to 2020, but area staff annually conducted this project to begin collecting baseline data on hatchery/wild salmon proportions in LCI fisheries and escapements while the TBLH and PGH were brought back online after being dormant for several years. The impetus to sample escapements as the hatcheries gradually increased production was to evaluate whether the proportion of hatchery produced pink salmon in streams near the hatchery Special Harvest Area (SHA) increased with increasing hatchery releases, and if so, what was the pattern of increase (e.g., linear, step-wise, or other). Staff were also interested to see if hatchery proportions in LCI streams exhibited a pattern similar to that observed in PWS, where hatchery proportions were higher in streams closest to hatchery release sites (Brenner et al. 2012). Sampling of the SGN catch was initiated in response to concerns by Nanwalek residents that commercial SGNs in Kachemak Bay were intercepting sockeye salmon returning to English Bay Lakes. From 1990 to 2015, CIAA back-stocked thermal-marked sockeye salmon fry into that system (Appendix A5).

Provisional results from this pilot study can be found in Hollowell et al. (2017 and 2019), Otis et al. (2018), and Otis et al. (*in prep*). The results are considered provisional because, except for 20% of the 2017 samples, lack of funding precluded our ability to re-read otoliths to assess reader accuracy and precision. Provisional catch sampling results indicate the average proportion of hatchery marks identified in CCP PS harvest samples was 48.7% (range: 5.3%–96.8%, n=2,730 fish from 30 sample events) and 51.0% (range: 5.5%–88.5%, n=2,010 fish from 23 sample events) for sockeye and pink salmon, respectively (Otis and Hollowell *in prep*). Hatchery proportions in SGN CCP harvest samples were generally lower for both sockeye (mean=18.7%, range: 0.0%–46.8%, n=6,018 fish from 67 sample events) and pink salmon (mean=28.1%, range: 25.0%–31.3%, n=192 fish from two sample events; Otis and Hollowell *in prep*).

Three noteworthy observations resulting from previous escapement sampling in LCI that warrant further study include: 1) in several years, hatchery proportions comprised 25% or more of the escapement samples collected from some wild stock streams with escapement goals that occur outside of hatchery SHAs (e.g., Barabara, Dogfish, and Port Chatham creeks), 2) lower than anticipated levels of LCI hatchery-origin pink salmon were encountered in pink salmon index streams in LCI, and 3) hatchery pink salmon from PWS often outnumbered LCI hatchery pink salmon in our samples (Otis et al. 2018).

OBJECTIVES

1. Estimate the overall[1] hatchery/wild stock composition and the hatchery of origin/release site for CCP harvests of pink and sockeye salmon in the Southern District each statistical week by using thermal mark recoveries, such that the overall hatchery proportion estimate is within 10% of the true proportion 95% of the time.
2. Estimate the overall[2] hatchery/wild stock composition and the hatchery of origin/release site for selected HCR harvests of pink and sockeye salmon in the Southern District by using thermal mark recoveries, such that the overall hatchery proportion estimate is within 10% of the true proportion 95% of the time.
3. Use thermal mark recoveries from spawned out carcasses to estimate the overall[1] hatchery/wild composition and the hatchery of origin/release site for pink salmon spawning escapements to select streams in the Southern and Outer districts, such that the overall hatchery proportion estimate for each stream is within 10% of the true proportion 95% of the time.
4. Collect pink salmon tissue samples, paired with otoliths, from select streams in LCI so unmarked fish can be used to develop a pink salmon genetic baseline.

Project tasks in 2021 will include:

1. conduct single-blind test with known origin otoliths to evaluate the ability of Homer readers to identify thermal marks and distinguish between marked/unmarked fish;
2. recover otoliths from LCI CCP pink and sockeye salmon harvests so the samples adequately represent the spatial and temporal breadth of the harvest;
3. recover otoliths from LCI HCR pink and sockeye salmon harvests so the samples adequately represent the spatial and temporal breadth of the harvest;
4. recover otoliths from spawned out pink salmon carcasses in select streams in Southern and Outer districts so the samples adequately represent the spatial and temporal breadth of the run;
5. send all CCP and HCR sockeye salmon otolith samples to CIAA for TM reading under an informal cooperative agreement;

[1] Our objectives focus on estimating the overall hatchery/wild composition within the specified precision for select harvests and escapements. Because unique thermal marks are applied to otoliths for each hatchery/release site, project results will also provide an estimate of the relative contribution each hatchery makes to the harvest/escapement sampled. This information will be reported and should be of interest to hatchery operators wishing to evaluate program performance. However, contribution estimates by individual hatchery/release site will likely not achieve the same level of precision as the overall hatchery contribution estimates.

[2] Ibid.

6. log pink salmon catch and escapement otolith samples into the Mark, Tag, and Age Lab's (MTAL) Southeast mark recovery database (SEMR);
7. mount pink salmon otoliths onto 27x46mm petrographic slides pre-labeled with bar code labels printed from MTAL software;
8. grind otoliths to expose the mid-sagittal plane, where thermal marks were laid down;
9. complete 1st readings of 100% of otoliths collected from the CCP harvest of pink salmon, entering results directly into the SEMR database; if funding is available, ≥30% of the otoliths will be read a 2nd time for quality assurance;
10. complete 1st readings of 100% of otoliths collected from the HCR harvest of pink salmon, entering results directly into the SEMR database; if funding is available, ≥30% of the otoliths will be read a 2nd time for quality assurance;
11. complete 1st readings of 100% of otoliths collected from spawned out pink salmon carcasses sampled from select streams in the Southern and Outer districts, entering results directly into the SEMR database; if funding is available, all marked otoliths from escapement samples will be read a 2nd time for quality assurance;
12. at the direction of the Gene Conservation Lab, when time and resources allow, collect genetic tissue samples, paired with otoliths, from pink salmon escapements to select LCI streams to develop a genetic baseline.

LONG TERM GOALS

The primary goals associated with this 1-year operational plan are described in the Purpose and Objectives sections above. However, a supplemental long-term goal of this project is for the data collected to be of value to broader scale policy discussions that take place at upper management levels. The department has a long history of conducting internal evaluations and collaborating with external research addressing hatchery-wild salmon interactions (e.g., Brenner et al. 2012, Grant 2012, Habicht et al. 2013, Jasper et al. 2013, Lescak et al. 2019) to ensure adherence to applicable state policies and regulations (McGee 2004, Evenson et al. 2018). This project takes advantage of a unique opportunity to monitor changes in hatchery proportions in commercial harvests and escapements within a 30-mile radius (>60 miles by water) of two pink salmon hatcheries that recently reopened and are currently increasing production to their full permitted capacities. By quantifying hatchery proportions in the catch and escapement within specified precision levels, over time the results from this project could help inform policy for both aquaculture operations and harvest management. For instance, it may be informative to conduct spatial/temporal analyses to evaluate whether hatchery proportions in streams correlate with hatchery release size, release location, or imprinting method and then, if warranted, design and implement focused research to identify causal mechanisms. Other future work could include spatial analysis of hatchery proportions in streams relative to distance from hatchery release sites to see if LCI results are similar to those observed in PWS (Brenner et al. 2012).

METHODS

Relevant details associated with catch and escapement otolith samples will be documented using standardized sampling forms (Appendix B). This project will adhere to relevant protocols established for the Alaska Hatchery Research Program (AHRP) by ADF&G's Mark, Tag, and Age Lab (MTAL) in Juneau for otolith collection, tracking, preparation, analysis, data entry and archiving (Agler et al. 2016, Appendix C). Similarly, we will follow the MTAL's quality assurance and quality control procedures to assess reader accuracy and precision (Agler et al. 2017, Appendix

D). Sampling CCP and HCR harvests and the analysis of the resulting data to estimate hatchery proportions will generally follow methods used by ADF&G staff in Cordova. Most of the CCP and HCR sampling and data analysis methods described below were adapted from those described by Haught et al. (2019). Obtaining random and representative samples from both escapement and harvest is crucial to the success of this project.

STUDY SITES

Otolith sampling to estimate hatchery/wild stock composition in CCP and HCR sockeye and pink salmon harvests will occur in the Southern District, where currently 100% of the pink salmon and >40% of the sockeye salmon hatchery releases in LCI occur (Figures 2 and 3). Hatchery sockeye salmon are also released in the Eastern (Bear Lake, Resurrection Bay; Figure 4) and Kamishak (Kirschner Lake) districts (Figure 1); however, current funding limitations preclude sampling harvests from those more distant districts.

Otolith sampling to estimate hatchery-origin proportions in escapements will occur at 11 pink salmon streams, 10 of which have sustainable escapement goals (SEG). Seven of the streams are in the Southern District and four are in the Outer District (Table 1, Figure 5). There are 18 pink salmon stocks with SEGs in the LCIMA, 15 of which occur in the Southern and Outer districts (Table 1; Otis and Hollowell 2019). None of these 18 index streams are road accessible; however, 10 are regularly visited by skiff and floatplane to conduct foot surveys to assess salmon escapement. This feature made them very cost-effective sampling sites for this project, which was a major consideration given current funding limitations. The seven streams to be sampled in the Southern District represent approximately 90% of the pink salmon natural production in that district based on available escapement data (ADF&G, unpublished data). The four streams targeted for sampling in the Outer District represent approximately 50% of that district's pink salmon natural production (ADF&G, unpublished data). If additional funding becomes available, we will add 2–3 floatplane accessible index streams in the Outer District (Rocky River, Windy Left and Windy Right creeks), which would result in ~80% of pink salmon natural production being sampled in that district. The English Bay River in the Southern District does not have an SEG, but it was selected for sampling because it supports a sizeable population of pink salmon (frequently >5,000 fish, ADF&G unpublished data) that has important subsistence value to the residents of Nanwalek (Wiita 2019). The English Bay River is outside of the Port Graham SHA, but it is the nearest pink salmon stream west of the Port Graham hatchery (Figure 3).

THERMAL MARK METHODOLOGY

This project will quantify the proportion of thermal-marked, hatchery-origin sockeye and pink salmon in select harvests and escapements in the Southern and Outer Districts of Lower Cook Inlet by determining the marked/unmarked ratio of sampled otoliths. However, not all hatchery-origin salmon are thermal marked. For instance, the Kitoi Bay Hatchery (KBH) on Afognak Island only recently (2013) began thermal marking sockeye salmon, and pink salmon released from KBH were unmarked until they began experimenting with a saltwater mark on 19% of the BY18 pink salmon released in 2019 and 100% of the BY19 pink salmon released in 2020 (2019 and 2020 KBH Annual Management Plans, respectively, available here: Annual Management Plans, Alaska Department of Fish and Game). If saltwater marking is successful, the marks should be similar enough to thermal marks that this project will be able to identify them. However, the Gulkana Hatchery (GH) in PWS marks their sockeye salmon releases using strontium chloride (SrCl; Stopha 2013), which requires a scanning electron microscope to detect (Haught et al. 2019), rather

than the compound microscope this project will use to identify thermal marks. Thus, the hatchery proportions estimated by this project represent the proportion of thermal-marked salmon, but not necessarily the proportion of all hatchery-origin salmon (unmarked or SrCl marked) that could possibly be present in our samples.

Single-Blind Tests of Thermal Mark Readers (Project Task 1)

Thermal marks will be classified by a uniform hatch code (Johnson et al. 2006; Table 3) that is annually assigned by ADF&G's MTAL (Oxman 2016, Oxman 2017, Oxman 2018, Oxman 2020). Relevant details associated with unique thermal marks assigned to each hatchery, brood year, and release location in Alaska, including images of voucher specimens, can be found in the MTAL's voucher summary reports, which are available online (https://mtalab.adfg.alaska.gov/OTO/reports/VoucherSummary.aspx).

Reader ability to accurately determine the origin of otoliths extracted from brood year 2019 pink salmon fry will be assessed using blind tests conducted at the ADF&G Homer office before production otolith reading commences. The extent to which readers agree with known-mark assignments will be measured and an identification matrix will be constructed to highlight misclassification tendencies for each reader and test. To facilitate this test, samples of known-origin wild (unmarked) and thermal-marked hatchery fish (vouchers) will be needed.

In the spring of 2021, CIAA staff will collect thermal-marked pink and sockeye salmon fry from incubators at the TLH, TBLH, and PGH hatcheries. These voucher specimens will be sent to the MTAL in Juneau so prescribed thermal marks can be verified and variant marks documented. Extracted otoliths (left otolith from the pair) will be mounted, sulcus side up, on a petrographic glass slide with thermoplastic glue. Otoliths will be ground to the mid-sagittal plane manually with 1200-grit or 4000-grit silicon carbide paper and viewed under a compound microscope and transmitted light at 200X or 400X. The prescribed thermal mark and any variant marks for each hatchery/release site will be documented, imaged, and uploaded to the MTAL's voucher summary report website, and then the mounted voucher otoliths will be sent to the Homer office for use in blind testing.

We do not currently have the funding to collect and process wild pink salmon fry from LCI streams, so we will use known wild-origin otoliths collected for this same purpose by Cordova staff in PWS. Mounted otoliths will be placed in slide boxes labeled by origin. Slides will be coded and randomized by Homer ADF&G employees not working in the otolith laboratory. Single-blind tests will be administered to all personnel whose duties include reading otoliths. First, readers will train with known-origin otoliths and pictures of known-origin marked otoliths. Next, readers will be tested by reading and identifying discrete sets of 100 coded and randomized otoliths. Code information will not be made available to any laboratory personnel until results of the tests have been analyzed by the Fishery Biologist III (FB III) project leader (Ted Otis).

The overall ability of readers to correctly identify otoliths will be determined by comparing readers' interpretations of marks to the known origins. The priority order of identifications for readers is as follows: 1) thermal-marked fish versus an unmarked fish, 2) hatchery of origin versus all other origins, and 3) the specific mark (e.g., program/release site) versus all other thermal marked or unmarked fish. Agreement between or among readers and trends in misclassifications will also be examined. If readers are misidentifying specific marks, they will have further training and practice and the test will be repeated with additional coded and randomized samples of 100 otoliths. The minimum score for each reader to pass the blind test is >90% correct identification

of the otoliths to mark (priority three listed above); however, blind test score minimums may be adjusted in the case of poor marks. For example, a poor mark with multiple variants that is misidentified consistently by all readers might only need a >80% correct identification to proceed with production reading. However, in the case of poor marks, readers still must correctly identify >90% of otoliths at the thermal-marked versus unmarked level in order to proceed. Scores required to advance to production reading in the case of poor marks will be determined in consultation with the project leader and the most experienced otolith reader.

Recovering Thermal Mark Otoliths from the Harvest (Project Tasks 2–3)

There are two legal gear types for commercially harvesting salmon in the Southern District of LCI - purse seine and set gillnet. Both sockeye and pink salmon are harvested in abundance by PS and SGN gear; however, run timing and magnitude differ by species. Peak sockeye and pink salmon harvests occur in July and August, respectively. We will collect samples from both species weekly for each gear type whenever they are available in sufficient abundance to facilitate achieving minimum sampling goals (96 otoliths) in a relatively efficient manner.

In the Southern District, the CCP SGN fishery opens by regulation on June 1 and typically consists of two 48-hr periods per week, beginning at 0600 hrs on Monday and Thursday. The CCP PS fishery in the Southern District opens by emergency order, generally in mid-June, and in recent years has consisted of three 16-hr periods each week, beginning at 0600 hrs on Monday, Wednesday, and Friday. Purse seine is the only gear type used for HCR fishing in the Southern District. Within a timeframe specified in regulation (5AAC 21.372 and 21.373), HCR fishing in the Southern District occurs at the discretion of the Area Management Biologist, CIAA, the processor that was awarded the contract, and the fisherman designated by the processor for cost-recovery operations that year. Generally, HCR pink and sockeye salmon harvests in the Southern District occur once per week throughout the run, although additional cost recovery harvests may occur as needed.

SGN fishing is allowed in portions of the Halibut Cove, Tutka, Barabara, Seldovia, and Port Graham subdistricts of the Southern District (Figure 6). One fishing tender services all the setnet sites throughout the Southern District, except those in the Halibut Cove and Port Graham subdistricts. In the past, Homer staff have coordinated with the tender operator to use brailer bags to keep harvests from each subdistrict separated until fish are sold to processors in Homer. SGN operators in Halibut Cove deliver their own fish to the processor so field crews will coordinate with them individually to sample the SGN harvest from that subdistrict. SGN deliveries occur on Wednesdays, which coincides with the middle of the statistical week, and Saturdays. A 2-person sampling crew will meet the tender (or Halibut Cove SGN operator) at the dock each Wednesday prior to offloading. One person will monitor the offload and label fish totes to assure that subdistricts targeted for sampling are tracked from the tender to the SGN processor in Homer. The 2nd person will station themselves by the guillotine on the processor line and collect a minimum of 96 fish heads from each subdistrict marked for sampling. Totes will be labeled for Halibut Cove, Tutka/Barabara, and Seldovia subdistrict harvests; harvest from the Port Graham subdistrict rarely occurs and the Tutka/Barabara subdistricts are pooled because the area open to SGN fishing straddles the boundary between these two subdistricts. When the total harvest from each subdistrict is large (e.g., >200 fish), the field crew will systematically sample proportional to harvest at a rate scaled to the total fish available for sampling to achieve the sampling objective of heads from $\geq$96 fish (e.g., every 5th fish passing through the header for harvests totaling ~500 fish). Each Wednesday, the SGN delivery will be sampled in this manner so samples are collected from

throughout the total delivery from each subdistrict each statistical week. Limited resources will preclude sampling Saturday SGN deliveries in 2021.

In recent years, over 20 permit holders have participated in the LCI PS fishery. However, only 10–15 vessels regularly fish the Southern District and >90% of their effort occurs in the following subdistricts/sections (in approximate rank order): Tutka Bay, Tutka Hatchery SHA, Neptune Bay, China Poot, Halibut Cove, and Port Graham. Seine-caught CCP pink and sockeye salmon landed in Homer usually come from multiple subdistricts and are typically transported immediately by truck to Seward for processing. Hence, systematic sampling at the processor is not feasible for this gear type. Consequently, otoliths will be recovered by ADF&G crews sampling directly from purse seine vessels and/or tenders actively fishing or tendering harvests in the Southern District. During CCP openings, a 2-person crew operating from a 20' skiff will survey fishing effort in the Southern District and target sample collections to assure adequate representation from each subdistrict contributing to that day's harvest. Working from a skiff on the fishing grounds will also ensure that location-specific sampling integrity is maintained, since individual seiners often move between subdistricts and mix the catch in their hold(s).

Once a seiner has completed a CCP or HCR set, and before fish are stored in the vessel's fish-hold (potentially getting mixed with harvest from other subdistricts), the crew will approach the vessel and ask to sample the catch. With the captain's permission, up to 100 salmon of the target species will be removed from the net and put in chilled seawater inside an insulated fish tote aboard the ADF&G skiff. The seine vessel can then continue fishing while the sampling crew logs the date, time, and location (latitude, longitude) of the harvest and assigns it a unique sample identification number. The ADF&G skiff will then anchor nearby to quickly extract up to 96 pairs of otoliths before delivering the sampled fish back to the seiner that harvested them. Brailer bags will be used to quickly transfer fish between the seiner and skiff to avoid unnecessarily delaying the vessel's ability to continue fishing. In recent years while conducting the pilot study, members of the seine fleet have been very cooperative in complying with this sampling strategy. Their continued cooperation is critical to ensure that all vessels have an equal chance of being sampled.

Otoliths collected from each vessel's harvest will be placed sequentially into plastic 96-well plate trays. Individual trays will be labeled with vessel name, species, fishing subdistrict and period, date and other descriptive information for data entry, cataloging, and storage purposes. Otoliths will be collected so that each vessel, species, fishing period, and subdistrict will be stored in their own tray with its own data sheet containing the descriptive and quantitative data for data entry and cataloging purposes (Appendix B1). All data forms and otolith trays from Homer samplers will be delivered to the crew leader's office at the end of each shift.

For each statistical week, a weighted sample of 96 otoliths will be selected from all the otoliths collected from each subdistrict during the two periods sampled that week. A proportional allocation scheme will be used where the total poundage harvested from each subdistrict will determine its contribution to the overall sample. Inseason harvest statistics will be obtained from daily processor reports (summarized in the ADF&G Mariner system). Because the delivered poundage determines the number of otoliths examined from each subdistrict, it will be important to have samples from each subdistrict contributing to that period's harvest and accurate reporting of the poundage by species. Otoliths will be selected systematically from cell trays sampled from each subdistrict to maintain a representative sample from all vessels sampled. If sufficient unsampled otoliths from each subdistrict are available after collecting one weighted sample, another weighted sample of 96 otoliths formed in a similar manner will be selected and stored for

possible analysis. The Bayesian sampling algorithm of Geiger (1994) may be used to decide if additional otoliths will need to be examined. However, for any sampled stratum, the sample size goal of 96 otoliths should provide a hatchery proportion estimate with at least a 95% chance that it is within 10% of the true proportion.

Recovering Thermal Mark Otoliths from the Escapement (Project Task 4)

To account for a worst-case sampling scenario (i.e., infinite population size and an overall hatchery proportion [P] of P=0.5), we will attempt to collect otoliths from 96 fish during each of three sampling events (288 total) from each index stream. This will facilitate estimating the overall percentage of thermal-marked hatchery-origin salmon in the stream within $\leq$10% of the true percentage, with $\geq$95% confidence (Thompson 1992). Historical escapement timing and ground survey schedules will be used to determine when sampling events will occur. Sampling events will coincide with the early, middle, and late segments of the run for each stock targeted. Stream sampling events with fewer than 50 otolith pairs collected will be excluded from our analyses.

As with the catch samples, otoliths collected from spawned out carcasses will be placed sequentially into plastic 96-well plate trays. Individual trays will be labeled with stream name, sample date, and other descriptive information for data entry, cataloging, and storage purposes. Results from the AHRP study in PWS suggest hatchery fish may not be randomly distributed in streams, so samplers will record the location (latitude and longitude) for groups of fish sampled along the stream. Samplers will first walk the stream to assess carcass distribution, so samples are collected in proportion to abundance. Otoliths will be collected so that samples from each stream will be stored in their own tray with its own data sheet containing the descriptive and quantitative data for data entry and cataloging purposes (Appendix B2). The stream survey crew that will accompany otolith samplers to each stream will provide live/dead counts of pink salmon in the stream and these values will also be recorded on the sample log form. All data forms and otolith trays from Homer samplers will be delivered to the crew leader's office at the end of each shift.

Thermal Mark Processing (Project Tasks 5–11)

Extracted otoliths (left otolith from each pair) selected for analysis will be cleaned and rinsed using MTAL protocols (Appendix C) prior to being mounted sulcus side up on a petrographic glass slide with a unique bar-coded label attached that contains sample details (Figure 5 in Appendix C). Otoliths will be ground to the midsagittal plane manually using 1200-grit or 4000-grit silicon carbide paper and viewed under a compound microscope with transmitted light at 250X or 400X. After determining the origin of an otolith, a bar-code scanner will record the slide identity to the MTAL otolith recovery database (Oracle) and a front-end application will be used to enter additional relevant data about the otolith (e.g., thermal mark ID, age, comments, etc.). Contribution estimates will be determined after identifying all otoliths from a sample.

Preliminary hatchery contribution estimates in a district-period-fishery-species stratum will be generated from the first reading of the otolith samples collected from seine and tender vessels on the grounds. Hatchery contribution estimates will be distributed as portable document format (PDF) files via email to CIAA and ADF&G personnel, and other stakeholders as they become available. Limited funding will preclude in-season analyses in 2021, but we hope to develop the capacity for more timely processing in the future to facilitate the use of otolith results for in-season management.

Second Reads of Harvest and Escapement Mark Interpretation (Project Tasks 9–11)

For quality control, readers typically reexamine ≥ 30% of previously examined otolith samples from CCP and HCR harvests (Haught et al. 2019), and up to 100% from spawned out carcasses (D. Oxman, ADF&G-MTAL, personal communication). Limited funding will likely preclude 2nd reads in 2021, but if resources are available, subsamples from each district-period-fishery-species combination (CCP and HCR samples), and from each index stream-sampling event combination (escapement samples) will be randomly selected and examined by another reader in Homer.

