Multidisciplinary Evaluation of the Feasibility of Parentage-Based Genetic Tagging (PBT) for Management of Pacific Salmon

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List of Acronyms and Abbreviations

AABM	aggregate abundance based management
ADC	adipose fin clip
ADFG	Alaska Department of Fish and Game
ASFEC	Ad-Hoc Selective Fishery Evaluation Committee
AWT	agency wire tag ("blank wire")
BPA	Bonneville Power Administration
BY	brood year
CDFO	Canadian Department of Fisheries and Oceans
CDFW	California Department of Fish and Wildlife
CRITFC	Columbia River Inter-Tribal Fisheries Commission
CWT	coded-wire tag (or tagging)
CWTIT	Coded Wire Tag Improvement Team
DIT	double index tagging
ETD	electronic tag detection
ExN	exonuclease-based sequencing
FPG	full parental genotyping
GBS	genotyping by sequencing
GS	General Schedule
GSI	genetic stock identification
GT-seq	Genotyping-in-Thousands by sequencing
IBD	identical by descent
ID	Idaho
IDFG	Idaho Department of Fish and Game
MM	mass-marking
MSF	mark-selective fishery
MVSH	Magic Valley Steelhead Hatchery
NMFS	National Marine Fisheries Service
NMT	Northwest Marine Technology, Inc.
NWIFC	Northwest Indian Fisheries Commission
ODFW	Oregon Department of Fish and Wildlife
PBT	parentage-based tag (or tagging)
PCR	polymerase chain reaction
PFMC	Pacific Fishery Management Council
PIT	passive integrated transponder (also called passive induced transponder)
PSC	Pacific Salmon Commission
PSCTR	Pacific Salmon Commission Technical Report
PSMFC	Pacific States Marine Fisheries Commission
PST	Pacific Salmon Treaty
QA	quality assurance
QC	quality control
qPCR	quantitative real-time polymerase chain reaction

RFP	request for proposals
RMIS	Regional Mark Information System
SFEC	Selective Fishery Evaluation Committee
SNP	single nucleotide polymorphism
TCCHINOOK	Chinook Technical Committee
TCCOHO	Coho Technical Committee
UID	unique identifier
USFWS	United States Fish and Wildlife Service
WDFW	Washington Department of Fish and Wildlife

Part I. Current Status of the CWT System and of the PBT Concept and Applications.

I.A. Update on the current status, operation, and concerns with the existing CWT system based on reports and experiences since publication of the 2005 Expert Panel Report on the Future of the Coded Wire Tag Recovery Program for Pacific Salmon.

The coastwide coded-wire tag (CWT) system provides information allowing the Pacific Salmon Commission (PSC) to meet its obligations under the Pacific Salmon Treaty (PST) to report annual catches, harvest rate indices, estimates of incidental mortality and exploitation rates for all Chinook fisheries and stocks harvested within the Treaty area (PSC 2014, TCCHINOOK (14)-1 V. 1) and to calculate coho exploitation rates constrained under abundance-based management regimes for naturally-spawning coho salmon originating from rivers along the Washington/British Columbia border (PSC 2013, TCCOHO (13)–1). Information derived from the CWT system is also crucial to management of multiple non-treaty stocks (e.g. Goldwasser et al. 2001; O'Farrell et al. 2012; Mohr and O'Farrell 2014) and is used as the basis for numerous scientific studies of aspects of salmon ecology including ocean spatial distributions (Norris et al. 2000; Weitkamp and Neely 2002; Trudel et al. 2009; Weitkamp 2010, Satterthwaite et al. 2013), maturation rates (Hankin et al. 1993), growth rates (Satterthwaite et al. 2012), and patterns in early marine survival (Satterthwaite et al. 2014a ; Kilduff et al. in press).