The correct mark may be difficult to determine for species with both base and accessory marks because the accessory mark may be ground away to reach the base mark. This issue should be noted during early production reads, and the Project PI or otolith crew leader will have to consider the issue when arbitrating differences. If there are significant differences between readers, the right otoliths may need to be examined for final determination on those specific samples.

Differences between first and second reads will be arbitrated by the Project PI or the most experienced reader. Reading errors found in the quality control process will be corrected in the database and the contribution estimates will be recalculated, as necessary.

Genetic Tissue Collections (Project Task 12)

When time and resources allow, escapements of live pink salmon from select locations will be sampled for genetic tissue, otoliths, sex, and length. Because tissues collected from dead fish do not generally yield sufficient DNA quality for genetic analysis, these samples will be collected as separate sampling events from carcass otolith sampling for determining the hatchery composition of escapements. For the genetic tissues, a piece of pelvic fin will be removed from each fish and placed on a 48 position Whatman paper card in its own grid space and then stapled in place (Appendix E1). Whatman cards with tissue samples will be placed in an airtight case with desiccant beads to preserve the tissue for DNA extraction. Each Whatman card will have a unique barcode and a numbered grid. To identify hatchery-origin fish in the sample and exclude them from future baseline analyses, both otoliths will be removed from the head of each fish and placed in a 96 well tray and analyzed for thermal marks post season (Appendix E2). The sex, length (mid-eye to fork), tissue sample Whatman card barcode and grid position number, and otolith tray number will be recorded for each fish on data forms specially designed for recording paired genetic tissue and otolith sample data (Appendix E3). The sampling goal for each location will be 96 natural-origin fish. Locations where hatchery-origin fish have been identified in previous year's escapements will be over sampled to meet the 96 natural-origin fish sampling goal. For example, the sampling goal for a stream that has had past escapements made up of 50% hatchery-origin fish would be 192 fish. Sampling locations will be determined in season and will be chosen based on the availability of live fish to sample and the cost of accessing the location. All streams targeted for otolith sampling will also be targeted for odd and even year genetic sampling (if funding is available), with the exception of Humpy Creek, which was sampled for paired otolith/genetic tissues in 2018 and 2019 (Table 1). Genetic tissue samples will be archived at the GCL for future genetic analysis and otolith samples will be sent to the MTAL to be analyzed for thermal marks.

DATA ANALYSIS

Identification matrices (Task 1)

An identification matrix will be produced to identify trends in errors in blind tests results. A matrix for each blind test set for each reader and species will be constructed with true and observed origin describing rows (*i*) and columns (*j*), respectively. Additional matrices will be constructed to identify trends in 2nd read results.

Success rates in voucher mark identification (Task 1)

This test will estimate the expected identification success rate for a group of 100 otoliths selected from the population of otoliths consisting of those marked salmon returning in 2021 for any reader at any time. Success rates will be estimated in two ways.

The first, $\hat{p}'_i$, is defined:

$$\hat{p}'_i = \frac{\sum_{j=1}^{b}\sum_{k=1}^{r}\sum_{l=1}^{t} p'_{ijkl}}{brt} \quad (1)$$

where

$$p'_{ijkl} = \frac{\sum_{m=1}^{n} x_{ijklm}}{n} \quad (2)$$

where x_{ijklm} =1 if the identification of the m^{th} otolith in box *j* identified by reader *k* for time *l* is correct according to the decision rule: 'Of origin *i*' versus 'Not of origin *i*', and 0 otherwise. The index *i* denotes hatchery of origin with values specific to hatcheries or release sites for each species. For example, for pink salmon, index *i* values would include: TBLH, PGH, and 'Hatcheries Combined'. For example, for $p'_{PGH\,123}$ = 0.95, the otoliths in box 1 assessed by reader 2 at time 3 were identified correctly 95% of the time based on the criteria that a successful identification occurs if the otolith in question is assigned a "PGH' or 'Not PGH' identity correctly.

The second definition, $\hat{p}''_i$, is defined similarly, except that the success criteria are restricted only to the otoliths of origin *i*. Thus, for p''_{PGH123} = 0.95, the PGH otoliths in box 1 assessed by reader 2 at time 3, were identified correctly 95% of the time.

Estimating Hatchery Contributions to CCP and HCR Harvests (Objectives 1–2)

The otolith-derived estimate of the contribution of hatchery *h* to district-period stratum *i*, C_{hi} is

$$\hat{C}_{hi} = \frac{o_{hi}}{n_i} N_i \quad (3)$$

where,

o_{hi} = Number of otoliths from hatchery *h* in sample n_i,

n_i = Number of otoliths sampled from stratum *i* (usually 96), and

N_i = Number of fish caught in stratum *i*.

The variance estimate of $\hat{C}_{hi}$ is

$$var(\hat{C}_{hi}) = {N_i}^2 \frac{1}{n_i - 1} \frac{o_{hi}}{n_i} \left(1 - \frac{o_{hi}}{n_i}\right) \quad (4)$$

Otolith-derived estimates of the contribution of hatchery *h*, C_{Sh}, to all sampled CCP (or other collections: HCR, special harvests, or broodstocks), will be calculated as

$$\hat{C}_{Sh} = \sum_{i=1}^{Q} C_{hi} \quad (5)$$

where,

Q = Number of recovery strata associated with CCP or other collection in which otoliths from hatchery *h* are found.

The variance estimate of $\hat{C}_{Sh}$ is

$$var(\hat{C}_{Sh}) = \sum_{i=1}^{Q} var(C_{h_i}) \quad (6)$$

The contribution of hatchery *h* to unsampled strata, Cu_h , will be estimated from contribution rates associated with strata sampled from the same district-period openings as the unsampled strata using methods similar to those used for coded wire tags (Riffe et al. 1996):

$$\hat{C}u_h = \sum_{i=1}^{U} \left[N_i * \left(\frac{\sum_{j=1}^{S} \hat{C}_{hj}}{\sum_{j=1}^{S} N_j} \right) \right] \quad (7)$$

where

U = Number of unsampled strata,

N_i = Number of fish in i^{th} unsampled stratum,

S = Number of strata sampled in the period in which the unsampled stratum resides,

C_{hj} = Contribution of thermal mark *h* to the sampled stratum *j*, and

N_j = Number of fish in j^{th} sampled stratum.

The variance estimate of $\hat{C}_{Uh}$ is

$$var(\hat{C}_{Uh}) = \sum_{i=1}^{U} \left[{N_i}^2 * \left(\frac{1}{\sum_{j=1}^{S} N_j - 1} \right) \left(\frac{\sum_{j=1}^{S} \hat{C}_{hj}}{\sum_{j=1}^{S} N_j} \right) \left(1 - \frac{\sum_{j=1}^{S} \hat{C}_{hj}}{\sum_{j=1}^{S} N_j} \right) \right] \quad (8)$$

If a statistical week was not sampled (an infrequent occurrence), the harvest from that week will be treated as unsampled harvest of the subsequent or previous week in the same district.

An estimate of the contribution by hatchery *h* to all strata, sampled and unsampled, will be calculated by

$$\hat{C}_h = \hat{C}_{Sh} + \hat{C}_{Uh} \quad (9)$$

The variance estimate of $\hat{C}_h$ is

$$var(\hat{C}_h) = var(\hat{C}_{Sh}) + var(\hat{C}_{Uh}) \quad (10)$$

If there are few unsampled strata, the variance associated with $\hat{C}_{Uh}$ will be assumed to be negligible.

The overall hatchery contribution by all hatcheries is calculated by

$$\hat{C}_H = \sum_h^H \hat{C}_h \quad (11)$$

The variance estimate of $\hat{C}_H$ is

$$var(\hat{C}_H) = \sum_h^H var(\hat{C}_h \quad) \quad (12)$$

Estimating Hatchery Proportion in Escapement (Objective 3)

Eleven pink salmon stocks in the Southern and Outer districts will be targeted for estimating the proportion of hatchery fish in the escapement in 2021 (Table 1, Figure 5). All but one (English Bay River) are stocks with sustainable escapement goals. Of the 11 streams in this study, six are accessible by skiff and five are floatplane access only.

The hatchery/wild estimate of pink salmon in our study streams will be computed as a weighted average:

$$\bar{q}_i = \frac{\sum w_{i,t} q_{i,t}}{\sum w_{i,t}} \quad (13)$$

where q_i is the overall proportion of thermal-marked, hatchery-origin fish for stream i, $w_{i,t}$ is the relative number of dead fish available to be sampled in stream i at time t (e.g., t_1=early, t_2=middle, and t_3=late segment of the run), and $q_{i,t}$ is the unadjusted hatchery proportion derived from the otolith sample collected at time t. An estimate of the relative number of dead fish available to be sampled during each event ($w_{i,t}$) will be used to weight the unadjusted hatchery proportion ($q_{i,t}$) observed during that event (t), so we can estimate the overall weighted average hatchery proportion in the stream for the entire run (q_i). Because carcass abundance and distribution along the stream can be affected by high water events, live counts and historical escapement/run-timing curves will be used to determine appropriate weighting factors for each sampling event. The live escapement/run timing curve will be lagged 18 days to account for the average streamlife (SL) for pink salmon in LCI (Otis and Hollowell 2019). The resulting "carcass availability" curve will yield an estimated proportion of the run that is expected to be dead on the date each sampling event occurs (Figure 7). Ideally, sampling events will occur 18 days after the dates that 25, 50, and 75% of the live escapement has entered the river so otoliths collected from spawned out carcasses will represent the early, middle, and late segments of the run, respectively. In this scenario, weighting factors of 0.25, 0.25, and 0.25 will be used to weight the unadjusted hatchery proportions to estimate the overall weighted average hatchery proportion in the stream for the entire run. To the extent possible, we will target recently deceased, spawned-out pink salmon for otolith sampling to ensure that they represent the current run segment rather than a prior run segment.

ADDITIONAL GUIDELINES AND PROCEDURES

Safety

Employees conducting data collection shall adhere to standard operating procedures (SOP) listed in relevant chapters/sections of ADF&G's SOP manual, including but not limited to Chapter 3, Section 700 (General Safety Policies/Procedures), to be found online at: https://stateofalaska.sharepoint.com/sites/DFG/Pages/SOPs.aspx. All employees shall maintain current First Aid/CPR certifications. New field crew members collecting otoliths from spawned out carcasses on streams shall participate in wildlife and firearms safety classes prior to going afield. Experienced field crew members shall attend annual wildlife and firearms safety refresher training. In order to be certified to carry a firearm, new and experienced crew members will need to pass the firearms proficiency test each year, as required under the new firearms policy. New field crew members shall be trained in safe boat operation by the Project Leader or field crew leader prior to being allowed to take command of motorized watercraft. Field crews accessing remote sample sites via floatplane are encouraged to take Underwater Egress Training when available to become knowledgeable and proficient in escaping aircraft that have crash landed in water. Field crews shall always carry emergency communications equipment capable of functioning effectively from the remote locations where they are working (e.g., cell phone, VHF radio, satellite phone, or an emergency satellite beacon/texting device such as DeLorme inReach).

Maintenance

Field crews assigned to this project use a variety of equipment and operate various watercraft (e.g., 22' Boston Whalers powered by twin outboard motors; 10' inflatable raft powered by oars and/or 4 HP outboard motor) to access streams to collect carcass otoliths. Hence, they are responsible for all in-season and post-season maintenance required to keep state equipment in good, serviceable condition. This includes but is not limited to all highway vehicles, boats, trailers, outboard motors, radios, satellite phones/messaging devices, handheld computers, waders, boots, shotguns, and other miscellaneous equipment.

Compliance with ADF&G Regulations

All employees are responsible for complying with local subsistence, sport fishing, and hunting regulations. Copies of State and Federal regulations will be available in the Homer office and should be carried while conducting field work where the public is likely to be encountered. Violations will be recorded on employee evaluations and may be cause for immediate dismissal.

Violations

If a fishing violation is observed, all information pertaining to the violation should be recorded and retained by the employee and the project leader must be notified immediately. If you have a camera, record as much as possible.

The use of the five Ws can aid in obtaining sufficient information pertaining to a violation.

1. What is the violation?
2. When did the violation take place?
3. Where did the violation occur?
4. Who is in violation and who are the witnesses?
5. Why was the violation committed?

If the violator refuses to cooperate with an employee without enforcement authority, no action should be taken, other than to relay all information and evidence collected to the Project Leader, who will contact the appropriate law enforcement authorities.

Emergencies

In the event of a medical emergency, administer first aid to stabilize the situation. If an injury is life threatening, immediately call for emergency response using a device appropriate for the remote survey location (e.g., satellite phone, inReach satellite messenger, VHF radio). If using a cell or satellite phone, call 911 or notify the US Coast Guard at 800-478-5555. The US Coast Guard can also be reached on SSB radio frequency 4.125 MHz or on VHF channel 16.

When contacting the U.S. Coast Guard, have the following information ready to pass along:

- Specific location of the emergency (latitude, longitude, if available),
- Name and phone number of supervisor,
- General nature of medical emergency,
- Number of patients,
- Specific information regarding the patient (name, age, primary complaint, and vital signs),
- Your assessment and treatment,
- Wind and weather conditions, and
- Other information pertinent to a possible medical evaluation.

SCHEDULE AND DELIVERABLES

1. Begin collecting CCP and HCR otolith samples targeting early run sockeye salmon stocks.
   Target date: On or around May 20.
2. Begin collecting CCP and HCR otolith samples targeting early run pink salmon stocks.
   Target date: On or around July 15.
3. Begin collecting otolith samples from spawned out pink salmon carcasses in select index streams.
   Target date: On or around August 1.
4. Collect weekly otolith samples from the CCP and HCR harvests of pink and sockeye salmon throughout their respective harvest periods in the Southern District.
   Target date(s): May 20–September 15.
5. Periodically collect otoliths from spawned out pink salmon carcasses on select streams in the Southern and Outer districts throughout their respective spawning runs.
   Target date(s): August 1–September 25.
6. Mount, grind, and read pink salmon otoliths, entering results directly into the MTAL otolith recovery database.
   Target date(s): September 15–January 15.
7. Estimate the proportion of thermal marked pink salmon (overall, and by contributing hatchery) in the Southern District SGN and PS commercial harvest by statistical week, and in the total escapement to select streams in the Southern and Outer districts.
   Target date(s): January 1–March 1.
8. Publish otolith results in fishery data series (FDS) report and provide copies to hatchery operators.
   Target date(s): Mar 1–May 15.

RESPONSIBILITIES

Project Leader (Fishery Biologist III, Research):

Duties: Supervise project, complete data analysis, assist with field data collection, primary author on report.

Project Leader (Fishery Biologist III, Management):

Duties: Assist with project supervision (e.g., aerial and ground surveys), field data collection, and reporting.

Otolith Field Project Leader (Fishery Biologist I or Fish and Wildlife Technician III):

Duties: Supervise and lead daily otolith collection and processing activities; train new personnel in sampling procedures, otolith reading, and data entry; assist with analysis and reporting.

Otolith Recovery Crew Member (Fish & Wildlife Technician II):

Duties: Assist in collection of field data. Routine data entry.

Otolith Recovery Crew Member (Fish & Wildlife Technician II):

Duties: Assist in collection of field data. Routine data entry.

Ground Survey Crew Leader (Fish and Wildlife Technician III):

Duties: Manage logistics and lead field operations to estimate salmon escapement; enter, edit and summarize escapement data.

Ground Survey Crew Member (Fish and Wildlife Technician II):

Duties: Assist with ground surveys to estimate salmon escapement; routine data entry.

Consulting Biometrician (Biometrician III):

Duties: Review data collection methodology and provide technical support for data analysis.

Consulting Geneticist (Fishery Biologist III):

Duties: Provide technical support for baseline genetic tissue sample collections.

Programmer and Database Analyst (Analyst/ Programmer V):

Duties: Provide technical support for database and write/support front-end application for access to database.

Commercial Fisheries Fish Ticket staff (Office Assistant I):

Duties: Collect, edit, enter, and summarize processor reports and fish tickets.

BUDGET

Table 3 provides a list of project personnel and estimated line 100–400 expenditures required to recover and analyze thermal marked otoliths in Lower Cook Inlet in 2021.

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TABLES

Table 1.–List of 18 pink salmon stocks in Lower Cook Inlet with sustainable escapement goals (SEG), and 1 additional stock without an SEG, 11 of which will be sampled to estimate the overall proportion of thermal-marked hatchery pink salmon in the escapement in 2021.

Species/Stock	District	Year Adopted	SEG Range Lower		Upper	Monitoring Method Aerial	Ground	Video	Weir
Pink Salmon									
SEG Streams (18)									
[1,2] Humpy Creek	Southern	2017	17,500	–	51,400				x
[1,2,3] China Poot Creek	Southern	2017	2,500	–	6,300				x
[1,2,3] Tutka Creek	Southern	2001	6,500	–	17,000				x
[1,2,3] Barabara Creek	Southern	2017	2,000	–	5,600				x
[1,2,3] Seldovia Creek	Southern	2017	21,800	–	37,400				x
[1,2,3] Port Graham River	Southern	2017	7,700	–	19,700				x
[1,2,3] Dogfish Lagoon Creeks	Outer	2017	800	–	7,100	x			x
[1,2,3] Port Chatham	Outer	2017	7,800	–	18,100				x
[4] Windy Creek Right	Outer	2017	3,400	–	11,200	x			
[4] Windy Creek Left	Outer	2017	5,400	–	27,100	x			
[4] Rocky River	Outer	2017	11,700	–	54,800	x			
[1,2,3] Port Dick Creek	Outer	2017	17,900	–	49,800	x			x
[1,2,3] Island Creek	Outer	2017	9,600	–	32,500	x			x
[1] S. Nuka Island Creek	Outer	2017	2,800	–	11,200	x			
Desire Lake	Outer	2017	1,500	–	18,000	x			
Bruin River	Kamishak	2017	17,800	–	103,000	x			
Sunday Creek	Kamishak	2017	4,400	–	24,900	x			
Brown's Peak Creek	Kamishak	2017	2,600	–	17,500	x			
Non-SEG Streams (1)									
[1,2,3] English Bay River	Southern	NA		–		x			x

[1] Streams sampled during a 2014–2017 pilot study to estimate the proportion of hatchery pink salmon in the escapement (Otis et al. 2018).

[2] Streams selected for continued monitoring in 2021 to estimate the overall proportion of hatchery pink salmon in the escapement.

[3] Pink salmon stocks that will be targeted for odd and even year genetic tissue collections if funding is available. Paired otolith/genetic tissue samples were collected from Humpy Creek in 2018/2019.

[4] Additional streams that may be sampled in the Outer District if funding is available for air charters.

Table 2.–Thermal mark codes at LCI hatcheries for the predominate brood years of pink and sockeye salmon returning in 2021.

Mark ID	Species	Agency	Facility	Brood Year	Hatch Code Target	Hatch Code Actual	RBr Actual
PORTGRAHAM19	PINK	CIAA	PORT GRAHAM	2019	5H3		
PORTGRAHAM19A	PINK	CIAA	PORT GRAHAM	2019	5H5		
TUTKA19PINK	PINK	CIAA	TUTKA BAY	2019	6,3H		
TUTKA19PINKA	PINK	CIAA	TUTKA BAY	2019	3,6H		
TUTKA19PINKB	PINK	CIAA	TUTKA BAY	2019	4,3H		
HAZEL17	SOCKEYE	CIAA	TRAIL LAKES	2017	3,3H3	3,3H3	1:1.3,2.3+3.3
PENINSULA17SOCKEYE	SOCKEYE	CIAA	TRAIL LAKES	2017	2,2H	2,2H	1:1.2,2.2
TRAILLAKES17A	SOCKEYE	CIAA	TRAIL LAKES	2017	3,3H		
TRAILLAKES17B	SOCKEYE	CIAA	TRAIL LAKES	2017	1,3H	1,3H	1:1.1,2.3
TRAILLAKES17C	SOCKEYE	CIAA	TRAIL LAKES	2017	6n,4H		
TUTKA17	SOCKEYE	CIAA	TRAIL LAKES	2017	4,2H	4,2H	1:1.4,2.2
HAZEL16	SOCKEYE	CIAA	TRAIL LAKES	2016	4,1,3H	4,1,3H	1:1.4,2.1,3.3
PENINSULA16SOCKEYE	SOCKEYE	CIAA	TRAIL LAKES	2016	1,3H	1,3H	1:1.1,2.3
TRAILLAKES16A	SOCKEYE	CIAA	TRAIL LAKES	2016	4H	4H	01:01.4
TRAILLAKES16B	SOCKEYE	CIAA	TRAIL LAKES	2016	2,4H	2,4H	1:1.2,2.4
TRAILLAKES16C	SOCKEYE	CIAA	TRAIL LAKES	2016	6,2H		
TUTKA16	SOCKEYE	CIAA	TRAIL LAKES	2016	2,5H	2,5H	1:1.2,2.5
HAZEL15	SOCKEYE	CIAA	TRAIL LAKES	2015	H2,2,2		
HIDDENLAKE15	SOCKEYE	CIAA	TRAIL LAKES	2015	3,2,1H	3,2,1H	1:1.3,2.2,3.1
PENINSULA15SOCKEYE	SOCKEYE	CIAA	TRAIL LAKES	2015	H2,2	H2,2	2:1.2,2.2
PORTGRAHAM15SOCKEYE	SOCKEYE	CIAA	TRAIL LAKES	2015	2,3,2H		
TRAILLAKES15A	SOCKEYE	CIAA	TRAIL LAKES	2015	4,2H	4,2H	1:1.4,2.2
TRAILLAKES15B	SOCKEYE	CIAA	TRAIL LAKES	2015	3,3,2H	3,3,2H	1:1.3,2.3,3.2
TRAILLAKES15C	SOCKEYE	CIAA	TRAIL LAKES	2015	4,6nH		
TUTKA15	SOCKEYE	CIAA	TRAIL LAKES	2015	3,5H	3,5H	1:1.3,2.5

Table 3.–Approximate budget required to recover and analyze thermal mark otoliths in Lower Cook Inlet in 2021, including line 100 details.

Line	Allocations	Expenditures	Encumbrances	Credits	Obligated	Balance	Comments
100-Personnel	$46,000.00	$0.00	$0.00	$0.00	$0.00	**$46,000**	see line 100 details below
200-Travel	$0.00	$0.00	$0.00	$0.00	$0.00	**$0**	training
300-Contractual	$3,000.00	$0.00	$0.00	$0.00	$0.00	**$3,000**	air charter and shipping costs
400-Commodities	$8,000.00	$0.00	$0.00	$0.00	$0.00	**$8,000**	field supplies, fuel, slides, etc.
500-Equipment	$0.00	$0.00	$0.00	$0.00	$0.00	**$0**	
Lines 200-500	$11,000.00	$0.00	$0.00	$0.00	$0.00	**$11,000**	
All Lines	**$57,000.00**	$0.00	$0.00	$0.00	$0.00	**$57,000**	

Line 100 Details

Job Description	Name	Position	Mos Worked	Salary/Mo	Total Cost to Project	Comments
Project Leader	Ted Otis	FB III	4.0		$0	on salary (F12034)
Project Leader	Glenn Hollowell	FB III	2.0		$0	on salary (F12033)
Consulting Geneticist	Andy Barclay	FB III	0.5		$0	on salary
Consulting Statistician	Xinxian Zhang	Biometrician III	0.5		$0	on salary
Analyst Programmer	Tim Frawley	AP V	0.5		$0	on salary
Otolith Field Project Leader	TBD[1]	FB I/FWT III	4.0	$7,220	$28,880	for otolith reads
Otolith Recovery Technician	TBD[2]	FWT II	1.5	$5,324	$7,986	for otolith collection
Otolith Recovery Technician	TBD[2]	FWT II	1.5	$5,695	$8,543	for otolith collection
Stream Survey Crew Leader	Tom Sigurdsson[3]	FWT III	2.5		$0	covered by F12037
Stream Survey Crew	Patrick Houlihan[3]	FWT II	2.5		$0	covered by F12037
Fish Ticket data entry	Mark Wayne	Office Asst. II	1.0		$0	covered by F12033
Premium Pay for field staff					$0	
					$45,409	

[1] Position currently vacant but will be filled by FB I or FWT III; five months of this position's time to oversee otolith collection is covered by project F12036; the four months listed here are for reading otoliths, including 2nd reads for quality control.
[2] Two 6-week FWT II positions needed to help collect otoliths will be filled by borrowing employees with PCNs who would normally be on SLWOP
[3] Both stream survey crew positions are covered by project F12037

FIGURES

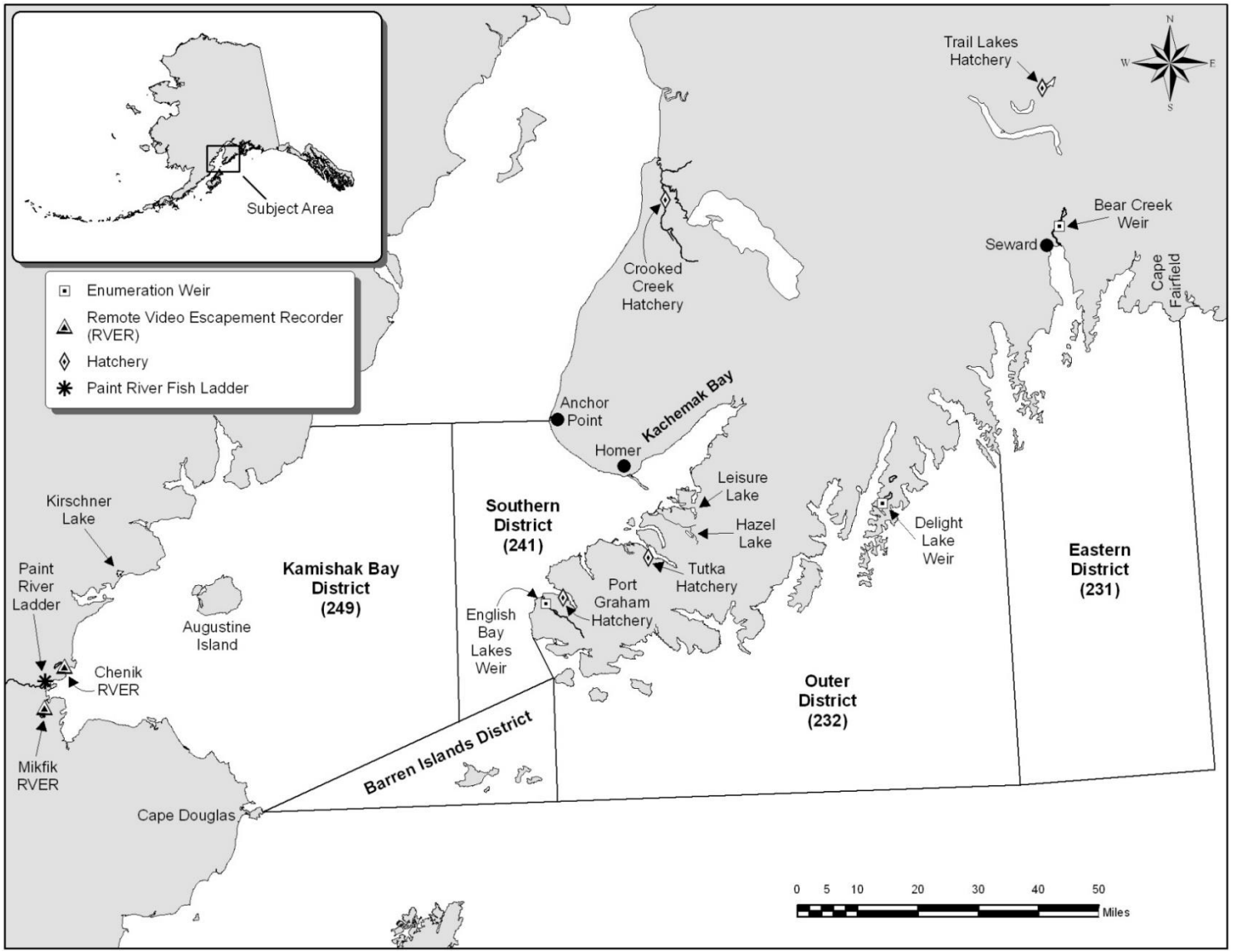


Figure 1.–Lower Cook Inlet management area showing commercial fishing districts, salmon hatcheries, hatchery remote release sites/programs, weir and fish ladder locations, and remote video salmon monitoring sites.

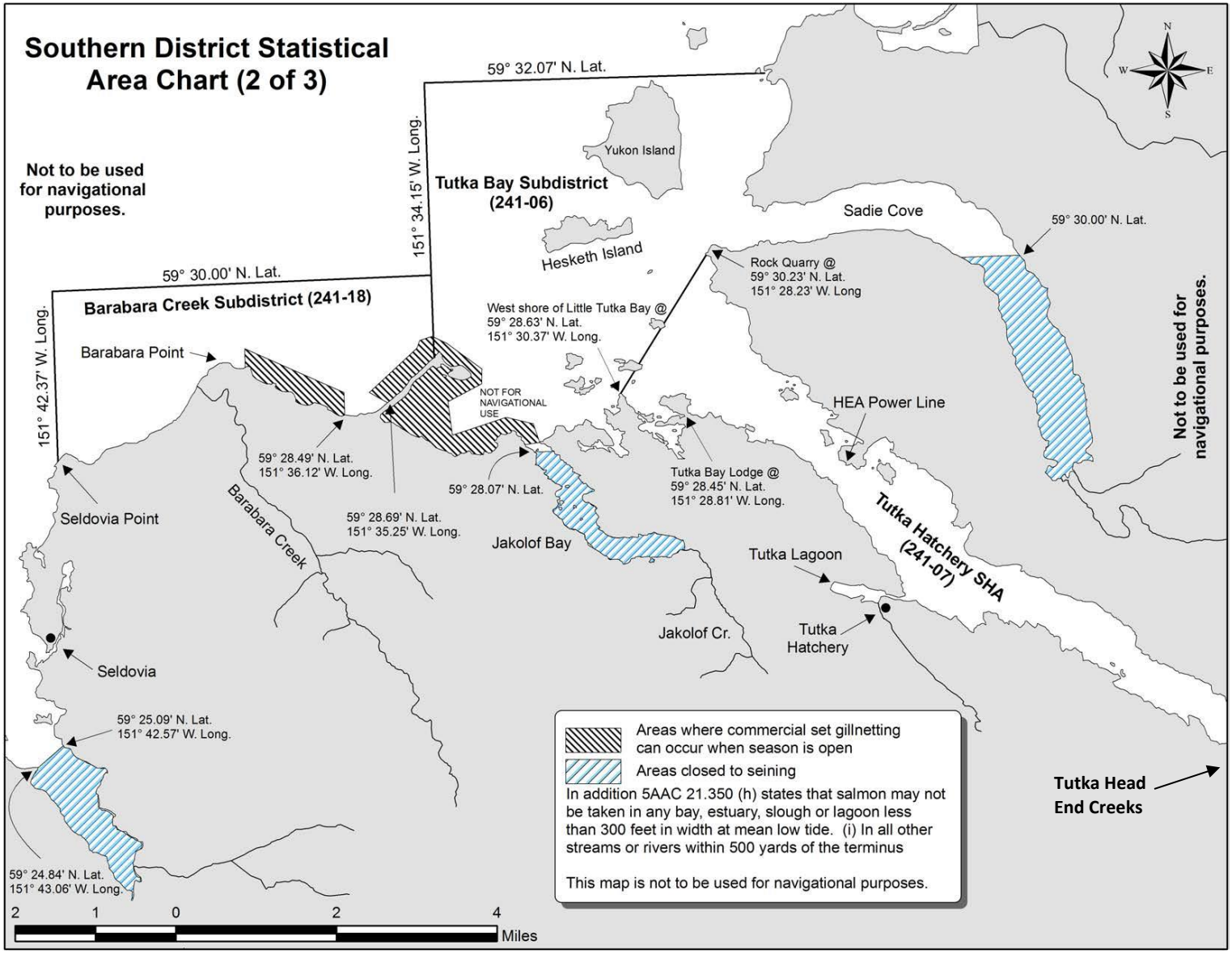


Figure 2.–Lower Cook Inlet management area, Southern District, Tutka Bay Lagoon Hatchery and special harvest area (SHA).

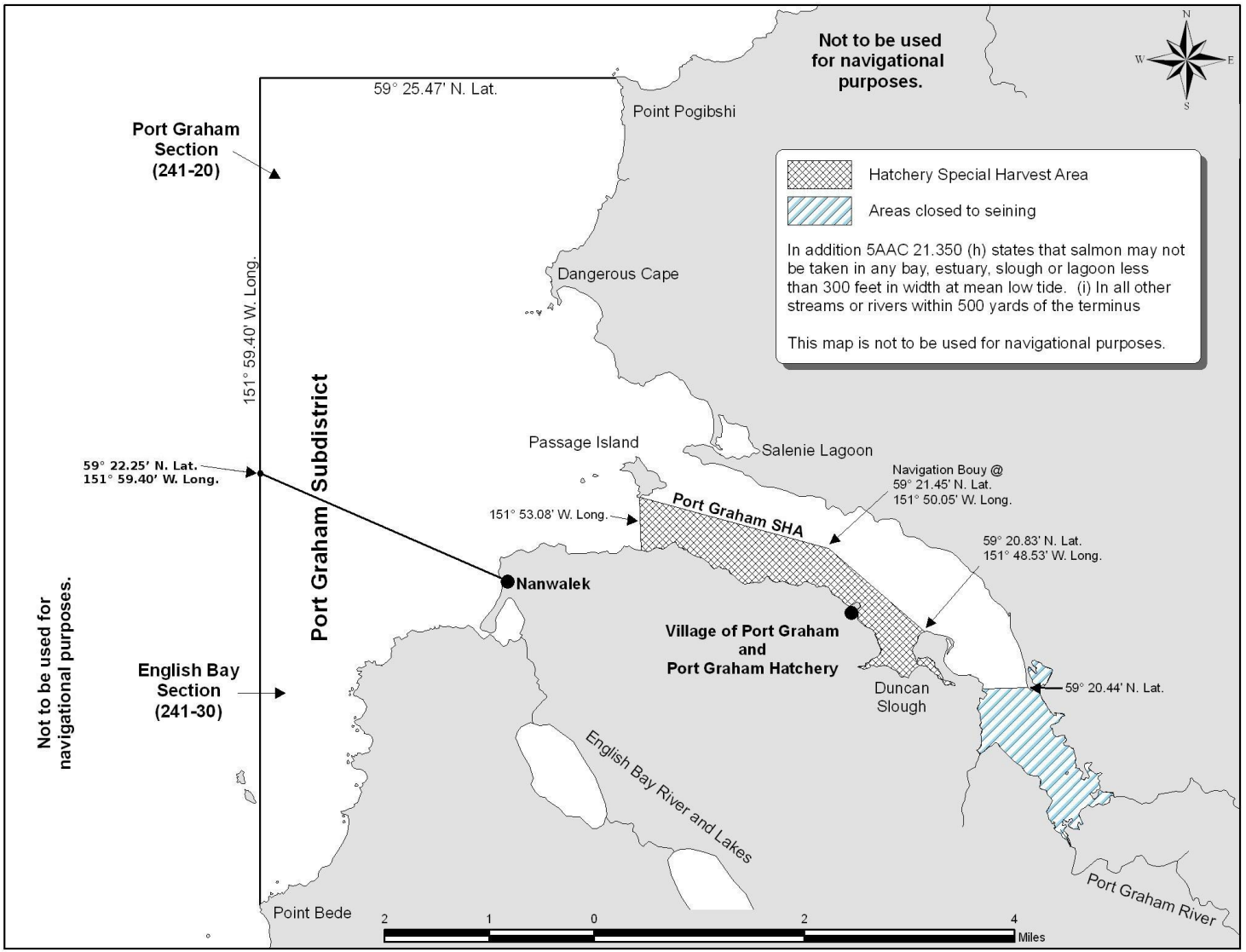


Figure 3.–Lower Cook Inlet management area, Southern District, Port Graham Hatchery and special harvest area (SHA).

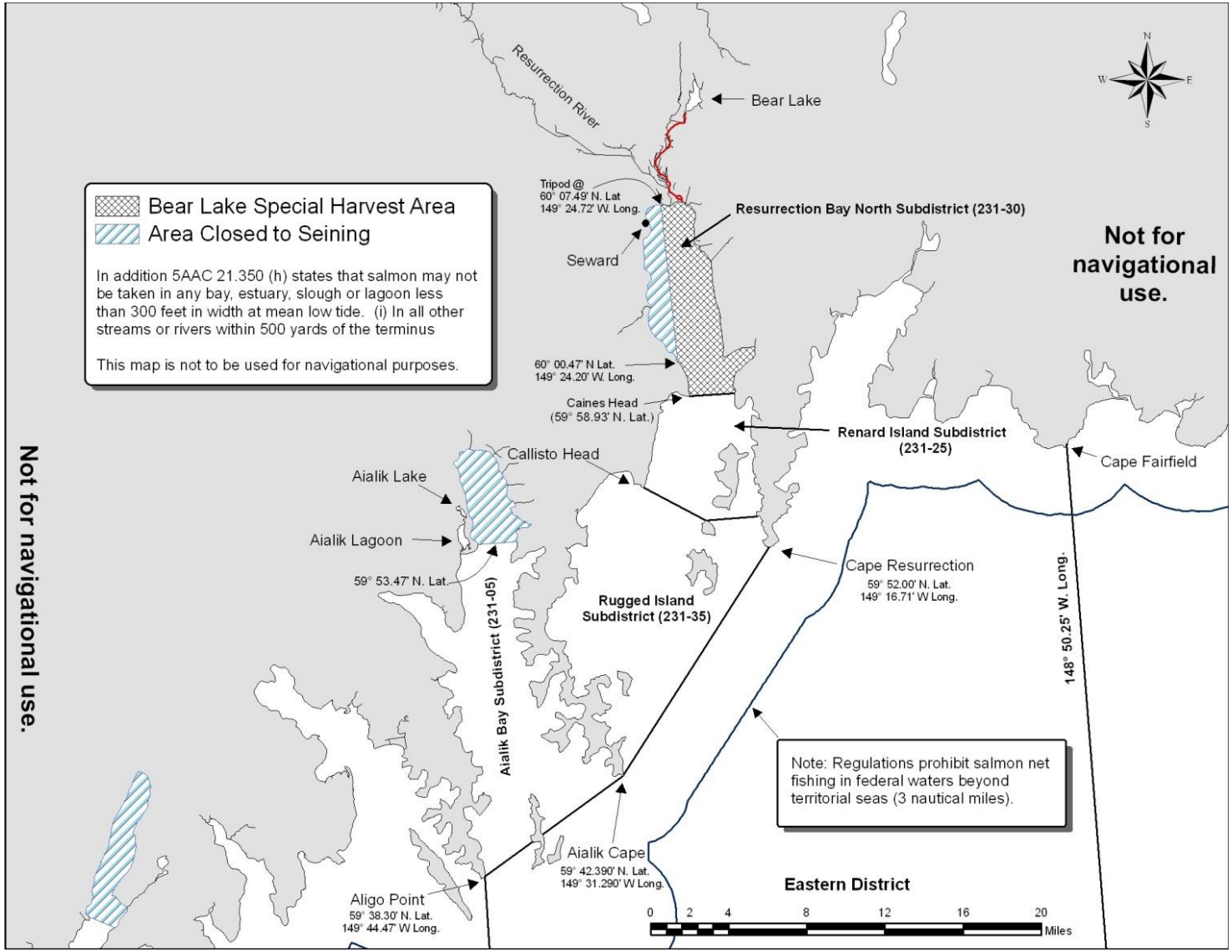


Figure 4.–Eastern District of Lower Cook Inlet management area showing commercial fishing districts, reporting subdistricts, and hatchery special harvest area (SHA), Aligo Point to Cape Fairfield.

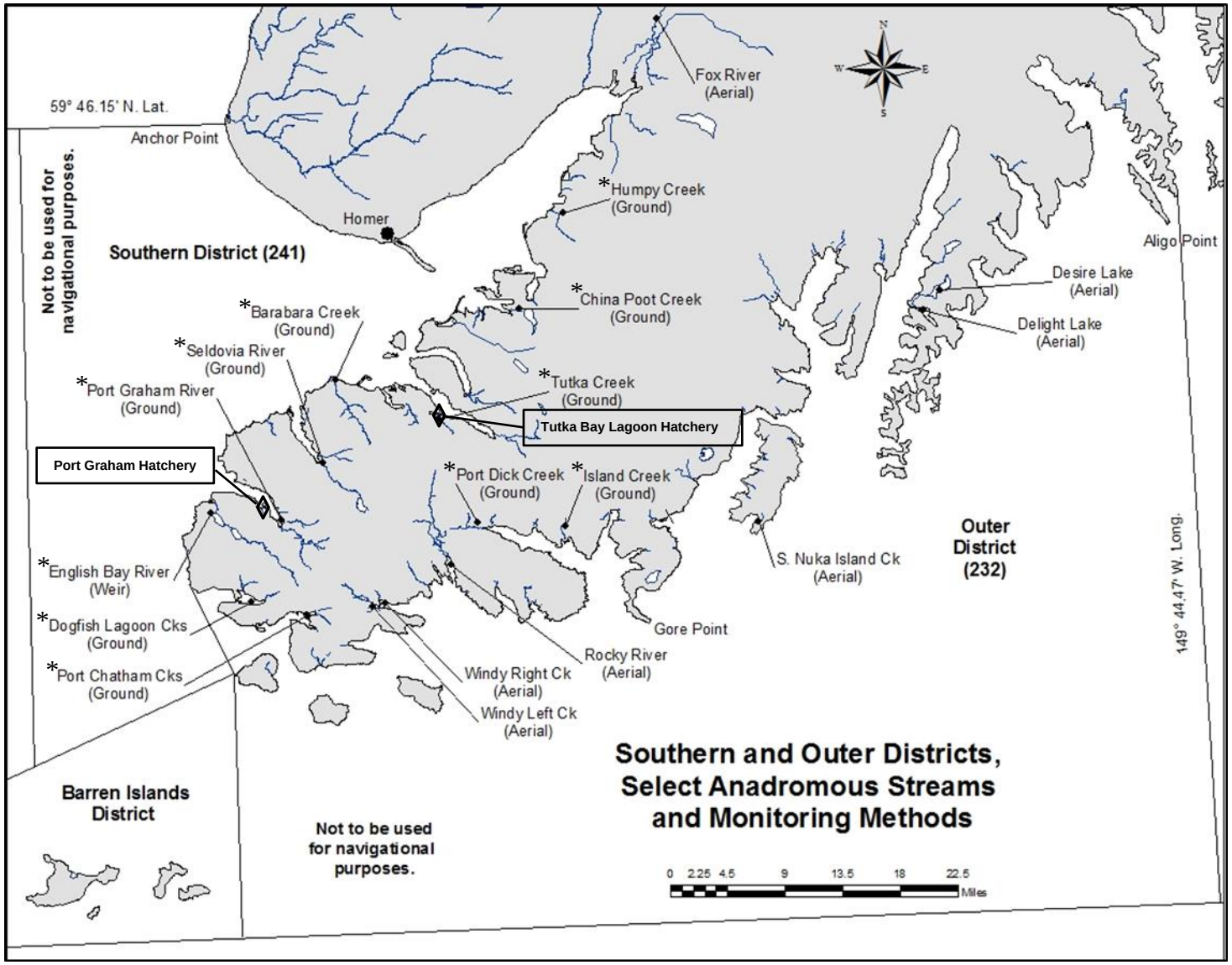


Figure 5.–Anadromous streams with escapement goals and their monitoring methods in the Southern and Outer districts, Lower Cook Inlet; streams with an asterisk (*) will be targeted for monitoring the proportion of hatchery pink salmon in the escapement in 2021.

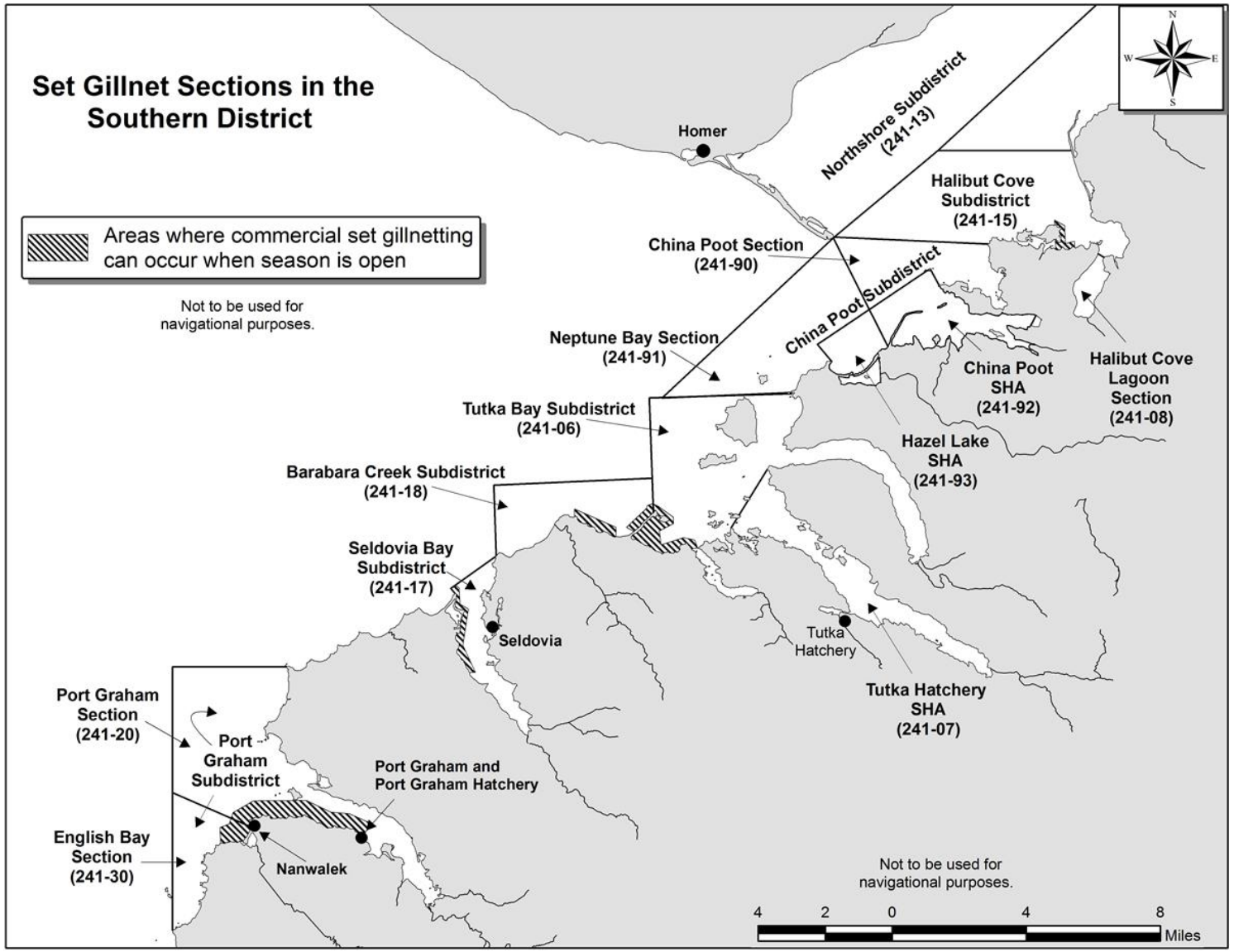


Figure 6.–Areas where commercial set gillnet fishing is allowed in the Southern District of the Lower Cook Inlet Management Area.