The CWT was introduced in the 1970s, and during the intervening period there have been numerous changes to ocean conditions and marine survival, marking and sampling programs, and management demands (PSC 2005, PSCTR 18). As a result, the PSC convened an expert panel to evaluate the CWT program and explore ways to augment or modify it to ensure its continued utility. This led to the establishment of a CWT Working Group to develop recommendations to correct deficiencies in data collection and reporting throughout the basic CWT system and to improve analysis of CWT recovery data, resulting in a report in 2008 (PSC 2008, PSCTR 25). In 2009, the CWT Improvement Program was established with both the U.S. and Canada pledging to provide $7.5 million each in their respective currencies over the next five years.

In the remainder of this section, we discuss each of the 19 key "Findings" identified by the Expert Panel, and then for each issue we provide an update on efforts taken by the CWTIT and other parties in response to them, as well as documenting relevant changes in the fishery and its ecological context since 2005.

The 19 Findings and related challenges identified by the Expert Panel are:

1. The CWT system is the only technology that is currently capable of providing the data required by the PSC's Chinook and Coho Technical committees. There is no obvious viable short-term alternative to the CWT system that could provide the data required for cohort analysis and implementation of PST management regimes for chinook and coho salmon. Therefore, agencies must continue to rely upon CWTs for several years (at least 5+ years), even if agencies make decisions for development and future implementation of alternative technologies.

This report explores the viability of parentage-based tagging (PBT) as an alternative, or supplement, to CWT. Some sort of transition period would certainly be necessary if PBT were to be pursued as a partial or complete replacement of the CWT system.

2. Historic shortcomings of the CWT recovery data system remain problems today. These problems include inaccurate or non-existent estimates of freshwater escapement, especially of stray (non-hatchery) escapement, and inadequate sampling of some fisheries (e.g., inadequate sampling of freshwater sport fisheries and direct sales).

This is primarily an issue of access to fish rather than the specific tagging methodology employed, and so we did not focus attention on this issue. For 2009-2014, 39% of Canadian funds and 11% of US funds for the CWT improvement program went toward addressing low sampling rates in terminal fisheries, low sampling rates in escapement, uncertainty in catch or escapement estimates, incomplete coverage of fisheries or escapement, or sport fishery sampling programs (PSC 2015, PSCTR 33). It is possible that voluntary recovery of PBT tags from recreational fisheries could be increased and the burden of sampling programs on commercial fishermen reduced compared to the current CWT program, since genetic samples require only a small amount of tissue be collected, in contrast to removing the head of a fish for extraction of a CWT.

3. Since the inception of the PST, the quality and quantity of CWT recovery data have deteriorated while increased demands have been placed on these data to provide guidance for protection of natural stocks at risk. Deterioration is due to a number of interrelated factors:
a. reduced fishery exploitation rates, sometimes coincident with periods of poor marine survival, have resulted in fewer fishery recoveries of CWTs;

While reduced exploitation rates and low survival would reduce recovery rates for any tag technology, they could be ameliorated by higher sampling rates, by higher tagging rates, or by larger release groups with the same tag rates. PBT has the potential to decrease the cost of tagging relative to CWT and so could increase tag rates, although some stocks or release groups within stocks are already tagged at or near 100% with CWT. For 2009-2014, 21% of Canadian funds and 36% of US funds for the CWT improvement program went toward increasing sampling rates in terminal fisheries, at escapement, or in highly mixed-stock fisheries (PSC 2015, PSCTR 33). Section II.A explores implications of PBT for tagging rates, marking rates, sampling schemes, and the quantity and quality of information that would result from various proposed systems.