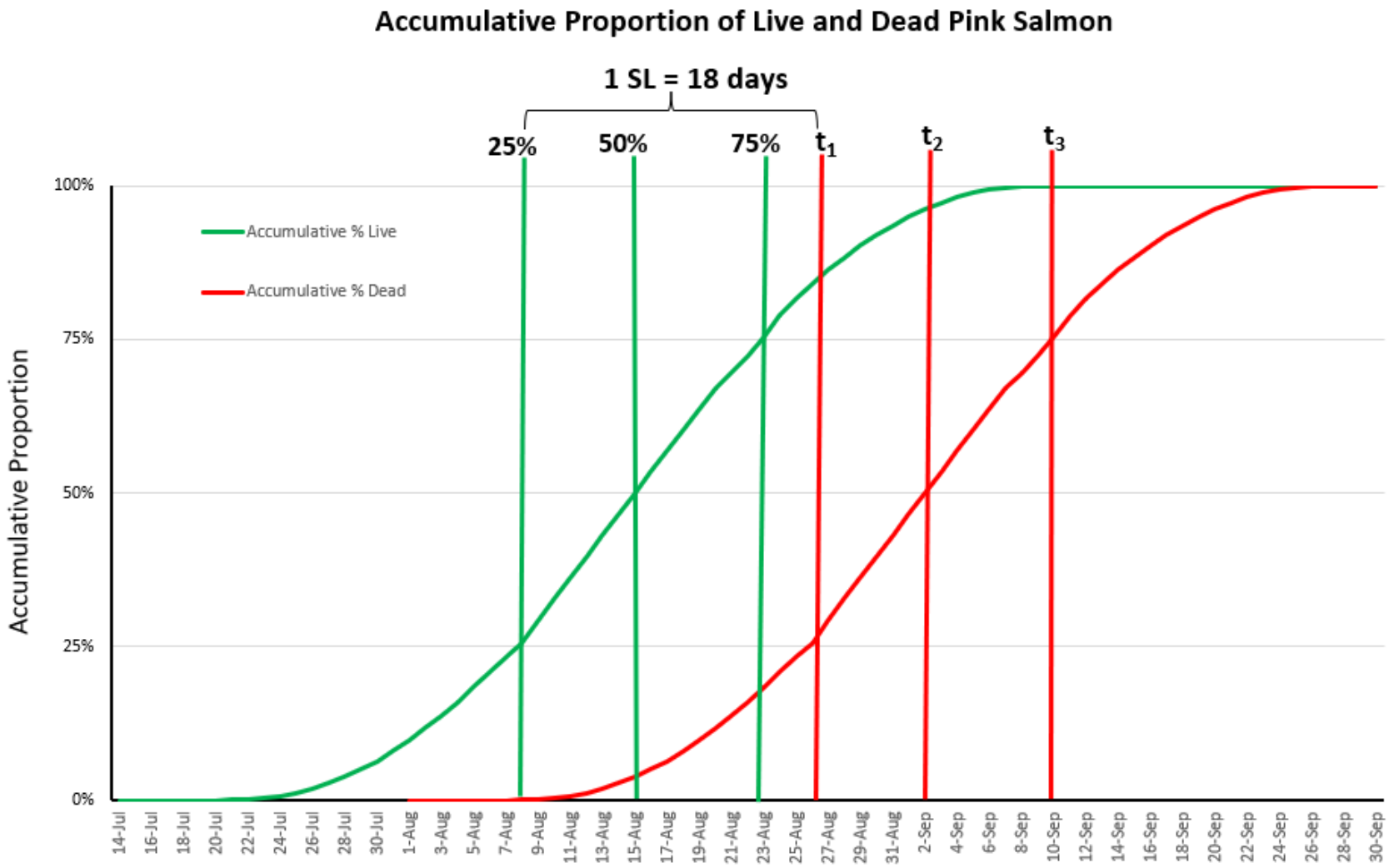


Figure 7.–Illustration of how historical escapement/run-timing curves (green lines) will be used to determine the corresponding "carcass availability curve" (red lines) based on the average streamlife (SL=18 d) for pink salmon in Lower Cook Inlet. Weighting factors for sampling events (t_1, t_2, t_3) will be derived from the accumulative proportion of "carcass availability" on each sampling date, which will ideally coincide with 25, 50, and 75% of the live run, resulting in weighting factors of 0.25, 0.25, and 0.25 respectively.

APPENDIX A: HATCHERY PRODUCTION AND RETURNS

Appendix A1.–Tutka Bay Lagoon Hatchery salmon releases, 1977–2020. Unless otherwise noted, annual releases were not thermal marked. Blanks indicate no releases.

Year released	Sockeye	Pink	Chum
1977	91,347	318,280	
1978	400,000	4,820,937	
1979		9,243,717	732,000
1980		6,795,244	5,872
1981		10,268,753	7,992
1982		15,475,435	15,440
1983		15,232,750	1,117,745
1984		18,142,463	140,500
1985		23,537,000	9,777
1986		26,234,600	18,000
1987		8,240,700	445,700
1988		15,589,360	3,211,200
1989		36,977,190	2,164,393
1990	355,347	36,684,662	1,508,557
1991		30,000,000	
1992		31,950,000	
1993		48,700,000	
1994		61,100,000	
1995		63,000,000	
1996	75,000	105,000,000	
1997	245,000	89,000,000	
1998		90,000,000	
1999	100,000	60,132,000	
2000		65,120,870	
2001		99,336,410	
2002		99,371,000	
2003		67,967,000	
2004		47,964,360	
2005	[a]		
2006	[a]		
2007	[a]		
2008	[a]		
2009	[a]		
2010	[a]		
2011	[a]		
2012	[a]	11,246,399	
2013		18,603,000 [b]	
2014		51,298,000 [b]	
2015		12,274,240 [b]	
2016		11,433,515 [b]	
2017		54,245,411 [b]	
2018		50,040,000 [b]	
2019		85,580,538 [b]	
2020		27,684,949 [b]	

[a] Sockeye salmon fry reared and thermal marked at Trail Lakes Hatchery, remote released as smolt at Tutka Bay Hatchery. Release numbers are included in releases for Trail Lakes Hatchery.

[b] Thermal marked.

Appendix A2.–Port Graham Hatchery salmon releases, 1991–2020. Blanks indicate no releases.

Year	Sockeye	Coho	Pink
1991	84,757		255,000
1992	144,982		1,810,487
1993	194,700		
1994	830,159		1,295,000
1995			358,000
1996	292,134		6,469,975
1997	199,000	29,963	918,000
1998			
1999	918,348		4,617,362 [a]
2000	906,057		1,142,726 [a]
2001			27,298,797 [a]
2002			6,600,985 [a]
2003	694,647 [a]		57,200,000 [a]
2004	159,616 [a]		36,282,671 [a]
2005	203,000 [a]		26,567,983 [a]
2006	422,060 [a]		13,883,682 [a]
2007			13,282,049 [a]
2008			
2009	[a,b]		
2010			
2011			
2012			
2013	[a,b]		[a,c]
2014			[a,c]
2015			2,200,060 [a]
2016			1,310,762 [a]
2017			6,059,800 [a]
2018			21,155,000 [a]
2019			10,144,850 [a]
2020			5,948,143 [a]

[a] Thermal marked.

[b] Remote releases from Trail Lakes Hatchery.

[c] Remote releases from Tutka Bay Lagoon Hatchery.

Appendix A3.–Trail Lakes Hatchery salmon releases, 1983–2020. Blanks indicate no releases.

Year released	Chinook	Sockeye	Coho	Chum
1983		2,310,751	1,039,673	
1984	406,755	1,236,864	1,283,815	
1985	398,586	1,805,792	1,538,361	455,809
1986	217,648	516,000	1,530,116	
1987	268,399	3,718,311	1,702,446	
1988	98,429	9,074,486	945,999	
1989		5,690,000	1,337,340	
1990		7,679,698	840,585	
1991		6,345,252 [a]	390,841	
1992		7,575,637 [a]	255,533	
1993		7,979,820 [a]	620,588	
1994		6,640,000 [a]	320,000	
1995		6,339,485 [a]	516,400	
1996		4,110,638 [a]	75,000	
1997		10,857,470 [a]	601,700	
1998		7,653,000 [a]	409,000	
1999		9,923,500 [a]	357,000	
2000		12,521,000 [a]	418,000 [b]	
2001		1,140,000 [a]	432,000 [b]	
2002		18,907,200 [a]	528,500 [b]	
2003		16,128,000 [a]	761,000 [b]	
2004		17,272,000 [a]	996,000 [b]	
2005		9,959,000 [a]	988,000 [b]	
2006		5,785,000 [a]	1,146,000 [b]	
2007		12,668,800 [a]	956,000 [b]	
2008		13,203,000 [a]	685,000 [b]	
2009		7,953,000 [a]	382,000 [b]	
2010		8,616,000 [a]	435,000 [b]	
2011		9,324,200 [a]	437,000 [b]	
2012		7,636,300 [a]	315,000 [b]	
2013		7,482,000 [a]	405,000 [b]	
2014		9,368,500 [a]	523,000 [b]	
2015		8,302,700 [a]	546,000 [b]	
2016		6,001,790 [a]	546,600 [b]	
2017		7,207,000 [a]	180,000 [b]	
2018		8,883,000 [a]	536,000 [b]	
2019		8,562,230 [a]	514,000 [b]	
2020		2,446,353 [a]	96,890 [b]	

[a] Thermal marking of sockeye salmon releases began in 1991 (BY 1990).

[b] Thermal marking of coho salmon releases began in 2000 (BY 1999).

Appendix A4.–Historical releases of pink salmon from hatcheries to Lower Cook Inlet, 1975–2020. Blanks indicate no releases.

	Southern District (241)					Eastern District (231)	Kamishak Bay District (249)
Year	Tutka Bay	Halibut Cove Lagoon	Halibut Cove-bight	Homer Spit	Port Graham Subdistrict	Resurrection Bay	Paint River
1975		50,916					
1976							
1977		318,280					
1978	4,820,937						
1979	9,243,717						
1980	6,245,103						550,141
1981	9,759,144						509,609
1982	15,070,927						404,508
1983	14,730,794						501,956
1984	18,142,463						
1985	23,537,000						
1986	22,228,600	4,006,000					
1987	4,385,600	3,001,400		594,500			
1988	12,003,878	3,022,491		310,016			
1989	30,091,053	6,229,062		331,695			
1990	23,689,702	6,000,000		603,845			
1991	23,657,112	6,039,062		303,826	255,000		
1992	25,700,000	5,950,000		300,000	1,810,487		
1993	48,700,000						
1994	61,100,000				1,295,000		
1995	63,000,000				358,000		
1996	105,000,000				6,469,975		
1997	89,000,000				918,000		
1998	90,000,000						
1999	60,132,000				4,617,362	48,329	
2000	65,120,870				1,142,726	24,216	
2001	99,336,410				27,298,797		
2002	99,371,000				6,600,985		
2003	67,967,000				57,200,000		
2004	47,964,360				36,282,671		
2005					26,567,983		
2006					13,883,682		
2007					13,282,049		
2008							
2009							
2010							
2011							
2012	8,100,399		3,146,000[a]				
2013	4,353,000				14,250,000		
2014	51,110,000				188,000		
2015	11,249,240				2,200,060		1,025,000
2016	11,433,515				1,310,762		
2017	54,245,400				6,059,800		
2018	50,040,000				20,850,000		305,000
2019	85,580,538				10,144,850		
2020	27,684,949				5,948,143		

[a] Released outside of Halibut Cove Lagoon, one kilometer east.

Appendix A5.–Historical releases of sockeye salmon from hatcheries to Lower Cook Inlet, 1981–2020. Blanks indicate no releases.

	Southern District (241)						Outer (232)	Kamishak District (249)					Eastern District (231)		
Year	Leisure Lake	Hazel Lake	Halibut Cove Lagoon	Tutka Bay Lagoon	English Bay Lakes	Port Graham Subdist.	Port Dick Lake	Chenik Lake	Paint River Lakes	Kirschner Lake	Bruin Lake	Ursus Lake	Bear Lake	Resurrection Bay	Grouse Lake
1981	1,094,713							1,096,718							
1982	1,527,876														
1983	2,113,239														
1984	2,110,000														
1985	2,018,000														
1986	2,250,303							839,000	820,026						
1987	2,022,000						704,900	1,005,000		866,700					
1988	2,100,000	783,000					221,700	2,601,000	2,207,300	521,000					
1989	2,000,000	1,000,000					430,000	3,500,000	2,000,000	250,000					
1990	2,000,000	1,500,000			855,347			3,250,000	2,000,000	250,000			2,577,962		
1991	2,000,000	1,300,000			255,071	84,757		2,100,000	750,000	250,000	250,000		1,604,922		
1992	2,000,000	1,000,000			290,298	144,982		2,750,000	750,000	250,000	250,000	250,000	1,482,489		
1993	2,000,000	1,000,000			755,692			1,400,000	750,000	250,000	250,000	250,000	1,810,261		
1994					820,174	9,985				208,000			170,000		570,000
1995	1,632,000	1,061,000						1,129,000	588,000	251,000	251,000	252,000	330,000		993,000
1996	1,490,000	1,030,000		75,000	292,134			951,000	500,000	250,000	250,000	250,000	780,638		217,605
1997	2,000,000	1,000,000		245,000	199,000					250,000			788,000		2,428,000
1998	1,877,000	1,218,000								234,000			772,000		1,514,000
1999	265,400	453,100		100,000	918,348					172,700			1,380,000		
2000	1,708,000	1,248,000			906,057					249,000			1,796,000		
2001	89,000												145,000		
2002	2,246,200	1,280,100							507,700	301,500			3,210,300		
2003	2,240,000	1,547,000			694,647					298,000			1,801,000		
2004	2,002,000	351,000			50,096	109,520				251,000			3,012,000		
2005	2,252,000	1,558,000		96,000	203,000					316,000			3,422,000		
2006	680,000			260,000		422,060							3,393,000		
2007	2,315,000	1,411,000		143,800						254,000			3,056,000		
2008	2,053,000	1,161,000		483,000	246,000					300,000			2,400,000	1,600,000	
2009	1,225,000	1,186,000		301,000		112,000							2,543,000	1,675,000	
2010	1,933,000	1,218,000		278,000	202,000					255,000			2,200,000	1,650,000	
2011	1,415,000	1,244,000		281,900	203,300					160,000			2,488,000		
2012	2,074,000	1,240,000		371,300	213,000					300,000			2,490,000	1,305,000	
2013	1,800,000	1,450,000		511,000	211,000	102,000							2,548,000	2,090,000	
2014	1,353,000	1,223,000		599,500	209,000					217,000			2,405,000	1,742,000	
2015	1,051,000	621,000		523,500	200,200					237,000			2,415,000	1,758,000	
2016				531,625						185,000			2,374,000	1,680,165	
2017	1,387,000	834,000		356,000		86,000				260,000			2,468,000	1,816,000	
2018	1,948,000	813,000		518,000						244,000			2,555,000	1,488,000	
2019	1,085,000	1,293,000		427,000						258,000			2,427,000	1,510,000	
2020	274,443	266,448		363,072						271,858			2,446,353		

APPENDIX B: SAMPLING FORMS

Appendix B1.–Sampling form for otoliths collected from Pacific salmon harvested in commercial common property (CCP) and hatchery cost recovery (HCR) fisheries, 2021.

Alaska Department of Fish and Game

Otolith Sampling Form- CATCH

Commercial Fisheries - Lower Cook Inlet

Sample Number | 2 | 1 | | | | | | | |

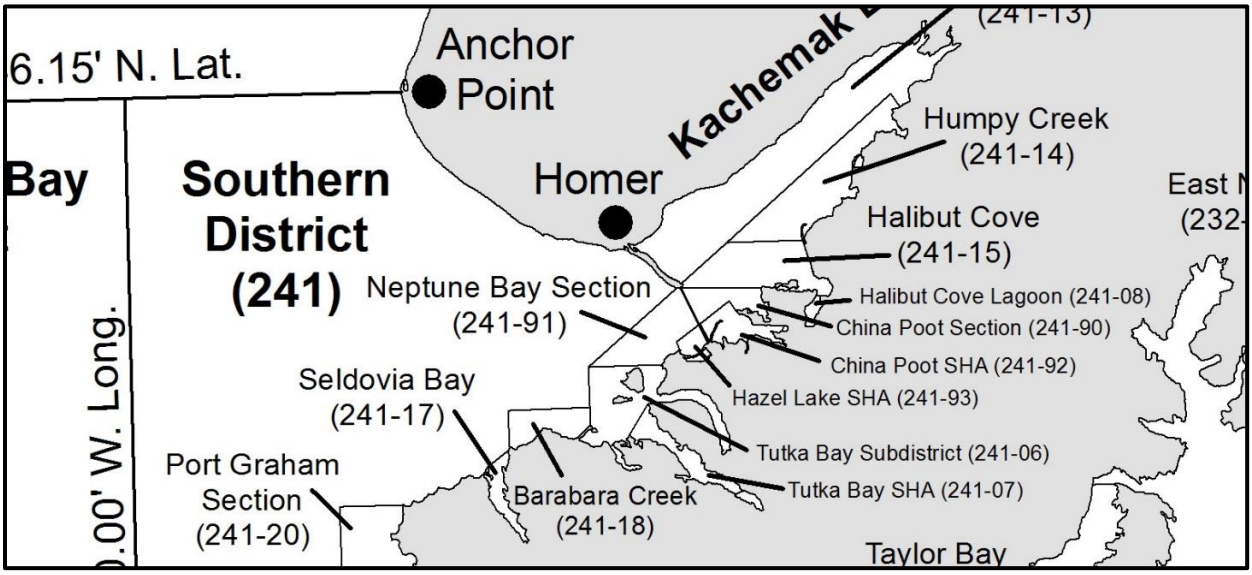
Harvest Type: CCP HCR **Page ____ of ____**

Sample Type: systematic grab **Sample Rate: _____**

Species: sockeye pink **Sample time: begin _____ end_____**

Survey Site: _____ **Date Sampled: _____**

Sampler(s): _____ **Date Caught: _____**



Catcher/Area Information

Processor:_____	**Gear type:** Gillnet	Seine		
Vessel/Tender:_____	241-14	241-15	241-08	241-90
Poundage:_____	241-91	241-92	241-06	241-07
Stat Week/Strata:_____	241-17	241-18	241-20	241-30

Other:_____Lat_____ Long_____

-continued-

Otolith Recovery Information

Total # sampled: _____ Shade the boxes of each sample taken

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Comments:

Appendix B2.–Sampling form for otoliths collected from spawned out pink salmon carcasses in select LCI index streams, 2021.

Alaska Department of Fish and Game

Otolith Sampling Form-ESCAPEMENT

Lower Cook Inlet Escapement Sampling

Sample Number | 2 | 1 | | | | | | | |

ONLY 1 DATE & DATA SHEET PER SAMPLE NUMBER

Stream Type: Index Non-Index **Species:** pink

Sample Type: grab **Sample Time:** **Begin Time:** _____ **End Time:** _____

Stream Name: _____

Sampler(s): _____

Date Sampled: _____ **Stat Week:**_____

Ground Survey Crew:_____

Pink Salmon Live Count:_____ **Dead Count:**_____

Sample Comments:

_____

_____

_____

-continued-

Otolith Recovery Information

Total # sampled: _____ Please indicate single otoliths or no otoliths where necessary

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Sample Locations (decimal degrees)

Cell A1 -____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Cell ____-____Lat_____Long_____Section_____

Comments:

APPENDIX C: TM RECOVERY PROCEDURES

Appendix C1.–Thermal mark recovery procedures of the ADF&G Mark, Tag, and Age Laboratory.

Alaska Hatchery Research Group **Technical**

Document:[1]
7

Title: Thermal Mark Recovery Procedures of the ADF&G Mark, Tag and Age Laboratory **Version:** 1.0
Authors: Agler, B., L. Wilson and M. Lovejoy
Date: August 9, 2016

Abstract

The Alaska Hatchery Research Program is designed to answer questions regarding concerns that hatchery fish released by private non-profit corporations in Prince William Sound (pink and chum salmon) and in Southeast Alaska (chum salmon) may have a detrimental impact on the productivity and sustainability of natural stocks. The study that was designed to answer these questions requires that samples and data collected by a contractor and by various Alaska Department of Fish and Game laboratories be combined to test hypotheses. One aspect of critical to this study is examining salmonid otoliths for the presence or absence of a thermal mark. This technical document describes the procedures used by the Alaska Department of Fish and Game, Mark Tag and Age Lab for thermal mark recovery. Procedures for thermal mark recovery include cleaning otoliths and trays, tracking trays and otoliths, otolith preparation (slide labeling, mounting the otoliths), grinding a prepared otolith to the core so that thermal mark presence or absence and thermal mark identification can be determined, and entering results to a database.

Background of AHRP

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 large 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 exists. To address these concerns ADF&G convened a Science Panel for the

[1] 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

Alaska Hatchery Research Program (AHRP) whose members have broad experience in salmon 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

To answer the above questions, we need to know the origin and pedigree of each fish captured in select streams across multiple generations. Origin refers to the type of early life-history habitat (hatchery or natural) that a fish experienced. Pedigree refers to the family relationship among parents and offspring. 'Ancestral origin' refers to the origin of an individual's ancestors (e.g., two parents of a single origin [hatchery/hatchery or natural/natural] or two parents of mixed origin [hatchery/natural]). These ancestral origins can be determined by combining information from three sources: identification of hatchery origin from otolith thermal marks, pedigree from genetic data, and age from scales (SEAK chum).

Question: How will we identify hatchery origin from otolith marks?

Salmonid otoliths are thermal marked by exposing them to repeated temperature cycles to create patterns of optically-dense bands (Volk et al. 1990). Because these can be applied accurately and identified quickly (Hagen et al. 1995), thermal marking (Figure 1) is an effective tool providing simple identifiers for hatchery salmon (Munk and Smoker 1991; Volk et al. 1990).

The North Pacific Anadromous Fish Commission (NPAFC) Working Group on Salmon Marking (WGOSM) coordinates the application of otolith mark patterns for hatchery-origin fish released in the North Pacific Rim countries; they work to minimize duplication of marks among release groups. Thus, thermal marks recovered from adult salmon can be used to identify release location of chum salmon releases in SEAK. The MTA Lab examines otoliths for thermal marks to identify origin of chum salmon in SEAK.

Goal

This technical document describes the procedures used by the MTA Lab for AHRP thermal mark recovery.

Methods

Sample types

For the AHRP project, there are two types of samples collected based on the surveys conducted: stream and pedigree. Samples collected at stream sites contain only otoliths. Samples collected at pedigree sites include DNA tissue and otoliths. Otoliths collected from stream sites are placed into

a shallow 96-well tray. When pedigree sites are sampled, an otolith pair and a tissue specimen are placed into the same cell of a 48 deep-well tray. The cells in a pedigree sample tray are filled with ethanol to preserve tissue.

For each fish sampled, both right and left sagittal otoliths are removed and placed in the appropriate trays. If enough samples to fill the tray are not obtained on a sampling trip, then some wells are left empty. If otoliths are lost in the field, missing ones are represented by glass beads. Thus, when one otolith is missing, a well contains one otolith and one bead to indicate a missing otolith. A well with no otoliths contains two beads, indicating that both otoliths are absent.

Prior to shipment of stream samples to the MTA Lab, stream trays are dried so that the otoliths remain within tray wells. If otoliths are wet, they may stick to the tray lid rather than stay in place. Trays are dried by leaving them uncovered overnight. To keep the otoliths in position, two acetate compression plates taped together with double-sided tape are placed between the tray and lid. The tray, compression plates, and lid are secured with three fresh #64 rubber bands (Figure 2). Only new rubber bands are used, because old rubber bands warp, break, crack, and stretch allowing otoliths to move out of place. Prior to shipment of pedigree trays to the Gene Conservation Lab (GCL), samples were refreshed with ethanol. Processing of pedigree trays follows methods detailed in Appendix A. After processing, the 48 deep-well tray (now only contains otoliths) are uncovered, dried, recovered, and shipped to the MTA Lab. The duplicate plate (contains only heart tissue) is uncovered, dried, recovered, and archived until DNA extraction. Archived location is entered into the GCL database, LOKI.

MTA Lab Procedures

Otolith processing procedures for the AHRP project begin with collection of stream and pedigree samples in SEAK streams and genetic tissues removed at the Gene Conservation Lab (Figure 3). These samples are then shipped to the MTA Lab where the trays are cleaned, logged in to an Oracle database to digitally track the samples, and mounting them to glass slides for reading. The mounted otoliths are ground and examined for thermal mark presence and identification by two independent readers, and any conflicts are resolved. Two independent reads are used to assess the accuracy of thermal mark presence and identification (see Thermal Mark Recovery Data Quality Assurance and Quality Control Technical Document). Mark recovery results are summarized on a public website, queried through the database, and reported by the AHRP project contractor.

Otolith cleaning and tray review

Upon receipt at the MTA Lab, the crew leader reviews the labels to ensure that the data recorded on the tray are legible and match the corresponding tray inventory. Discrepancies are resolved by contacting the contractor. Otoliths and trays are rinsed with a 5% chlorine solution to clean and bleach the otoliths. Trays are subsequently rinsed 0.7% thiosulfate and water to stop the bleaching process. Cleaning removes remaining tissue; otherwise, this tissue may prevent adherence to the

petrographic slides or it may obscure visibility of the otolith core. The wells in each tray are checked for missing otoliths, and glass beads are added to represent absent specimens (Figure 4).

Otolith tray log-in

All data associated with each tray are entered into the Southeast Mark-recapture (SEMR) database using custom data entry applications. The data includes tray number, species, life stage, statistical week, source, gear type, location, and stream code. The number of otoliths in each tray is recorded by selecting the last well position. For pedigree trays, an additional number (deep-well plate identification number) is recorded to coordinate genetics data with otolith data. Other information on the tray label, such as collectors, comments, and shipping method, is also entered. After samples are entered into the database, each fish can be located using sample, specimen, tray, and well number.

Otolith mounting

After trays are logged-in, labels with a unique bar code are printed and affixed to one-by-two inch petrographic glass slides. The labels contain information for quick reference, such as tray, well, sample, and specimen number (Figure 5). Maintaining proper tray orientation while mounting otoliths keeps specimens in order, which is important because otolith data are associated with other information, such as genetics and scale-age data.

Trays from stream sites, which hold 96 otoliths, are positioned so that the white, pre-painted corner (painted before a project begins) is to the upper left, indicating the starting position (Figure 4). This ensures that the correct otolith is removed from the correct well. Otoliths are removed from left to right by rows. Thus, the first otolith is removed from well "A1" in row "A" and the next otolith is removed from well "A2." This continues until all otoliths are pulled and mounted from row "A "through well "A12." Once complete, otoliths are removed from the next row down starting with well "B1."

Trays from pedigree sites, which have 48 deep wells, are placed in an apparatus designed to ensure proper tray orientation and allow only one space to be open for otolith selection from the tray at one time. This apparatus is helpful because otoliths are harder to see in a deep-well plate than in a shallow 96-well tray. A notch on the bottom left corner provides a visible reference starting position (Figures 6 and 7), and these trays are oriented differently. Otoliths are pulled from top to bottom by columns. Thus, after the first otolith is removed from well "A1," the technician removes and mounts otolith from well "A2" continuing until all otoliths are pulled from the first column, column "A." Once complete, otoliths are pulled from the next column starting with "B1" and so on.

After the correct otolith is selected, the left otolith is mounted on the un-labeled side of the glass slide sulcus-side up (Figures 5 and 8) with thermoplastic cement, so that the label is protected when the otolith is ground. The right otolith remains in the tray for age and brood year determination, if necessary and is available to be used if the left otolith is unreadable. Mounted

slides are stored in 100 specimen slide boxes labeled with district, subdistrict, species, sample date, statistical week, sample number, and box number. After mounting, otoliths are handled by box; the sample and box numbers on the box label are used for assessment of otolith mark recovery reads (see Thermal Mark Recovery Data Quality Assurance and Quality Control Technical Document).

Otolith Preparation and Mark Recovery

Prior to reading chum salmon otoliths, all readers review and study examples of thermal marks expected to be recovered during that sampling period. For this project, these marks include chum salmon released in Southeast Alaska from brood years that correspond with fish returning at age 0.2, 0.3, 0.4, and 0.5 (European age notation) in each recovery year. Please see the "Personnel Training" section of the Thermal Mark Recovery Data Quality Assurance and Quality Control Technical Document for a description of the pre-season thermal mark review process.

To examine a salmonid otolith for the presence or absence of a thermal mark, a reader first enters the specimen number directly into the SEMR Oracle database by scanning the bar code on the slide label. This connects the reader to that record in the database. Once the specimen number is entered, the reader grinds the otolith using a variable speed grinder and 800 grit grinding paper until the primordia at the otolith's core are visible under 200 x magnification on a compound microscope. If needed, the otolith can be fine-polished manually on wet nine µm grinding paper to enhance growth patterns at the otolith's core. The reader then places the slide otolith side up on a compound microscope and examines it using the 25 x and 40 x objectives to determine whether the otolith is thermal marked (hatchery-origin) or not marked (natural-origin). The reader enters the result in the SEMR Oracle database using a touch screen monitor and a custom data entry application. If a specimen is thermal marked, the reader enters the hatch code (unique thermal mark pattern), thermal mark identification (a name assigned to each hatch code that provides information regarding brood year and release site), and age (ADF&G 2011). All specimens receive a status code (readable or not readable). This status code is also used to track progress on a project. If a specimen is not readable, a reader enters a code providing a reason why an otolith could not be examined (e.g.; no otolith, crystalline, morphology problem, over-ground, or wrong species). Once a specimen is read, the slide is placed back in the slide box and stored in the MTA Lab. Accuracy of results are assessed using a variety of methods, all of which include independent re-examination of ground otoliths (see Thermal Mark Recovery Data Quality Assurance and Quality Control Technical Document).

Otolith archives

All thermal mark data processed at the MTA Lab, including the reference collection and adult recoveries, are housed in the SEMR database (Frawley et al. 2015 for details regarding AHRP data flow).

Reporting

Thermal mark read results are reported as follows:

(1) A public report, which includes the number of otoliths received, prepared, and read, the number marked, the number unmarked, and the mark identifications. Data are listed by fishery name, species, source, statistical week, statistical area, stream code, harvest type, sample date, gear, and survey site. This report can be accessed and generated via the web at: http://mtalab.adfg.alaska.gov/OTO/reports/MarkSummary.aspx
(2) Results stored in the SEMR Oracle database are integrated with results from other ADF&G labs and the project contractor in an ADF&G statewide data warehouse. This data flow between the contractor, who collects the specimens and records the sampling event data, and the MTA Lab is described in the AHRP Data Flow Technical Document (Frawley et al. 2015).
(3) Specialized reports can be developed using Microsoft Access to query the SEMR Oracle database. This is utilized for data quality control or specific reporting.

References

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Frawley, T. R., E. Lardizabal, and S. Johnson 2015. Alaska Hatchery Research Program Technical Document 10: AHRP Data Flow

Hagen, P., K. Munk, B. W. Van Alen, and B. White. 1995. Thermal mark technology for inseason fisheries management: a case study. Alaska Fishery Research Bulletin 2(2):143-155.

Munk, K., and W. W. Smoker 1991. Temperature-induced mass-marking of pink salmon otoliths. Production trial at Gastineau Channel. University of Alaska Fairbanks, Juneau, Alaska

Vercessi, L. 2014. Alaska salmon fisheries enhancement program 2013 annual report Alaska Department of Fish and Game, Anchorage http://www.adfg.alaska.gov/FedAidPDFs/FMR14-12.pdf

Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. American Fisheries Society Symposium 7:203-215.

Figures

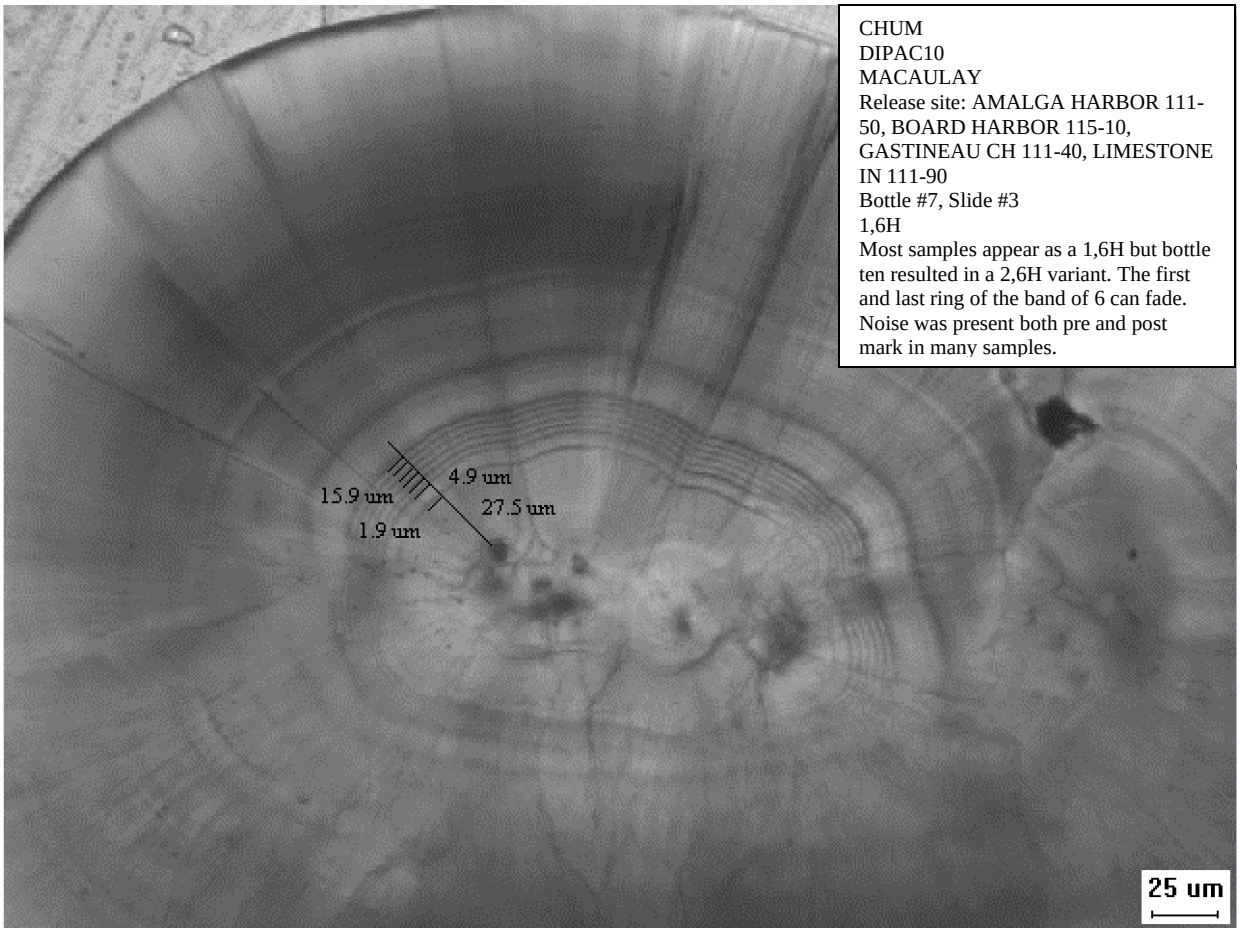


Figure 1.–Image of a thermal mark from a voucher specimen. This mark is from Macaulay Hatchery, brood year 2010, and has thermal mark identification "DIPAC10." It has a thermal hatch code of 1,6H. This hatch code indicates that from the otolith's core there is a band with one dark ring, a space, followed by a band of six rings, prior to the hatch mark (the blurry, wider dark area beyond the thermal mark). Measurements on the annotated transect line include the distance from the otolith's core to the first band, the width of the first band, the space between the first and second bands, and the average distance between rings in each band. All thermal mark images are published online and are available through the North Pacific Anadromous Fish Commission (NPAFC) Working Group on Salmon Marking (WGOSM) website:

http://wgosm.npafc.org/MarkSummary.asp

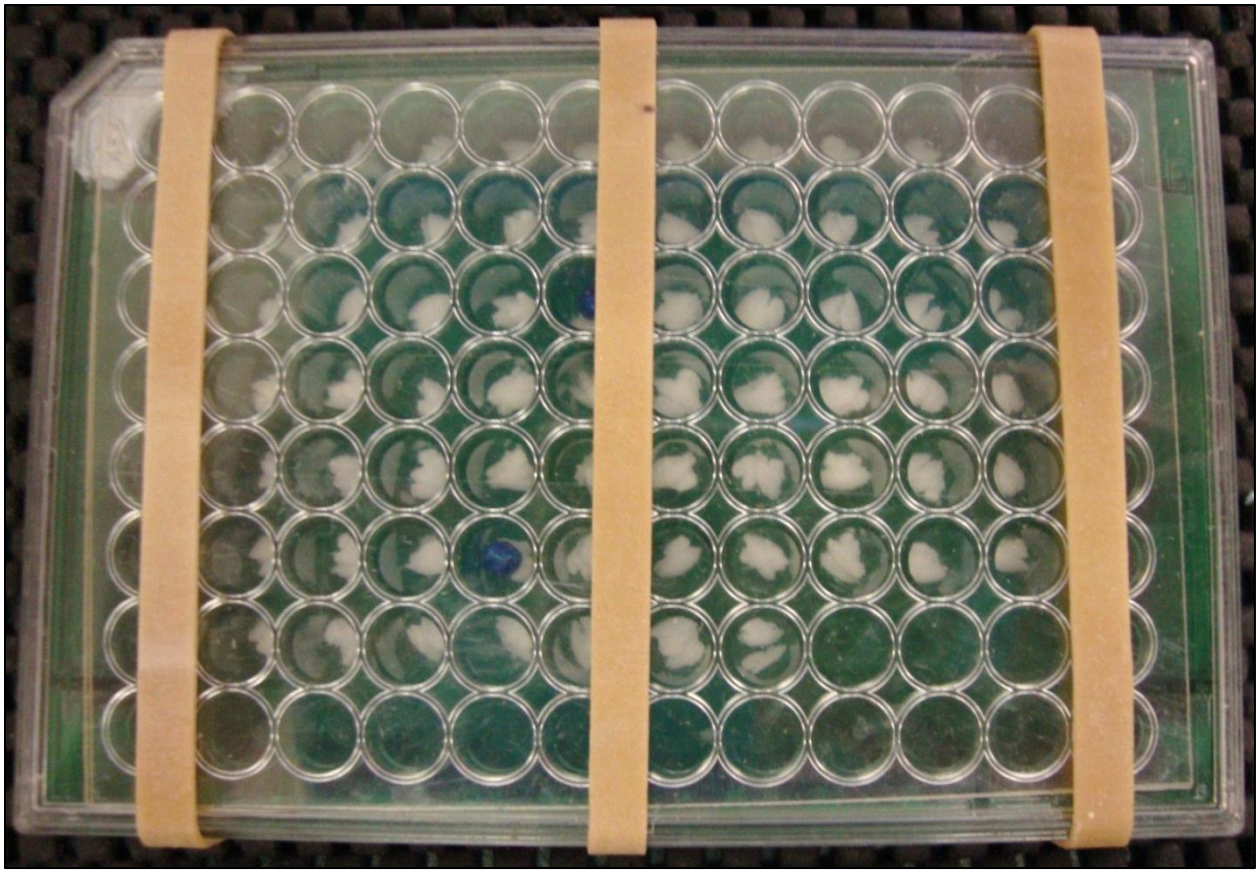


Figure 2.– Otolith tray from a stream sampling site prepared for shipment. Tray includes two acetates taped together and placed between the tray and lid. Lid is secured with three “fresh” #64 rubber bands. Note the white paint added to notched corner (upper left) to aid in identifying correct orientation of tray.

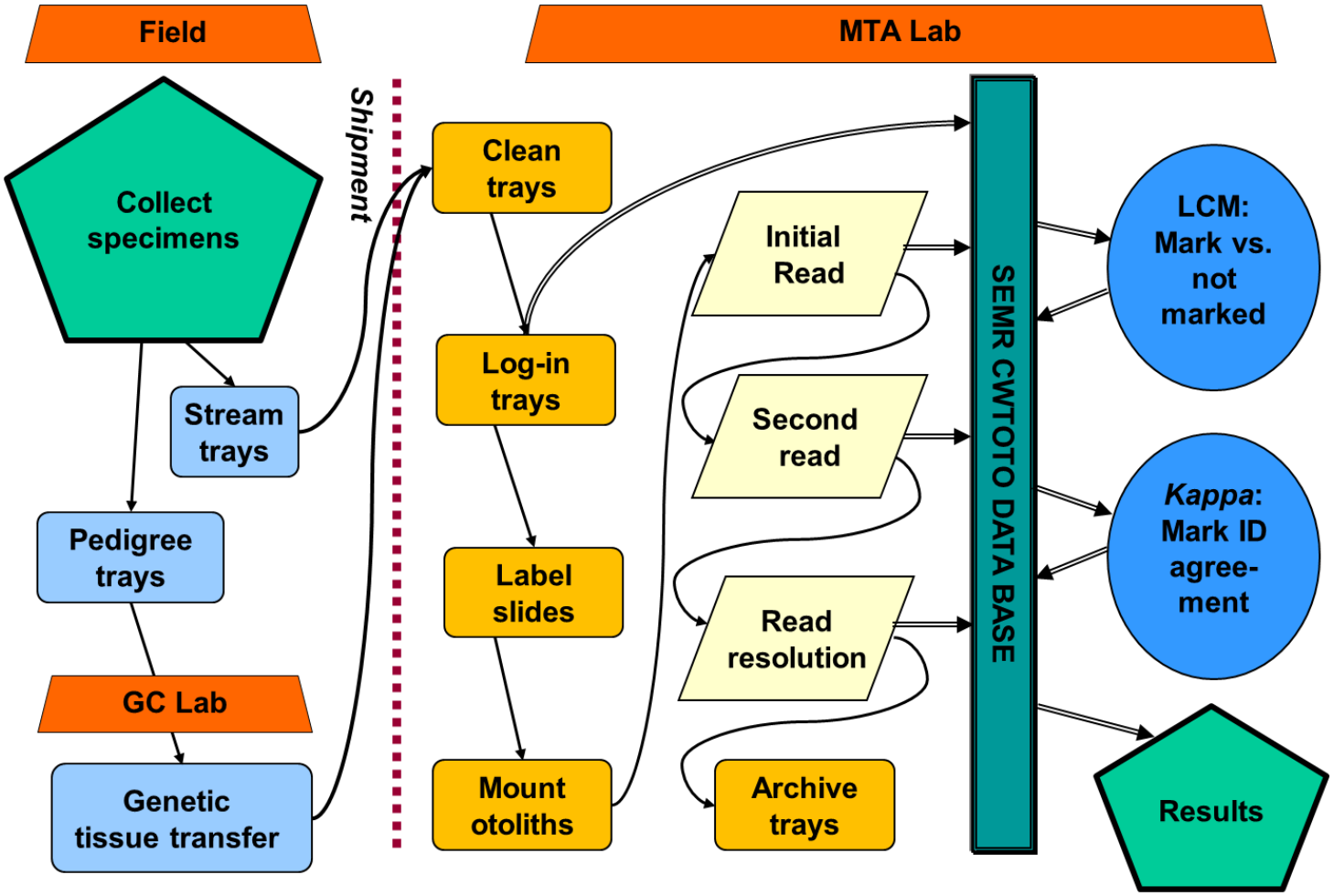


Figure 3.– Flow diagram of Southeast Alaska otolith processes for the Alaska Hatchery Research Program. Shapes indicate different processes: hexagons are the beginning and end of flow, trapezoids are location of process, rounded rectangles are tray or otolith preparation, parallelograms are otolith data collection, ovals are statistical examination of results. Solid arrows indicate specimen flow, double arrows indicate data flow. See text for descriptions of each process and the QA/QC technical document for descriptions of statistical methods. LCM: Latent class model, Kappa is Fleiss's Kappa statistic. Both are usedfor QA/QC.

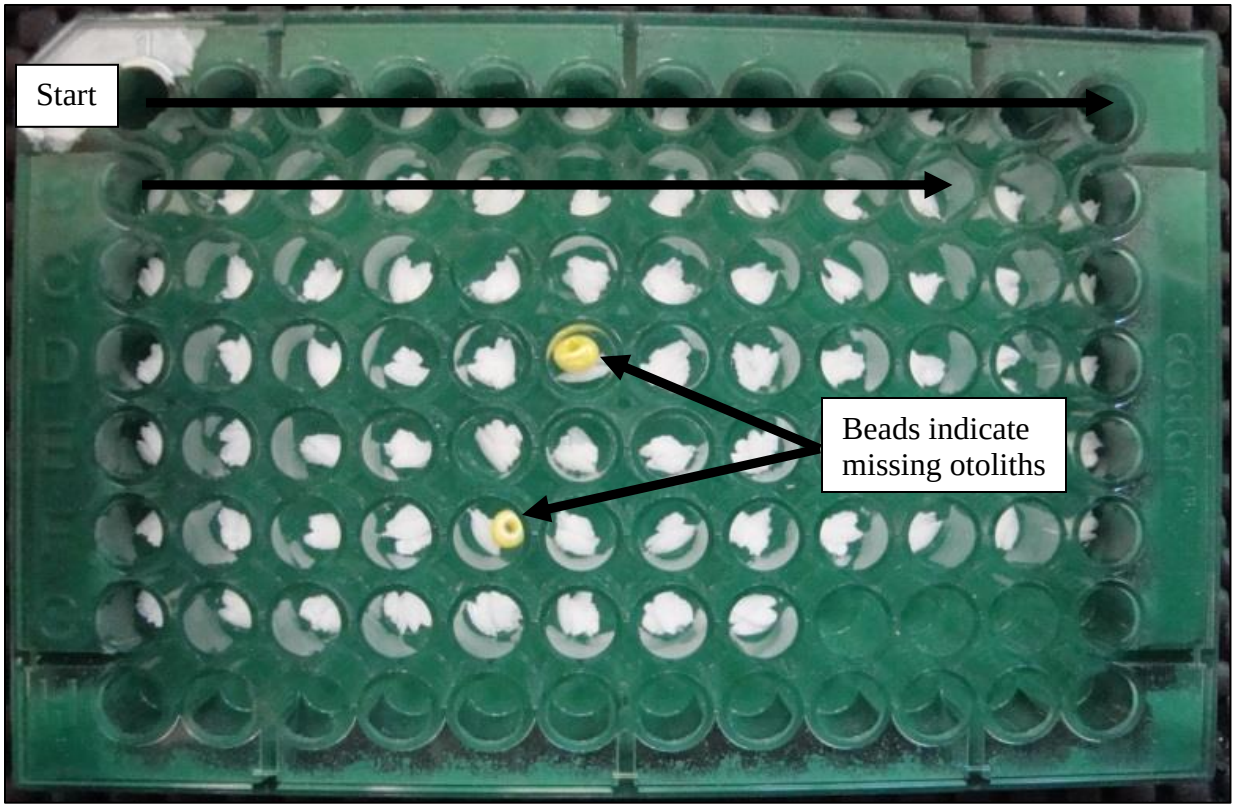


Figure 4.– Illustration of otolith placement in a tray from a stream sampling site. The tray is positioned so that the white, painted notch is in the upper left corner. Raised letters are visible on the left side of the tray; numbers are viewable across the top of the tray. Otoliths are added left to right by rows. Thus, samplers fill the first well with an otolith pair (A1, then A2). Beads indicate missing otoliths.

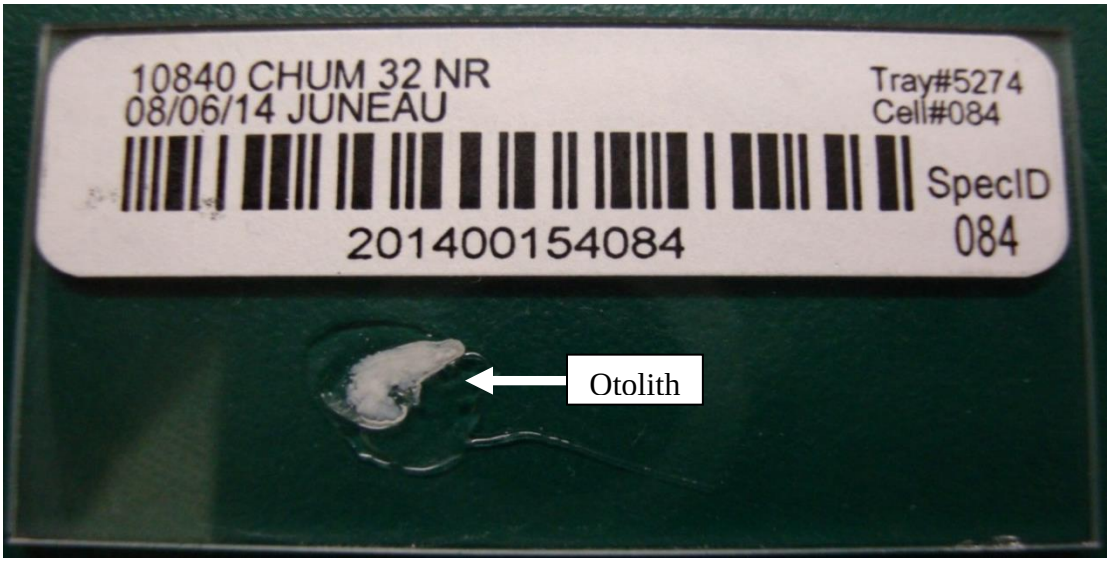


Figure 5.– Example of a petrographic glass slide (1 x 2 in) labeled with a unique bar code. Slide shows a left otolith mounted to the back using thermoplastic cement. Information includes: sub-district (108-40), statistical week (32), sample date (8/6/14), species (chum), tray number (5274), cell number (084), sample number (201400154), and specimen number (084).