The CWT Improvement Program also funded the development of PlanIt! (Morishima et al. 2012), a decision-theoretic tool for planning CWT experiments for Chinook salmon in the light of tagging and sampling considerations. Similar considerations would apply to PBT experiments.

b. fishing regulations such as minimum size limits and non-retention fisheries have resulted in significant non-landed (catch-and-release) mortality that is infrequently, or cannot be, directly sampled;

We are unaware of major efforts to address this problem. Since collection of tissues for genetic analysis is non-lethal, there would potentially be increased opportunities for collecting samples from non-landed fish in a PBT-based system. However, a sampling framework for non-landed fish is not currently in place and would face numerous challenges in obtaining access to an adequate number of fish. Given access to fish, sampling would be more straightforward under PBT, but if some increase in non-landed mortalities was deemed acceptable, heads could be collected to extract CWT from a subsample of discards, likely with electronic tag detection (ETD) used to assure that only heads with tags were taken (although this would have implications for the extent to which unmarked double-index tagged (DIT) fish which might be lethally sampled in the discards were representative of all unmarked fish, with untagged fish not subject to lethal sampling as discards).

c. changes in the economics of commercial fisheries in at least Washington have resulted in an increased percentage of the catch sold in dispersed locations that are difficult to sample;

This is an issue of access to fish that is independent of tagging technology, so we did not focus attention on this issue. It could potentially be easier to obtain cooperation in obtaining small tissue samples for genetic samples rather than requiring head removal to extract CWTs.

d. increased escapement rates, a reflection of reduced ocean fishery exploitation rates, have increased the proportions of total adult cohorts that return to poorly sampled or unsampled natural spawning areas;

This is an issue of access to fish that is independent of tagging technology, so we did not focus attention on this issue. For 2009-2014, 5.4% of Canadian funds and 0.1% of US funds for the CWT improvement program went towards increasing sampling rates in escapement (PSC 2015, PSCTR 33). Additionally, 8.2% of Canadian funds and 1.7% of US funds went to "incomplete coverage of fisheries or escapement".

e. an increased proportion of the total catch is occurring in sport fisheries which are more difficult to sample than commercial fisheries;

This is largely an issue of access to fish that is independent of tagging technology, so we did not focus much attention on this issue. For 2009-2014, 5.7% of Canadian funds and 0% of US funds for the CWT improvement program went toward voluntary sport fishery sampling programs (PSC 2015, PSCTR 33). It is possible that voluntary recovery of PBT tags from recreational fisheries could be easier than recovery of CWT since genetic samples require only a small amount of tissue be collected, in contrast to removing the head of a fish for extraction of a CWT.

f. competing demands for agency budgets have reduced support for CWT tagging efforts and CWT recovery programs in some jurisdictions.

Budgetary constraints are a reality for any tagging technology. This motivated the economic comparison of CWT and PBT presented in this report.

4. Fishery managers are becoming more concerned with obtaining information that cannot be readily obtained through direct observation or data provided by the CWT system. CWTs are not likely to be an effective tool to answer management questions that require identification of the origin of all fish encountered (e.g., stock-age composition of encounters of sub-legal sized fish) or the survival and migration routes of individual fish (e.g., migration patterns of released fish, catch-and-release mortality rates).

This remains an issue for the CWT system. A PBT system would be compatible with, but not require, collection of genetic data from unmarked/untagged fish allowing genetic stock identification (GSI). PBT in combination with GSI could identify almost all fish to their genetic reporting group (which may not correspond to a management stock boundary) of origin, with supplemental age information provided by scale-aging that could be partially validated through known-age fish identified with PBT. Genetic methods also allow nonlethal sampling, which could facilitate catch-and-release sampling of non-landed fish.

5. Although there appears to be substantial empirical support for the critical assumption that exploitation rates and patterns of hatchery indicator stocks are the same as those of associated natural stocks, there are few peer-reviewed, published studies on this topic, especially for chinook salmon. Much pertinent agency-collected data remains unanalyzed.