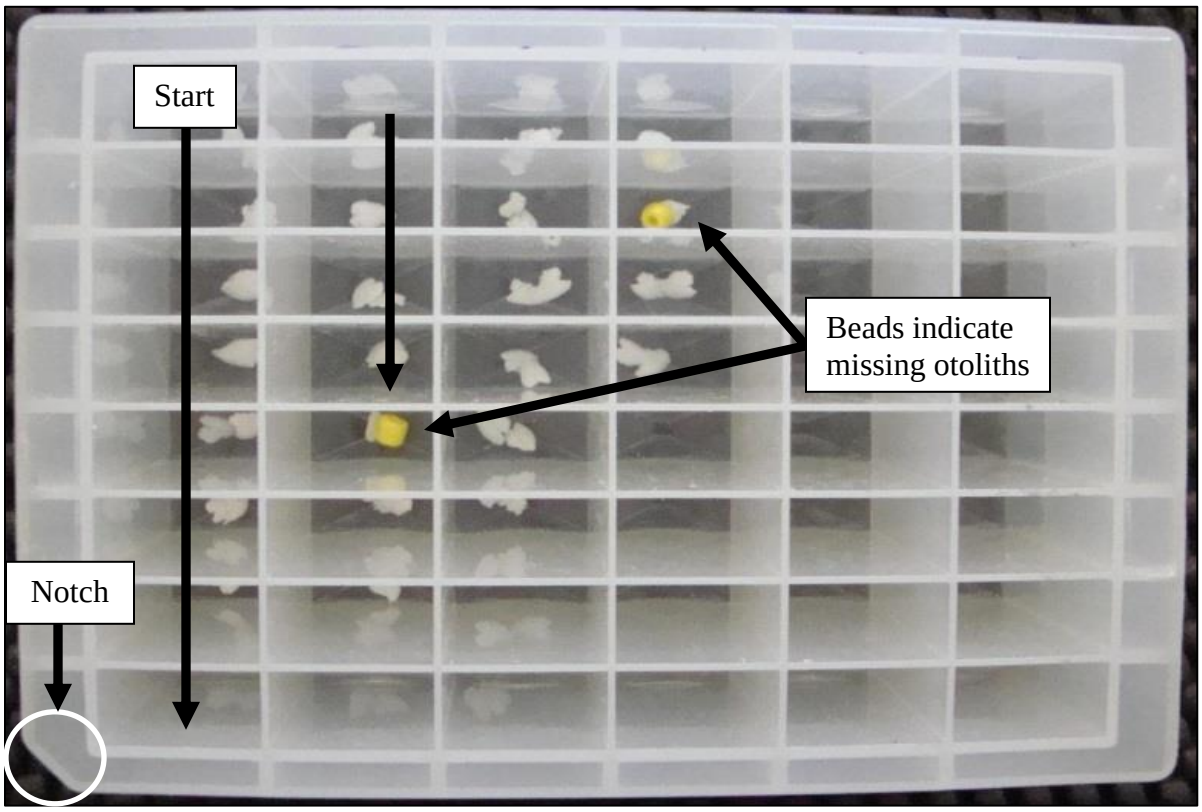


Figure 6.– Otolith location in a deep-well plate used to collect pedigree stream samples. The tray is positioned so that the notch is in the lower left corner. Otoliths are placed top to bottom by columns. Thus, the first otolith is in the upper left well, and second otolith is in the well below the first otolith. Beads indicate missing otoliths.

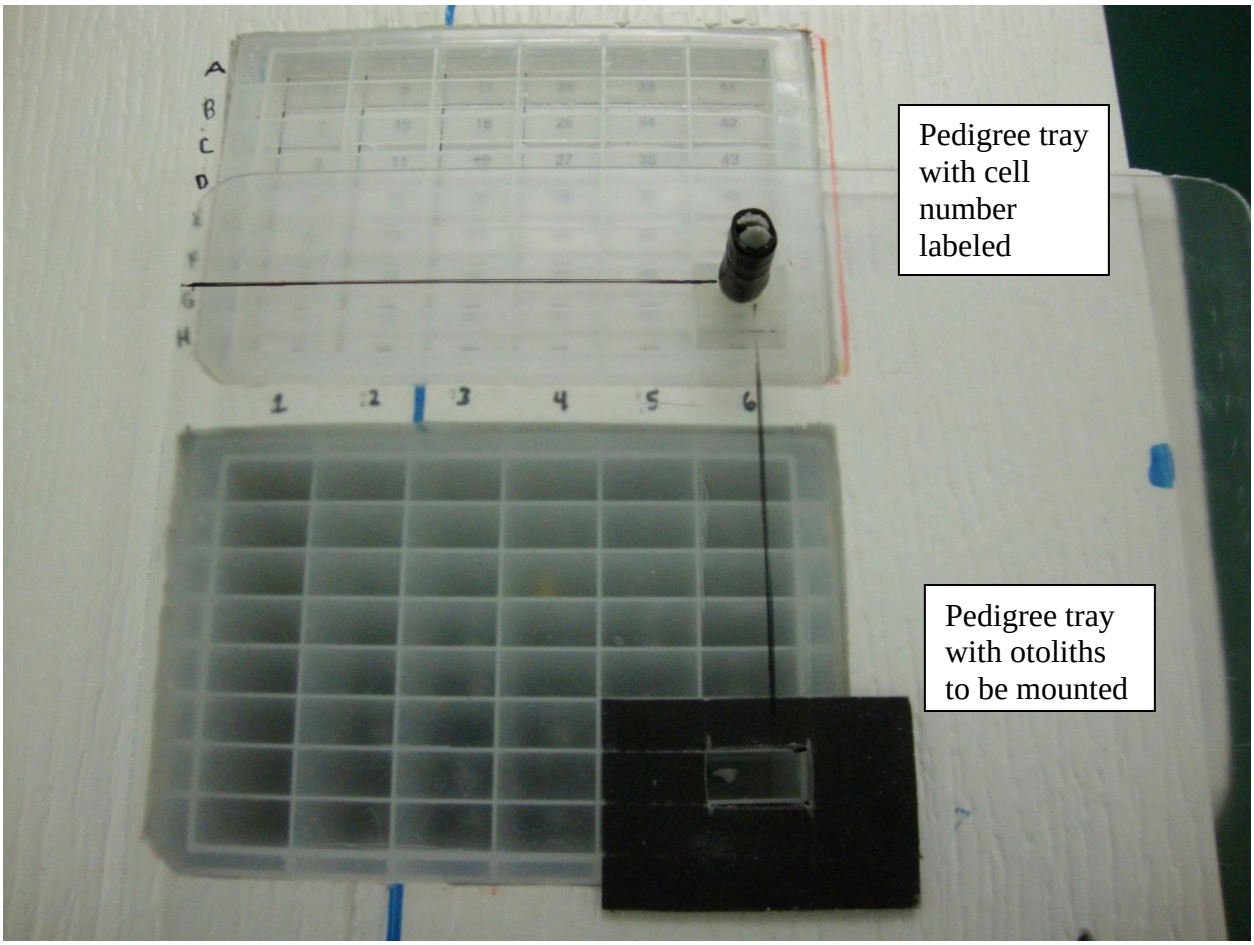


Figure 7.– Mounting apparatus for pedigree stream deep-well plates to ensure the correct otolith is selected. The apparatus permits only one cell number and well position open at a time.

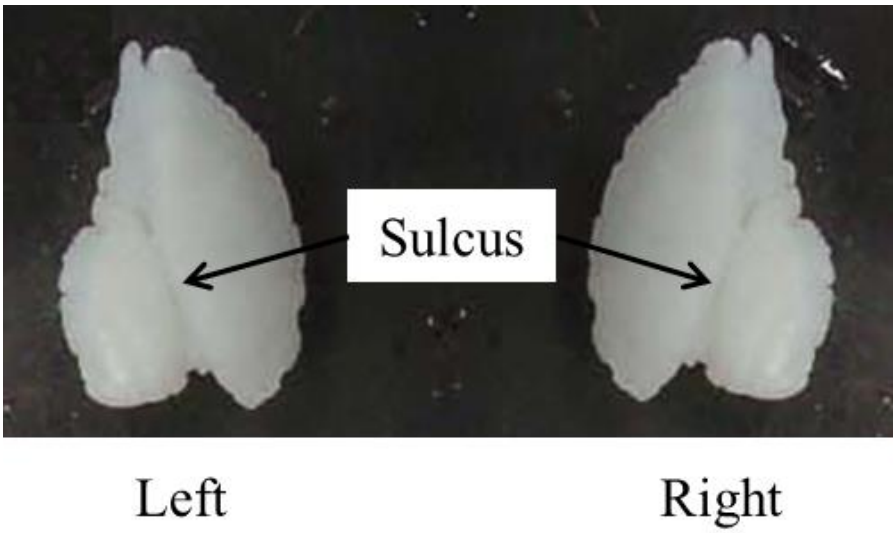


Figure 8.– Left and right sagittal otoliths, sulcus side up.

Appendix A. Tissue Transfer Protocol for 48 Deep-Well Plates

Setup:

1. Mark each original 48 well plate mat using solvent resistant marker with information from the plate: (a) project name and (b) plate number. Label a duplicate plate with an identical barcode label.
2. Mark position 1 (A1) of the original mat with a marker, so mat is returned to original plate with the same orientation after the transfer is complete.
3. Ensure you have a clean split-mat cover for each plate (Figure 1).

Transfer:

1. Remove mat from original plate and set aside.
2. Use the 48-well-plate-transfer guide to set up the original and duplicate plates (Figure 1)
    a. Guide will automatically orient both plates with the notch key.
    b. Have a colleague double-check that the labels match.
3. Position the sliding white cover with the rectangular opening over position 1 (A1): the guide will automatically position on A1 in the corresponding plate. Cover columns 3–6 on both plates with the split-mat cover.
4. Proceed with transferring the genetic tissue to its corresponding well in the duplicate plate
    a. Visually confirm that an otolith is not stuck to the genetic tissue. If not sure, gently rinse the tissue with ethanol over the original well before depositing tissue into the duplicate plate.
5. Continue transferring each genetic tissue, repositioning the sliding white cover over each well to ensure accuracy of transfer, moving down A1, B1, C1, etc. before proceeding to the next column. (See Figure 2 for example of E1 setup).
6. For each well in row H, the sliding white cover will need to be flipped so that the cover's keys fit into row G wells. These keys keep the guide from sliding.
7. Continue transfer proceeding down and over columns 2–6, repositioning the split-mat covers on either side of the active columns until the plate is complete. (See Figure 3 for example of C3 setup)
8. Replace mats on both duplicate and original plate (in the same orientation as before

Figure 1. 48-well-plate-transfer-guide

Figure 2. Example setup for tissue transfer from well El.

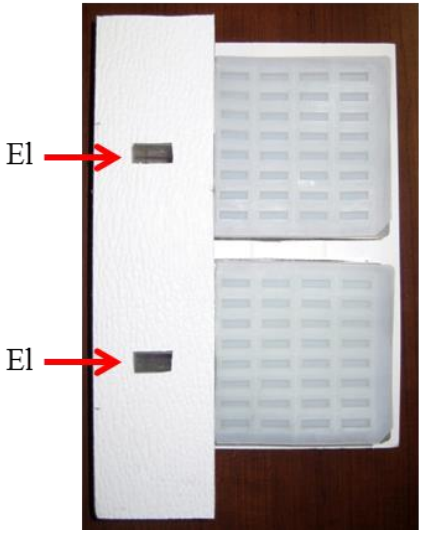
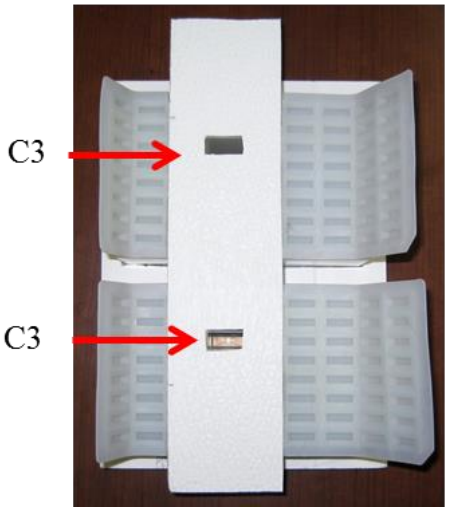


Figure 3. Example setup for tissue transfer from well C3.



APPENDIX D: QA/QC PROCEDURES

Appendix D1.–Thermal mark recovery data quality assurance and quality control procedures by the ADF&G Mark, Tag, and Age Laboratory

Alaska Hatchery Research Program **Technical Document:**[1]

Title: Thermal Mark Recovery Data Quality Assurance and Quality Control Procedures by the ADF&G Mark, Tag and Age Laboratory **Version:** 1.0
Authors: Agler, B., L. Wilson, and M. Lovejoy
Date: May 25, 2017

Abstract

Origin of Pacific salmon (*Oncorhynchus* spp.) sampled for the Alaska Hatchery Research Program can be determined by examining otoliths (ear stones) for thermal marks. Thermal mark presence indicates that a fish originated from a hatchery; whereas, thermal mark absence indicates wild origin. Identification of such marks provides information about a fish's age, hatchery of origin, and release location. The Mark, Tag and Age Lab, Alaska Department of Fish and Game is responsible for conducting mark recovery operations for a variety of statewide management and research projects. Thermal-marked fish typically are not given a secondary mark, so multiple readings among readers and across geographic areas are used to estimate reader ability to detect a thermal mark and to calculate agreement of thermal mark identifications. Thus, we compare first and second reads with an agreement matrix to determine whether there are any significant problems in reader training or challenging marks that might be re-examined. We then use the *kappa* statistic to examine overall agreement between readers as well as agreement by specific thermal mark. At the end of each project, we estimate the error rates of each reader using latent class models, because although useful, *kappa* statistics are influenced by the true proportion of marked fish. Analyzing the thermal mark read results in this manner provides a method to ensure quality control among projects and a measure of accuracy of thermal mark recoveries of fish sampled for the Alaska Hatchery Research Program.

Background of AHRP

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 large 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 exists. To address these concerns ADF&G convened a Science Panel for the

[1] 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

Alaska Hatchery Research Program (AHRP) whose members have broad experience in salmon 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

An important consideration in fisheries management is the ability to identify the origins of captured and harvested fish. The development of mass-marking techniques, such as thermal manipulation of water temperature to mark otoliths, permits millions of hatchery-incubated juvenile salmon to be marked simultaneously. These techniques have successfully applied species-specific, thermal mark patterns to otoliths (ear stones) of hatchery-reared salmon throughout Alaska and the Pacific Rim over the past 26 years (Hagen et al. 1995; Volk et al. 1990). For the AHRP, accurate mark interpretation is vital to the assessment of stray rates associated with hatchery-reared salmon and provides validation of genetic stock identifications.

There are many potential sources of error in any research project, and the extent that these errors can be minimized increases confidence in a study's findings and conclusions. There are two categories of reliability with respect to data collectors: reliability across multiple data collectors, or *inter-rater* reliability, and reliability of a single data collector, or *intra-rater* reliability. Presented with the same situation and phenomenon every time, the assumption is that a laboratory staff would react the same way every time; however, Gwet (2014) provided examples of where this was false and affected intra-rater reliability. Reader reliability is affected by the fineness of discriminations required by the samples. If a variable only has two possible states, and the states are sharply differentiated, reliability is likely to be high. For example, if the outcome variable is that a fish either survived or did not, or the otolith is marked or not marked, there is likely to be high reliability in data comparisons between readers. On the other hand, if readers are required to make judgements or determinations regarding the width and amount of thermal mark rings, both inter- and intra-rater reliability declines. Careful training of laboratory staff is critical to reader reliability.

To determine the presence or absence of a thermal mark in an otolith, laboratory staff use pattern recognition and image matching (Blick and Hagen 1998). Although hatcheries follow strict rearing protocols to produce consistent thermal marks, natural variation in otolith development and growth patterns can obscure these patterns and interfere with the ability to detect a mark, reducing mark identification. In addition, stress on fish at the hatchery caused by temperature

fluctuations, water quality, rearing density, noise and light fluctuations, lot size, maintenance procedures, and handling protocols can affect mark consistency and clarity (Hagen et al. 1995**)**.

Examination of accuracy rates of thermal mark identifications, including correct assignment of age, hatchery, and release site, provide useful knowledge regarding reliability of mark recoveries to assess stray rates and validate genetic stock identifications. However, otolith thermal marks are typically applied without a secondary mark, such as a coded-wire tag or a passive integrated transponder (PIT) tag, thus there is no reliable method to assess the true accuracy of thermal mark presence and identification. Consequently, the Mark, Tag, and Age (MTA) Lab uses latent class models (LCMs) to estimate a reader's ability to distinguish between hatchery and wild fish. In addition, *kappa* statistics (Cohen 1960) are used to assess reader agreement among individual mark patterns. Agreement matrices combined with the *kappa* statistic assist in identifying problematic mark patterns.

Goal

Our goal is to describe the methods used by the MTA Lab in Juneau, Alaska to find errors in thermal mark classification and correct them. We describe the methods used to assess the accuracy of reader's ability to correctly ascertain presence and absence of a thermal mark and to identify specific mark patterns.

Methods

Hatcheries apply thermal marks to incubating salmon eggs and fry by raising and lowering the water temperature at set intervals. Cycling temperature, or thermal marking, leaves patterns of optically-dense rings in the otolith (Volk et al. 1990). A thermal mark consists of rings, which are optically dark circles visible in the otolith and bands, which consist of one or more rings separated by a space from other rings (Figure 1). We describe the thermal mark with a specialized notation termed the "hatch code" (Josephson et al. 2006). For example, a hatch code of 4,2,2H describes a set of three bands: the first band is composed of four rings, the second band includes two rings, and the last band contains two rings. The capital "H" indicates the mark was applied before hatching. In this example, all three bands occur prior to the hatch mark (Figure 1). Varying the number and spacing of the induced rings produces unique patterns used to distinguish among similarly treated hatchery fish and wild stock (Hagen et al. 1995).

Thermal mark reference collection

Initially, laboratory personnel are trained to dissect, prepare, and process otoliths from reference specimens or representative samples of salmon eggs, fry, and smolt obtained from the hatchery and preserved in alcohol before release. Upon receipt, five otoliths from each sample are dissected, mounted to glass slides (see "Otolith mounting" section of AHRP MTA Processing Tech Doc 7), and examined with a compound microscope. These reference specimens become the standard or authoritative mark pattern for that thermal mark after laboratory staff compare the observed marked with the assigned mark. Laboratory staff then measures the specimens,

because mark locations and ring spacing can vary among individuals of the same thermal mark group due to variability of fry developmental stage during marking. When a thermal mark is applied during different developmental stages, the distance from the core of the otolith to the initial band varies among fish making the mark challenging to identify. Successful thermal mark application at hatcheries is the first step to correct determination of fish origin and is fundamental to the success of this project.

Thermal marks are described in the Mark Characteristic Report (available online – see below). This report includes: brood year, release year, thermal mark identification, species, brood stock, release site(s), assigned (target) mark, actual mark observed, mark quality assessments, number of samples received, measurements of the otolith, information about temperature profile (if available), various comments, and an authoritative image of the mark as well as images of any variants of the mark. Measurements include minimum, maximum, and average distance (µm) from the core of the otolith to the first band; minimum, maximum and average width of each band; and distance among bands (Figure 2). The authoritative image, which represents the mark pattern observed in the majority of voucher samples, is annotated with measurements and a comment about the thermal mark (Figure 1). Occasionally, thermal marking procedures can produce errant mark patterns or multiple pattern variants of the planned mark (Figure 3). When this occurs, images of these mark variants are included in the reference collection. The Mark Characteristic Report and the thermal mark reference collection are both available online:

http://www.taglab.org/OTO/reports/VoucherSummary.asp

Reader Training

Prior to each field season, laboratory staff (or "readers") gain familiarity with the thermal mark patterns likely to appear in AHRP samples by studying the physical and online reference collection of marked otoliths maintained at the MTA Lab. Familiarization with thermal mark patterns is important because growth rings in otoliths of wild salmon can occasionally appear to be similar to marks create during the thermal marking process. This review of known marks helps to minimize the chance of labeling an otolith as marked when it is actually wild as well as helps to increase reader accuracy and precision with regards to mark identification.

Laboratory personnel are trained to process adult otoliths using surplus otoliths to practice grinding to visually enhance the core or the "primordia" of the otolith. Staff learns to reduce processing time by controlling the pressure exerted during grinding and by becoming familiar with variations in otolith patterns and shapes. After approximately two to four weeks of training, laboratory staff begins to examine samples containing a mixture of marked and unmarked otoliths. Experienced personnel work with new staff members until their reader agreement is at least 95%.

First and Second Reads

All chum salmon (*O. keta*) otoliths are examined twice. In other words, these samples are read independently by a first reader and then read a second time by a different reader. The second reader typically knows who read the first sample but has no knowledge of the previous read results. Thus, we consider these to be a blind second read. The AHRP stream and pedigree samples are stratified into four areas (Figure 4). Disagreements between first and second readers are resolved by a third reader examining the otolith. The third read is not independent. The third reader knows who conducted both first and second reads and is cognizant of the results of each read. Second reads are performed as first reads are completed, and readers review the results. If disagreements occur, these are discussed, increasing familiarity with challenging patterns.

Study Design

Samples are assigned to readers by sample location (area) and over time. The MTA Lab currently uses four readers, thus there are six reader-pair combinations, which is critical for data analysis using a latent class model (see below). For the AHRP, the stream strata include four geographic areas in Southeast Alaska (Figure 4). Four streams were chosen for the pedigree sites, and each pedigree stream is treated as one stratum.

Read Assessment Methods

The MTA Lab uses three methods to assess a reader's ability to determine the presence or absence of a thermal mark. These methods include two agreement measures (agreement matrix and *Kappa*) and a latent class model, part of a family of models that allow estimation of reader classification error through the use of spatial data and multiple independent readings.

1) Agreement Matrices

As otoliths are examined, a preliminary review of results is conducted by cross-tabulating the first read and second read results (Table 1). Common in reliability studies (Blick and Hagen 1998), this matrix highlights results to review in detail. The matrix also highlights thermal marks that are mistakenly termed wild fish, as well as thermal mark identifications with a high percentage of disagreement. The first reader's results are listed on the rows, while the second reader's results are listed in the columns. Table 1 shows the number of thermal marked fish as well as the number not marked (e.g. wild) and unreadable. The numbers on the diagonal between the rows and columns indicates the number of thermal marks upon which the two readers agreed. Numbers off the diagonal highlight the disagreements (Table 1). For example, reader one and two agreed that 34 otoliths were TM3, but reader one called two otoliths TM4 and reader two labeled them TM3. Discrepancies in whether the otoliths are marked or unmarked are located on the edge of the matrix, and differences in readability may also be found by examining the matrix. For example, six otoliths were labeled TM4 by reader one but were called "wild" by reader two, and three otolith were called wild by reader one but labeled TM3 by reader two. Examination of the matrix provides a preliminary analysis during a project and allows biologists to target areas for review. Deviations from the diagonal are reviewed, and

sometimes otoliths are read a third time to ensure consistency. This matrix has been a useful tool for highlighting when a reader missed a mark. Often such errors are caused by incorrect sample preparations. If an otolith is not ground enough, the thermal mark will not be visible. In such cases, the sample is simply ground some more until the core is visible. Conversely, if an otolith is ground too much, the mark will be removed. In this instance, the other otolith can be prepared for mark recovery since both left and right otoliths will exhibit a thermal mark.

2) Latent Class Model

Latent class models (LCMs) provide an alternative approach to estimating agreement (Hui and Walter 1980). LCMs incorporate an estimate of reader classification error, so that the variability of reader agreement may be estimated. These models hypothesize the existence of unobservable (i.e. "latent") variables about which information can only be obtained through measurements on observable (i.e. "manifest") variables (Blick and Hagen 1998). LCMs use categorical variables for the latent and manifest variables. For the AHRP, the latent variable is whether an otolith is hatchery or wild; whereas, the manifest variables are a reader's classifications. Because the true error rate for each reader is unknown, latent class models provide a method to assess the accuracy of thermal mark results. Blick and Hagen (1998) demonstrated that LCMs could be successfully applied to thermal mark results by setting additional constraints or collecting additional information.

The most economical LCM method is to separate the study area into strata and use two readers. Use of three or more readers would give more degrees of freedom (*df*) and improve model results, but the cost of the project would increase. Maximum likelihood models are the preferred method for estimating LCMs. Assuming readings are independent among readers and among otoliths, the likelihood function is as follows:

$$\prod_{i=H,W}\prod_{j=H,W}\prod_{k=H,W}\left\{p\pi_{i|H}^{(1)}\pi_{j|H}^{(2)}\pi_{k|H}^{(3)}+(1-p)\pi_{i|W}^{(1)}\pi_{j|W}^{(2)}\pi_{k|W}^{(3)}\right\}^{n_{ijk}}$$

where

H = hatchery (thermal marked)

W = wild (unmarked)

n = sample size

$\pi_{i|j}{}^{(k)}$ = probability that reader k classifies an otolith as i when its true state is j

p = proportion of hatchery fish

The likelihood functions used to estimate the above parameters are maximized using Solver in Microsoft Excel. Standard errors are estimated using the jackknife method (Haddon 2001).

When there are only two readers, neither is a standard, and there are five parameters to estimate $\pi_{H|H}^{(1)}$, $\pi_{H|H}^{(2)}$, $\pi_{W|W}^{(1)}$, $\pi_{W|W}^{(2)}$ and p, which gives only three *df* (four data points – one due to fixed sample size, n). To prevent overparameterization, constraints on the parameters or more data are needed. Possible constraints include: 1) considering two parameters as known (e.g.; $\pi_{W|W}^{(1)} = \pi_{W|W}^{(2)} = 1$, both readers will call a wild stock correctly); or 2) considering two sets of parameters equal (e.g.; $\pi_{H|H}^{(1)} = \pi_{H|H}^{(2)} = \pi_{W|W}^{(1)} = \pi_{W|W}^{(2)}$, the accuracy rates are the same for both readers). These constraints are likely unrealistic, thus more data are necessary. One way to generate more information is to have a third independent reader (Walter 1984). Three readers provide seven parameters: $\pi_{H|H}^{(1)(2)(3)}$, $\pi_{W|W}^{(1)(2)(3)}$, and p, thus there are $2^3 - 1 = 7$ *df*, so all parameters may be estimated. On the other hand, adding a third reader is usually logistically unfeasible given the financial constraints of a project.

Hui and Walter (1980) proposed an alternative method to generate information. They suggested that if there are two or more strata with different hatchery proportions in each strata (Blick and Hagen 1998), then reader results could be stratified temporally or spatially. We can then assume that $\pi_{H|H}^{(k)}$ and $\pi_{W|W}^{(k)}$ remains constant across strata (Blick and Hagen 1998), reducing model parameters to eight with 12 *df*. Thus, a two reader – four strata model would have 4 *df* extra for goodness-of-fit, preventing overparameterization of the model.

The following is the likelihood function for the two independent reads with S strata (Hui and Walter 1980):

$$\prod_{g=1}^{S} \prod_{i=H,W} \prod_{j=H,W} \left\{ p_g \pi_{i|H}^{(1)} \pi_{j|H}^{(2)} + (1-p) \pi_{i|W}^{(1)} \pi_{j|W}^{(2)} \right\}^{n_{gij}}$$

To estimate the latent variable for each reader, the stream samples collected during the AHRP project were separated into four spatial strata (Figure 4). These spatial strata included: (1) Southern Southeast waters; (2) Lynn Canal and Stephens Passage; (3) Chatham and Icy Straits; and (4) Northern Outside waters. Samples were apportioned fairly equally across area. In addition, these areas provided both geographic coverage and geospatial separation. Pedigree samples were separated into strata based on the four creeks used in the project: Fish, Prospect, Admiralty, and Sawmill creeks. Care was taken to distribute readings evenly among readers, across areas, and by time. Samples were distributed among readers equally because we have observed that when the LCM was heavily weighted by one individual, it performed poorly.

We have also observed that "reader drift" can occur over time as readers observe more marks and sometimes altered their initial perception of a mark pattern (intra-rater reliability). To ensure that the LCM analyses included this potential scenario, we assigned readers samples from across the entire study period.

A critical assumption for both the LCM estimates of reader ability to detect a mark and *kappa* agreement values (see below) is that readings are independent, meaning that the reading of each otolith by a reader is independent of any other reading by the same reader and independent of readings by other readers for a given otolith. To support these assumptions, otolith first and second reads are provided to readers in random order by box. Another assumption is that individual accuracy rates are known to be greater than the error rates (Blick and Hagen 1998). Historically, reader agreement associated with mark recoveries conducted during the commercial sockeye fishery exceed 95%, so we believe this assumption is likely valid for the MTA Lab.

3) Kappa

The *kappa* statistic (Fleiss 1981) is frequently used to test inter-rater reliability. Rater reliability represents the extent to which the data collected in a study represent the variables measured. The *kappa* statistic provides examination of overall agreement between readers as well as agreement by specific thermal mark and an associated standard error (Fleiss 1981). Individual *kappa* statistics can be calculated for each category and pooled from different trials. Traditionally, inter-rater reliability was measured as percent agreement, calculated as the number of agreement scores divided by the total number of scores. Cohen (1960) critiqued the use of percent agreement due to its inability to account for chance, thus percent agreement tends to be higher when a category being rated has a high probability of occurrence. He introduced the Cohen's *kappa* (1960), which is chance corrected or accounts for the possibility that raters guess on some variables due to uncertainty.

Kappa is calculated by correcting the observed agreement for the degree of agreement expected by chance alone ($P_O = (n_{HH} + n_{WW})/n$). Overall *kappa* is weighted and is defined as:

$$\hat{\kappa}_w = \frac{P_o - P_e}{1 - P_e} \tag{3}$$

where P_e is the proportion of expected agreement = $(n_H n_H + n_W n_W)/n^2$ (Cohen 1960; Blick and Hagen 1998; Fleiss 1981). The weighted version of *kappa* has the same properties discussed above, but it is adjusted by giving lower weight to disagreements over marks with small numbers and full weight to disagreements over marks where agreement is high (Hagen et al. 1995). This better reflects agreement on what is marked and unmarked and reduces the influence of mark identifications with only one or two otoliths. Overall $\hat{\kappa}$, which assesses overall agreement between readers, is a weighted average of individual $\hat{\kappa}$ for each individual thermal mark identified and is equal to the sum of the individual p_0 - p_e (i.e., the sum of the numerators of the individual $\hat{\kappa}$) divided by the sum of the individual 1 - p_e differences (i.e., the sum of the denominators of individual $\hat{\kappa}$, Fleiss 1981).

The standard error for $\hat{\kappa}_w$ is estimated by:

$$SE(\hat{\kappa}_w) = \frac{\sqrt{A+B-C}}{(1-p_e)\sqrt{n}} \quad (4)$$

where

$$A = \sum_{i=1}^{n} p_{ij}\left[1 - \left(p_i + p_j\right) + (1 - \hat{\kappa}_w)\right]^2, \quad (5)$$

$$B = (1 - \hat{\kappa}_w)^2 \sum\sum p_{ij}(p_i + p_j)^2 , \quad (6)$$

and

$$C = [\hat{\kappa}_w - p_e(1 - \hat{\kappa}_w)]^2 \quad (7)$$

for readers *i* and *j* who have read *n* samples.

Although *kappa* is a commonly used inter-rater reliability statistical test, it has limitations. Judgments about what level of *kappa* is acceptable are often questioned. As in most correlation statistics, *kappa* values range from -1 to +1, where $\hat{\kappa}_w$ = 1 indicates complete agreement and $\hat{\kappa}_w$ = -1 indicates complete disagreement. If observed agreement is greater than or equal to chance agreement, $\hat{\kappa}_w \geq 0$, and if observed agreement is less than or equal to chance alone, $\hat{\kappa}_w \leq 0$ (Landis and Koch 1977). Landis and Koch (1977) suggested that $\hat{\kappa}_w > 0.61$ indicates substantial agreement beyond chance. Values between 0.41 and 0.60 represent moderate agreement, and $\hat{\kappa}_w < 0.40$ represent slight to poor agreement (Landis and Koch 1977). Although Landis and Koch (1977) interpreted a *kappa* score of 0.41 as acceptable, this might be considered too lenient for a project like AHRP.

At the MTA Lab, we use *kappa* to ascertain amount of agreement among marks between readers. Overall *kappa* among a suite of marks can be high (>0.80), but sometimes *kappa* scores for individual marks can be low (<0.50). This occurs for a variety of reasons: 1) the mark was rarely observed in a sample, usually older-aged fish; 2) otoliths were over- or underground; 3) mark application was incomplete or differed among incubation groups, causing recovering to be challenging; and 4) duplication of mark patterns among brood years required that otoliths be aged to differentiate between years. Once we have determined why errors occurred, we determine whether a higher proportion of the sample need to be second read or whether we need to have some samples re-examined to determine whether marks were missed (i.e.; mount right side of otolith and examine for thermal mark by a third reader). In the last instance, we work with staff to improve thermal mark identification proficiency.

Thermal marks with poor *kappa* values are examined and discussed among readers during each year of the project. They are also targeted for study prior to each project year. If a sample has a poor overall *kappa* value, then those otoliths are examined further to determine the cause (i.e.;

multiple poor marks or a sample coordination errors). *Kappa* values are archived on the local network.

Because *Kappa* is an index, it is important to remember that interpretation can be affected by the values of the underlying parameters (Blick and Hagen 1998). Thus, direct comparison of $\hat{\kappa}$ across populations with different underlying proportions is not appropriate. Although agreement measures may be subject to some ambiguity, they are useful in monitoring results for potential errors and pinpointing areas for the Lab to re-examine.

Discussion

Fisheries research often requires that trained individuals classify data according to a strict but somewhat subjective set of rules. In many situations, there is no standard available with which to confirm classifications, and it is necessary to apply some other method to determine the accuracy of the determinations. Distinguishing thermal-marked fish from wild fish is a good example of this type of problem because: 1) most thermal-marked salmon do not receive a secondary mark, so cross-validation is not possible; and 2) the ability to read otoliths for thermal mark presence and identification requires training and experience because natural variation in growth rings observed in chum salmon otoliths can appear similar to thermal mark patterns. In the absence of samples of known origin, it is common to collect multiple, independent observations of the same samples and assume that percent agreement among readers serves as a proxy for read accuracy. Agreement indices (matrices and *kappa*) are easy to compute and indicate read discrepancies in mark recovery and identifications**.** For the AHRP project, these QA/QC methods provide additional direction for validation of reader accuracy and precision. They also provide some quantitative indication of reader accuracy.

In addition, we use the agreement measures described above to highlight results in need of closer examination and suggest potential areas for critical review. When agreement measures indicate that results require evaluation, we examine the data to determine whether we need to: 1) conduct additional reader training when an individual is under- or over-grinding and missing marks, 2) read samples a third time by another independent reader when marks are especially difficult to discern, and 3) examine potential issues in greater detail during the next season's training period if a particular mark or brood year is expected to return.

Although these indices are fairly easy to calculate and are useful indicators of reading problems, it is important to remember that some of these indices are not directly comparable. It is difficult to compare *kappa* statistics across populations with different underlying proportions. Because of this, even when a suite of *kappas* is consistent, it may not be clear how reader agreement/disagreement influences the contribution estimate. In addition, these indices do not provide inferences about the relative ability of one reader over another to determine a particular set of patterns. Latent class models, however, provide readily interpretable qualities that can be easily calculated. Classification accuracies or errors provide direct, meaningful parameters,

unlike the use of an index of agreement alone. In addition, LCMs provide estimates of hatchery proportions (*p*).

We feel that the procedures described above provide a combination of approaches to provide a comprehensive examination of error rates and accuracy of reads conducted in the MTA Lab. The matrices and *kappa* statistics point out areas for review, and the LCM provides direct, meaningful parameters that can be compared from year-to-year.

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Figures

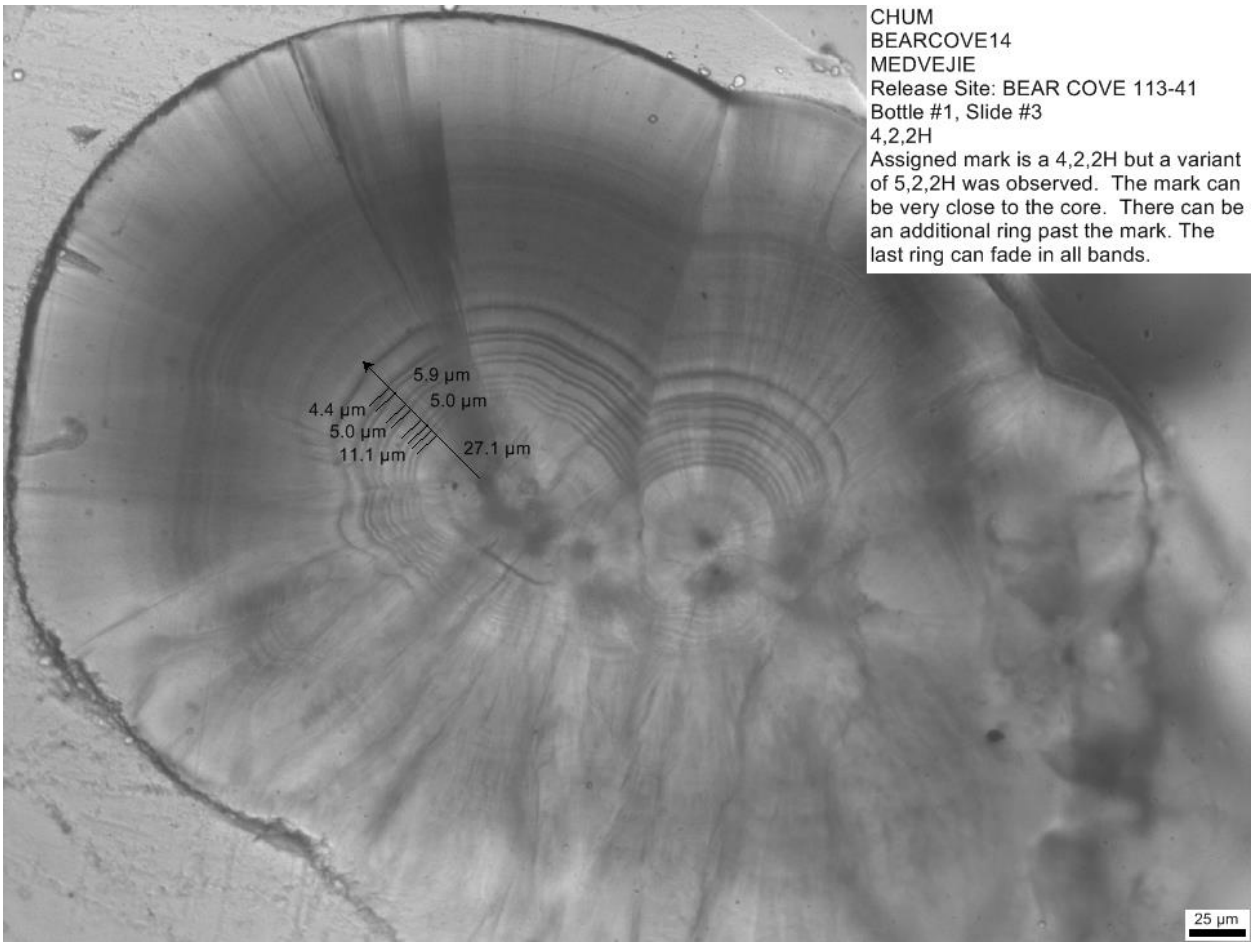


Figure 1. Image of a thermal mark reference specimen. From Medvejie Hatchery, this brood year 2014 mark (BearCove14) has a hatch code of 4,2,2H. The code indicates that the first band from the otolith's core contains four dark rings, then there is a space, followed by a band with two rings, followed by another space and a final band with two rings prior to the hatch mark (blurry, wide, dark area). Annotated measurements on the transect line include distance from otolith core (primordia) to first band, width of first band, space between first and second bands, and average distance between rings in each band. All thermal mark images are available online through the North Pacific Anadromous Fish Commission (NPAFC) Working Group on Salmon Marking (WGOSM) website: http://wgosm.npafc.org/MarkSummary.asp

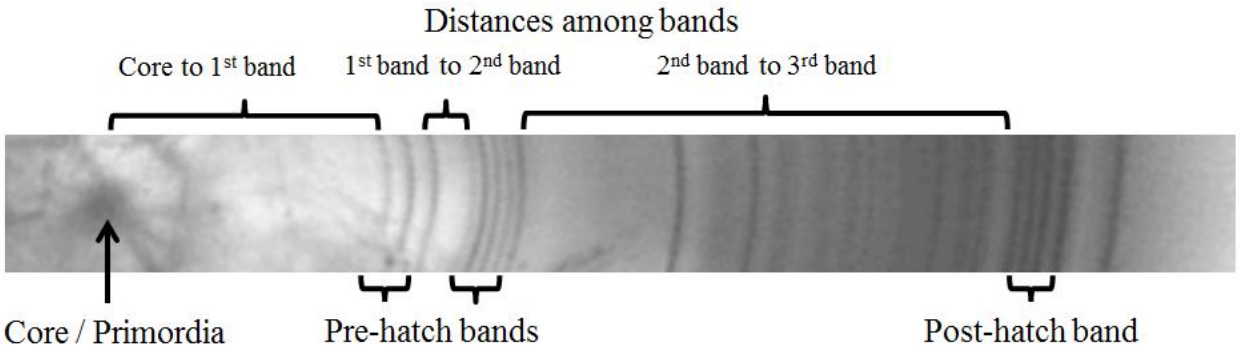


Figure 2. Thermal mark image with measurements shown in the Mark Characteristic Report. This figure shows a 3,5nH4 mark with a pre- and post-hatch mark. Thus this mark has two bands prior to hatch (the first with three rings and the second with 5 rings) and one band after the hatch containing 4 rings. The individual rings are the dark lines in each band, and in the second band, the spacing among the rings is narrower than that in the other bands so the 5 is followed by an "n."

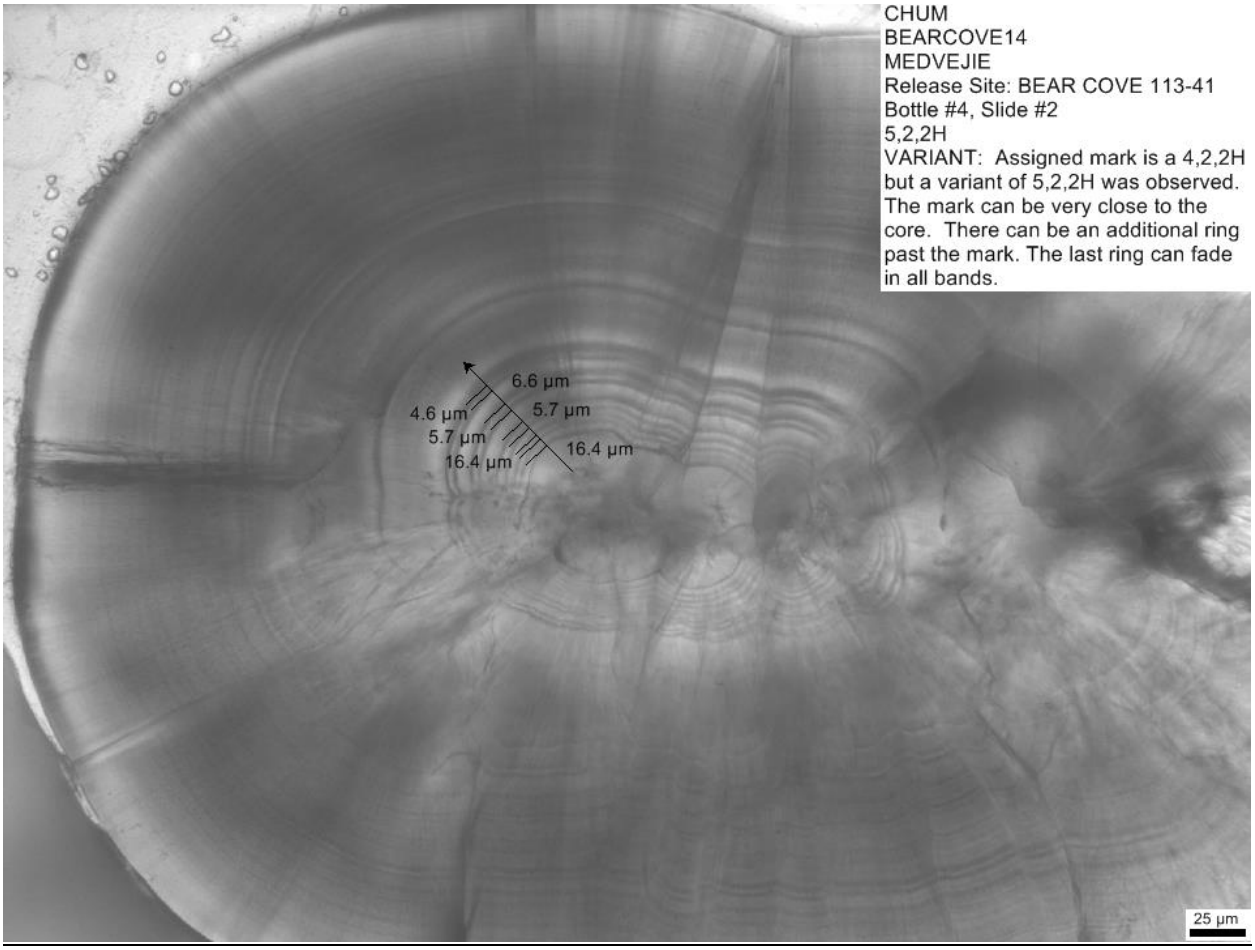


Figure 3. Image of a thermal mark variant of a reference specimen. This figure shows another image of Figure 1, thermal mark ID BearCove14. This fish, assigned a target thermal mark of 4,2,2H, which indicates that the first band from the otolith core contains four dark rings, a space, then a band with two rings, a space, and a band with two rings followed by the hatch mark (the blurry, wider, dark area). Instead, this otolith shows a 5,2,2H or a variant, meaning that the first band has five rings instead of the planned four rings.