This remains an important issue for CWT or any tag that is primarily deployed in a hatchery setting. For 2009-2014, 0.1% of Canadian funds and 19% of US funds for the CWT improvement program went toward representation of production regions (PSC 2015, PSCTR 33). Since the completion of the response to the expert panel in 2008 (PSC 2008, PSCTR 25), a total of 14 additional CWT indicator stocks have been developed, including four natural-origin smolt tagging programs in Alaska, although two new indicator stock programs in BC have been discontinued due to funding shortfalls (PSC 2015, PSCTR 33). Section II.F of this report evaluates the potential for PBT to tag natural-origin stocks. Recent (since PSC 2008, PSCTR 25 was released) publications of some relevance to the suitability of Chinook salmon indicator stocks include Weitkamp (2010), Bernard et al. (2014), and Satterthwaite et al. (2014b). Weitkamp (2010) documented similar broad-scale spatial patterns in recoveries of CWTs deployed in a limited number of natural-origin stocks when compared to nearby hatchery stocks with the same run timing. Satterthwaite et al. (2014b) used GSI data to infer similar spatial distributions early in the year of California Coastal Chinook (natural-origin stock) and Klamath River Chinook (mix of hatchery [partially tagged, used as an indicator] and natural-origin fish), but divergence toward their respective source rivers later in the year

for these fall-run stocks. Bernard et al. (2014) proposed a diagnostic tool to provide insight into the possible degree of mismatch between exploitation and maturation rates on an indicator stock versus the associated natural-origin stocks and applied it to three scenarios with mixed results.

6. The Panel concurs with previous ASFEC findings that MM and MSFs together pose serious threats to the integrity of the CWT recovery data system. In particular, under MSF, recovery patterns for adipose-clipped fish are no longer suitable indicators of recovery patterns for unmarked natural stocks, and under MM there are significant practical and statistical issues raised by the need to find adipose-clipped and coded wire tagged fish (Ad+CWT) from among the much larger number of fish released with adipose clips only. As MSF increase in number and intensity, the discrepancy between the fates of adipose-clipped fish and untagged fish will increase. The seriousness of these threats was previously pointed out to the PSC in the 1991 memorandum reproduced as a frontispiece for this report and in the 1995 report of the ASFEC.

Mass marking (MM) and mark-selective fisheries (MSF) remain serious challenges to fishery sampling and impact estimation. Since adipose fin clips no longer indicate with certainty that a fish is tagged, either some marked but untagged fish need to be processed, or ETD must be deployed. CWT improvement funds have been used to pay for ETD equipment in many jurisdictions, and Northwest Marine Technology, Inc. (NMT) has worked with agencies to reduce costs and improve availability of ETD equipment (Bilateral CWTIT Progress Report 2015). As sometimes proposed, PBT does not have a direct analog to ETD, although "blank wire" or agency wire tags (AWT) could be used to "mark" fish tagged with PBT, or alternative marks could be considered (see section II.A). In addition, tag deployment is likely cheaper via PBT, such that essentially all adipose fin clipped (ADC) hatchery fish might be tagged in a coastwide PBT-based system. However, this would not solve the challenges that MM poses to rare stock enrichment (see section II.A), unless an alternative mark were used. The current status of MM is described in detail below (section I.A.1).

The estimation challenges posed by MSF are not unique to the tagging technology employed (see II.A). Current attempts to estimate the impact of MSF rely on DIT groups in which marked (ADC) and tagged (CWT) fish are paired with a group of tagged but unmarked fish in the same release group, with recovery of the tagged but unmarked fish dependent on ETD. In most cases, aggregate impacts of all MSF that might impact a stock are estimated based on the ratio of marked versus unmarked members of DIT release groups in the escapement (PSC 2014, TCCHINOOK (14)-1 V.1). Methods have been proposed for more stratified estimates, but are highly uncertain (Zhou 2002) and/or over-parameterized without numerous simplifying assumptions (PSC 2005). Employing the current methodology with PBT would require a way to recover the tagged but unmarked fish from DIT groups. Use of AWT along with ETD is one straightforward solution, or alternative marks (e.g. ventral fin clips, PSC 2005, PSCTR 18) could be explored, or else unmarked fish would need to be genotyped in sampling strata where DIT groups were likely to be present. Increasing the unmarked component of DIT groups would allow for a lower genotyping rate of unmarked fish than of marked fish in the

sample, assuming current rates are adequate. The current status of MSF is described in more detail below (section I.A.2).