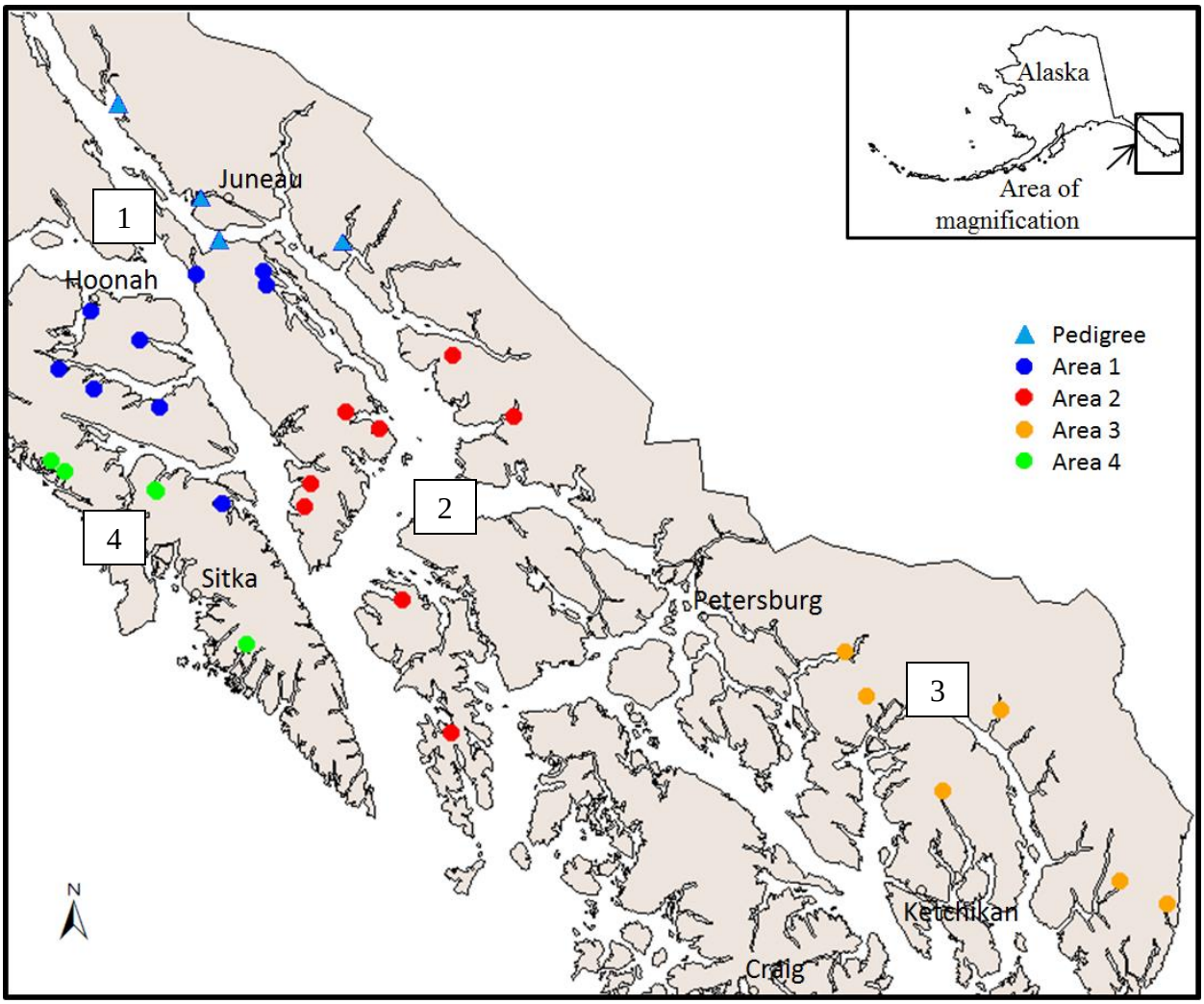


Figure 4. Four strata used for assessing the accuracy of thermal mark readings of chum salmon otoliths recovered from streams in Southeast Alaska during 2013 and 2014 for the Alaska Hatchery Research Project**.**

Tables

Table 1. Example matrix comparing thermal mark reader agreement. Row and column names represent potential thermal marks identified by each reader (TM1 through TM6), otoliths classified as wild, and otoliths classified as unreadable (ND). The number of otoliths where both readers agree is in bold font along the diagonal between the row and columns.

	2nd Reads								
1st Reads	TM 1	TM 2	TM 3	TM 4	TM 5	TM 6	Wild	ND	Total
TM 1	**0**	1							1
TM 2	1	**12**							13
TM 3			**34**						34
TM 4			2	**9**			6		11
TM 5					**26**				26
TM 6						**4**			4
Wild			3				**357**	1	358
ND							1	**3**	4
Total	1	13	36	9	26	4	358	4	451

APPENDIX E. PAIRED GENETIC TISSUE AND OTOLITH SAMPING INSTRUCTIONS AND DATA FORM

Appendix E1.– Genetic tissue sampling instructions.

Adult Finfish Tissue Sampling for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

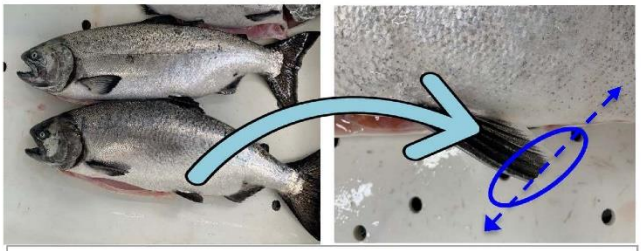
I. General Information

We use fin tissues as a source of DNA to genotype fish. Genotyped fish are used to determine the genetic characteristics of fish stocks or to determine stock compositions of fishery mixtures. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible.

Preservative used: Silica desiccant bead packet dries and preserves tissues for later DNA extraction. Quality DNA preservation requires **Dry storage** in Pelican box with desiccant packs.

II. Sampling Method

Record the Otolith Tray number in the box provided on each Whatman card.



At tip edge, cut small clip of pelvic fin ½-1" max (dotted line) and place on WGC card. One fish only per numbered grid.

III. Sampling Instructions

- **Prior to sampling**: Set up work-space, fill out required collection information on each card, fold back landscape cloth, and place Whatman genetics card (48WGC) on clipboard, secure with clip; ready to sample.
- **Sampling:**
  - Wipe fin prior to sampling.
  - Briefly wipe or rinse scissors between samples reducing cross contamination.
  - Using scissors, cut one fin clip per fish.
  - Place one clipped fin tissue onto # 1 grid space. Follow numerical sampling order (#'s 1-48) printed on card - **do not deviate**. If large tissue sample, center tissue diagonally on grid space.
  - **Only one fin clip per fish into each numbered grid space**.
  - **Staple** each sample to 48WGC (see photo).
  - Sampling complete, fold the landscape cloth "rain fly" over samples to the papers **edge** protecting tissue samples for storage/transport. DO NOT STAPLE landscape cloth closed!
- **Loading the Pelican Case:**
  - First card: Remove blotter papers and desiccant packs (remove vacuum pack plastic) from Pelican Case. Place first card in Pelican Case with tissues facing up. Next, place blotter paper directly over card and place 2 desiccant packs on top. Close and secure lid so drying begins.
  - Up to 4 cards can be added per case. Add them so the tissue samples always face the desiccant pack through blotter paper: 2nd card facing down between desiccant packs; 3rd card facing up between desiccant packs; and 4th card facing down on top of second desiccant pack. Close and secure Pelican Case after inserting each card.
  - All Whatman cards **remain in Pelican 1150 case** to dry cards flat.
- **Post-sampling storage:** All cards with tissue samples will remain inside of Pelican case or file box with desiccant packs at room temperature for duration of sampling and for return shipment to Anchorage genetics lab. Two desiccant packs are allocated for Pelican case. Be sure to **remove plastic vac wrap** before using desiccant packs for best drying results.
- **Shipping at end of the season:** Pack and seal Pelican case and place inside priority mailer box to accommodate Pelican box and supplies. Tape box shut, fix a return address on box and drop in mail.

IV. Supplies included in sampling kit:

1. Scissors - for cutting a portion of selected fin.
2. Whatman genetics card – holds 48 fish/card.
3. Bostitch stapler – staple secures fin clip to card.
4. Pelican Case - 1st stage of drying/holding card with samples.
5. File box – long term dry storage with desiccant packs for all cards.
6. Desiccant packs – removes moisture from samples.
7. Pre-cut blotter paper – covers full sample card for drying.
8. Shipping box – put sealed Pelican case inside a box.
9. Clipboard – holds Whatman genetics card while sampling.
10. Zip ties – to secure the Pelican case for return shipment.
11. Laminated "return address" labels.
12. Sampling instructions
13. Pencil

V. Shipping: Address the sealed mailer box for return shipment to ADF&G Genetics lab.

Return to ADF&G Anchorage lab: ADF&G – Genetics
333 Raspberry Road
Anchorage, Alaska 99518

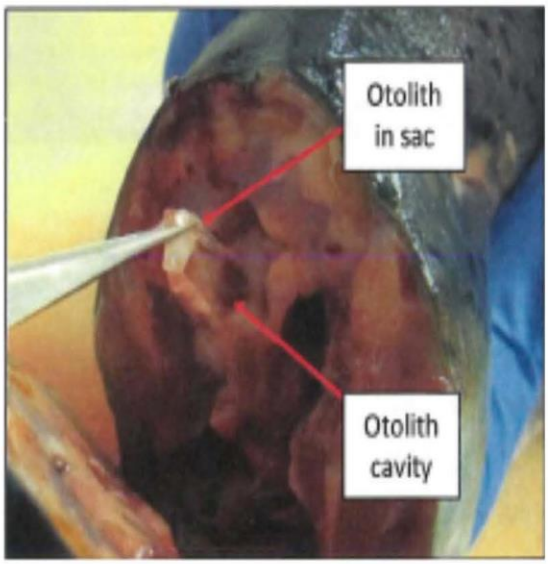
Lab staff: 907-267-2247
Judy Berger: 907-267-2175
Freight code: _____

Appendix E2.– Otolith sampling guide.

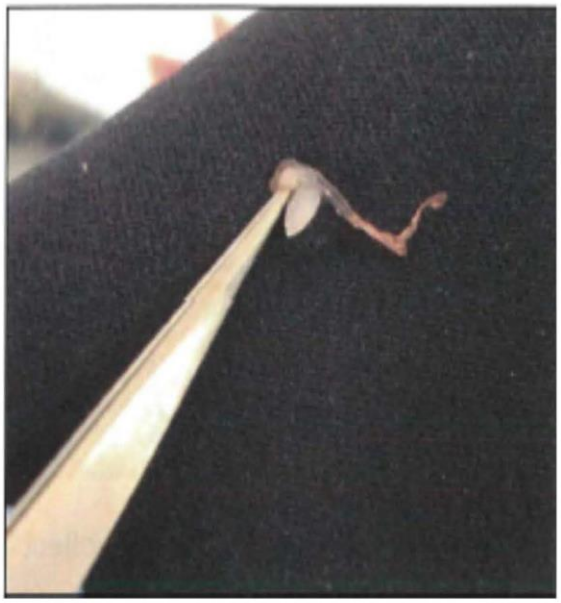
Otolith Sampling Guide

Step 1: Using pencil, fill out the label information located on bottom of an **empty** tray including sample date, location, collector name, and the two Whatman Barcode numbers without leading zeros. Also, write the otolith tray number on the corresponding Whatman card. Each 96-well otolith tray will have samples from two 48-grid Whatman cards (see back page for the tray filling order). Next, set up the tray for sampling by removing the lid and acetate (plastic sheet) located directly under the lid.

Step 2: Using the tweezers, remove both otoliths from brain cavity.

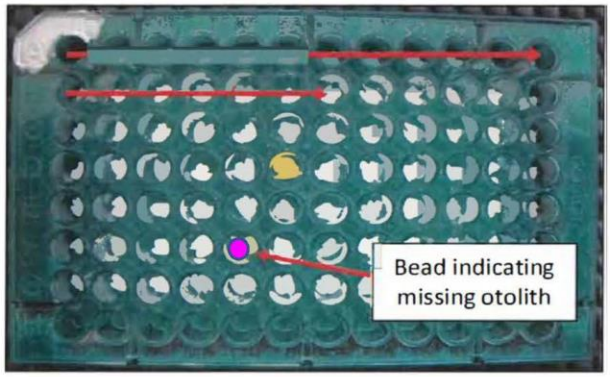


Step 3: Hold otoliths with forceps and drag across textured surface like a glove to remove tissue and free the otoliths from their sac.



Step 4: Place both otoliths from one fish in a single well. Replace any missing otoliths with beads – one bead per otolith (if both missing, use two beads). Fill trays left to right starting in well A1 (fill A1→A12, B1→B12, etc.). A1= grid #1 on Whatman card 1, E1=grid #1 on Whatman card 2 (see diagram on back side of sampling guide).

- If traveling from sampling site; place acetate sheet between tray and lid, secure lid to tray with 3 rubber bands. Remove acetate at overnight location for drying.
- When done sampling; leave filled tray uncovered overnight to dry otoliths. Next day, place acetate sheet between tray and lid and secure lid to tray with 3 rubber bands (see details on back page).



-continued-

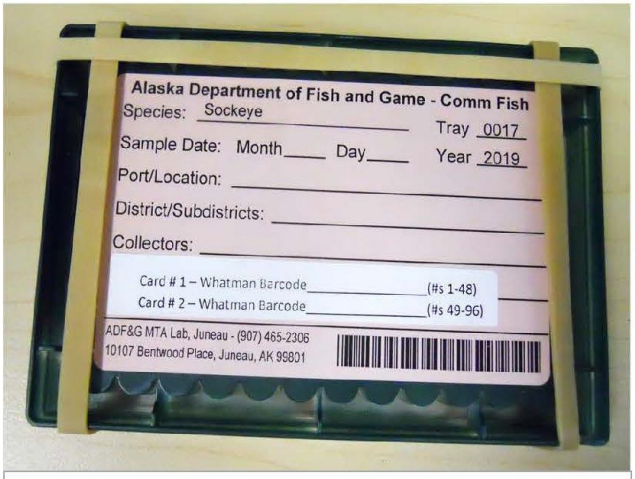
Order of filling otoliths into 96 well tray from two 48-grid Whatman cards

Fill trays left to right starting in well A1 (fill A1→A12, B1→B12, etc.)

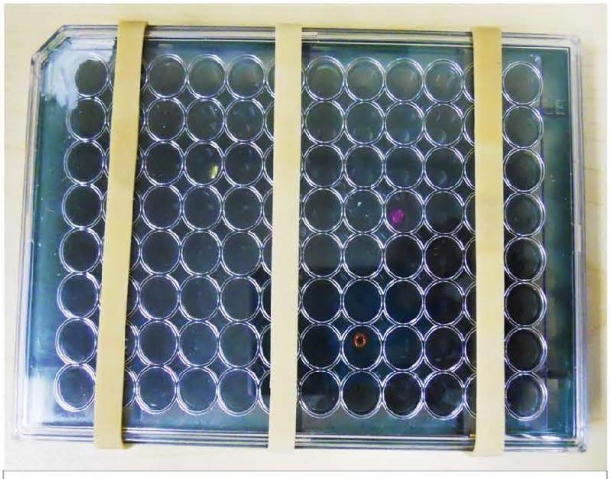
A1= Grid # 1 on Whatman Card 1, E1= Grid # 1 on Whatman Card 2

Start at notch

	1	2	3	4	5	6	7	8	9	10	11	12
A	Grid 1	Grid 2	Grid 3	Grid 4	Grid 5	Grid 6	Grid 7	Grid 8	Grid 9	Grid 10	Grid 11	Grid 12
B	Grid 13	Grid 14	Grid 15	Grid 16	Grid 17	Grid 18	Grid 19	Grid 20	Grid 21	Grid 22	Grid 23	Grid 24
C	Grid 25	Grid 26	Grid 27	Grid 28	Grid 29	Grid 30	Grid 31	Grid 32	Grid 33	Grid 34	Grid 35	Grid 36
D	Grid 37	Grid 38	Grid 39	Grid 40	Grid 41	Grid 42	Grid 43	Grid 44	Grid 45	Grid 46	Grid 47	Grid 48
E	Grid 1	Grid 2	Grid 3	Grid 4	Grid 5	Grid 6	Grid 7	Grid 8	Grid 9	Grid 10	Grid 11	Grid 12
F	Grid 13	Grid 14	Grid 15	Grid 16	Grid 17	Grid 18	Grid 19	Grid 20	Grid 21	Grid 22	Grid 23	Grid 24
G	Grid 25	Grid 26	Grid 27	Grid 28	Grid 29	Grid 30	Grid 31	Grid 32	Grid 33	Grid 34	Grid 35	Grid 36
H	Grid 37	Grid 38	Grid 39	Grid 40	Grid 41	Grid 42	Grid 43	Grid 44	Grid 45	Grid 46	Grid 47	Grid 48
				Whatman Card 1				Whatman Card 2				



Prior to sampling, use a pencil to fill out the label information located on bottom of an **empty** tray (shown above).

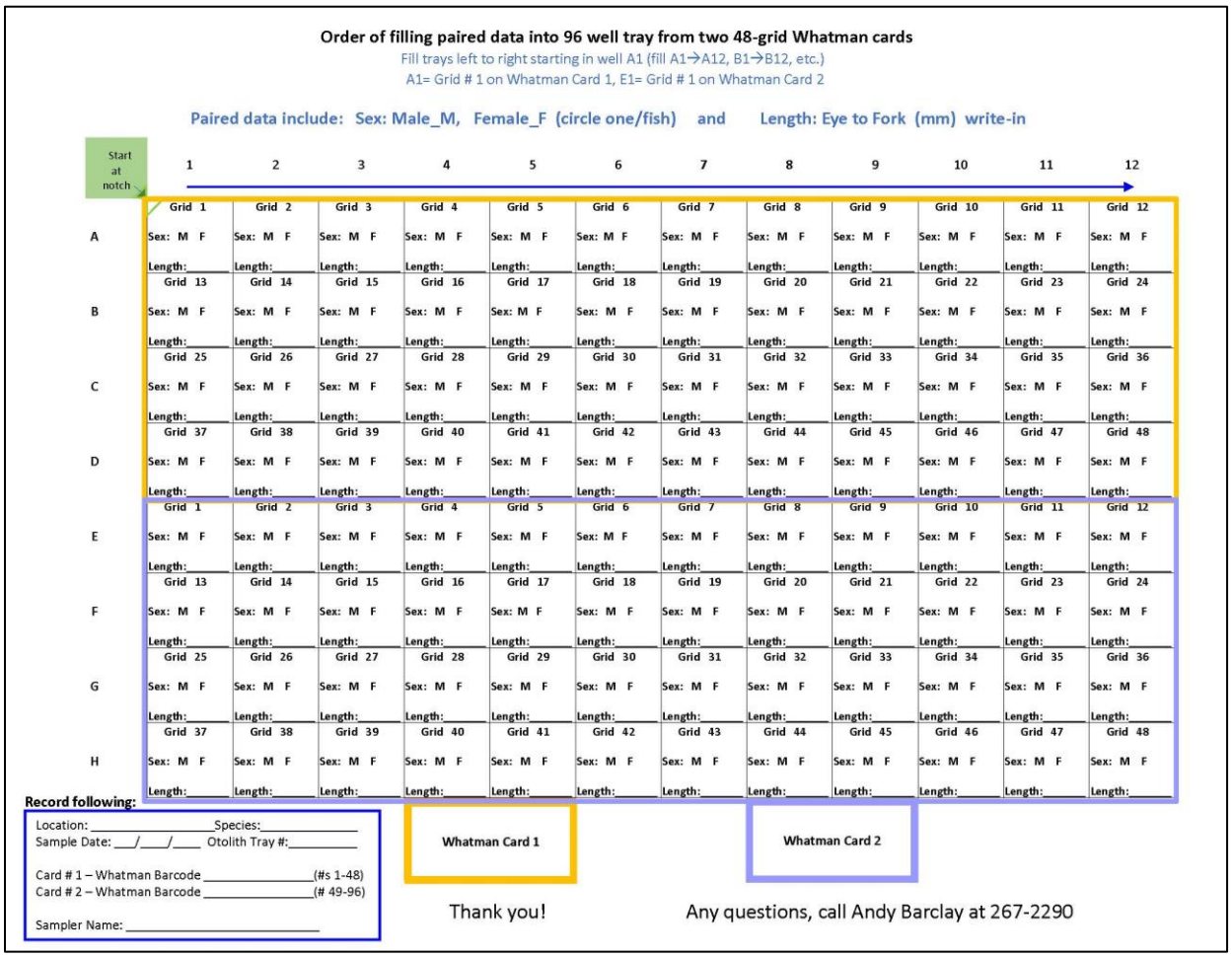


Sampling complete and otoliths dry; place one acetate and lid on top of otolith tray wells. Secure the lid for storage and shipment with 3 rubber bands (shown above).

Thank you!

Any questions, call Andy Barclay at 267-2290

Appendix E3.– Paired genetic tissue and otolith sample data form.



Order of filling paired data into 96 well tray from two 48-grid Whatman cards

Fill trays left to right starting in well A1 (fill A1→A12, B1→B12, etc.)

A1= Grid # 1 on Whatman Card 1, E1= Grid # 1 on Whatman Card 2

Paired data include: Sex: Male_M, Female_F (circle one/fish) and Length: Eye to Fork (mm) write-in

Start at notch

	1	2	3	4	5	6	7	8	9	10	11	12
A	Grid 1 Sex: M F Length:	Grid 2 Sex: M F Length:	Grid 3 Sex: M F Length:	Grid 4 Sex: M F Length:	Grid 5 Sex: M F Length:	Grid 6 Sex: M F Length:	Grid 7 Sex: M F Length:	Grid 8 Sex: M F Length:	Grid 9 Sex: M F Length:	Grid 10 Sex: M F Length:	Grid 11 Sex: M F Length:	Grid 12 Sex: M F Length:
B	Grid 13 Sex: M F Length:	Grid 14 Sex: M F Length:	Grid 15 Sex: M F Length:	Grid 16 Sex: M F Length:	Grid 17 Sex: M F Length:	Grid 18 Sex: M F Length:	Grid 19 Sex: M F Length:	Grid 20 Sex: M F Length:	Grid 21 Sex: M F Length:	Grid 22 Sex: M F Length:	Grid 23 Sex: M F Length:	Grid 24 Sex: M F Length:
C	Grid 25 Sex: M F Length:	Grid 26 Sex: M F Length:	Grid 27 Sex: M F Length:	Grid 28 Sex: M F Length:	Grid 29 Sex: M F Length:	Grid 30 Sex: M F Length:	Grid 31 Sex: M F Length:	Grid 32 Sex: M F Length:	Grid 33 Sex: M F Length:	Grid 34 Sex: M F Length:	Grid 35 Sex: M F Length:	Grid 36 Sex: M F Length:
D	Grid 37 Sex: M F Length:	Grid 38 Sex: M F Length:	Grid 39 Sex: M F Length:	Grid 40 Sex: M F Length:	Grid 41 Sex: M F Length:	Grid 42 Sex: M F Length:	Grid 43 Sex: M F Length:	Grid 44 Sex: M F Length:	Grid 45 Sex: M F Length:	Grid 46 Sex: M F Length:	Grid 47 Sex: M F Length:	Grid 48 Sex: M F Length:
E	Grid 1 Sex: M F Length:	Grid 2 Sex: M F Length:	Grid 3 Sex: M F Length:	Grid 4 Sex: M F Length:	Grid 5 Sex: M F Length:	Grid 6 Sex: M F Length:	Grid 7 Sex: M F Length:	Grid 8 Sex: M F Length:	Grid 9 Sex: M F Length:	Grid 10 Sex: M F Length:	Grid 11 Sex: M F Length:	Grid 12 Sex: M F Length:
F	Grid 13 Sex: M F Length:	Grid 14 Sex: M F Length:	Grid 15 Sex: M F Length:	Grid 16 Sex: M F Length:	Grid 17 Sex: M F Length:	Grid 18 Sex: M F Length:	Grid 19 Sex: M F Length:	Grid 20 Sex: M F Length:	Grid 21 Sex: M F Length:	Grid 22 Sex: M F Length:	Grid 23 Sex: M F Length:	Grid 24 Sex: M F Length:
G	Grid 25 Sex: M F Length:	Grid 26 Sex: M F Length:	Grid 27 Sex: M F Length:	Grid 28 Sex: M F Length:	Grid 29 Sex: M F Length:	Grid 30 Sex: M F Length:	Grid 31 Sex: M F Length:	Grid 32 Sex: M F Length:	Grid 33 Sex: M F Length:	Grid 34 Sex: M F Length:	Grid 35 Sex: M F Length:	Grid 36 Sex: M F Length:
H	Grid 37 Sex: M F Length:	Grid 38 Sex: M F Length:	Grid 39 Sex: M F Length:	Grid 40 Sex: M F Length:	Grid 41 Sex: M F Length:	Grid 42 Sex: M F Length:	Grid 43 Sex: M F Length:	Grid 44 Sex: M F Length:	Grid 45 Sex: M F Length:	Grid 46 Sex: M F Length:	Grid 47 Sex: M F Length:	Grid 48 Sex: M F Length:

Record following:

Location: _____Species:_____
Sample Date: ___/___/___ Otolith Tray #:_____

Card # 1 – Whatman Barcode _____(#s 1-48)
Card # 2 – Whatman Barcode _____(# 49-96)

Sampler Name: _____

Whatman Card 1

Whatman Card 2

Thank you!

Any questions, call Andy Barclay at 267-2290