7. For both coho and chinook salmon, it appears possible to generate approximately unbiased estimates of total non-landed mortalities at age in all MSFs from a full age-structured cohort analysis of paired DIT releases of CWT groups. The accuracy of these estimated total non-landed mortalities may be poor unless very large numbers of fish are released in DIT groups. Estimates of total non-landed mortalities in all MSFs combined would not, however, meet requirements of current PSC regimes to estimate age- and fishery- specific exploitation rates.
a. There does not appear to be any unbiased method to allocate estimated total non-landed mortalities over a set of individual mark-selective fisheries. That is, overall non-landed mortality impacts may be unbiasedly estimated, but impacts in individual MSFs may not be.

These points have largely been addressed above. In theory, PBT tags could be employed in DIT release groups in the same manner as CWTs are currently, with similar utility and challenges. In addition, because genetic samples can be collected non-lethally, there may be increased potential for sampling of discarded fish in MSFs via genetic methods. This could allow direct estimation of stock-specific contact rates (and then impact rates if discard mortality rates are known or assumed) in MSFs, but would require substantial new sampling infrastructure. Mark-recapture designs could also be possible.

8. We have serious methodological and sampling concerns regarding application of the DIT concept:
a. We have been unable to find convincing theoretical or empirical evidence that DIT approaches can generate precise, unbiased estimates of age- fishery-specific exploitation rates for natural stocks of chinook or coho salmon (represented by unmarked DIT release groups) in the presence of sub-stocks and multiple mark-selective ocean fisheries. Methods for analysis of DIT recovery data remain incompletely developed for: (a) complex mixtures of non-selective and mark-selective fisheries with varying exploitation rates and different catch-and-release mortality rates, and (b) the full age-structured setting required for chinook salmon.
b. The potential utility of DIT is undermined by the reluctance of some agencies to recover CWTs for both marked and unmarked DIT groups. This reluctance can be attributed in part to the additional sampling burdens and costs associated with the use of the adipose fin clip both as a mass mark and as a visual indicator for the presence of a CWT.

These points have been largely addressed above. As noted previously (PSC 2015, PSCTR 33), availability and use of ETD has increased somewhat since 2005. As noted above, MM and MSF remain common.

9. Concerns have been raised regarding "reliability in practice" of electronic wanding of salmon (especially large chinook) for presence of CWTs, but empirical evidence brought to our attention has consistently suggested that electronic wanding detection of CWTs is

very reliable. Problems reported with electronic wanding appear to be operational in nature, centering on purchase and maintenance costs of equipment, availability of back-up detection equipment, training and supervision, increased sampling costs, etc.

PBT has the potential to reduce reliance on ETD, however ETD might remain necessary to maintain DIT groups and/or rare stock enrichment (see section II.A), if an alternative mark is not used for these purposes. Recently introduced "T-wands" are more effective than older equipment at detecting wire electronically (PSC 2015, PSCTR 33).

10. Based on recent proposals, many chinook and coho salmon stocks affected by PST regimes may be impacted by increasingly complex mixtures of non-selective and MSFs. The overall impact of MSFs will be stock-specific, depending on migration and exploitation patterns. The potential complexity of these fisheries and the limitations of existing assessment tools have significant ramifications for fishery management:
a. Management agencies have not yet developed a framework to address the increased uncertainty that would result from the initiation of significant MSFs.
b. Improved coordination of sampling and analysis will be required to maintain stock assessment capabilities.

These points have largely been addressed above. A sufficient framework for addressing multiple MSFs has not been developed for the CWT system, nor have systems for alternative tagging technologies. PBT might open the door to alternative sampling or analysis schemes for analyzing MSFs, which would require their own framework for addressing uncertainties and assuring coordination.

11. Some existing technologies can complement the existing CWT system. These technologies include at least otolith thermal marking and Genetic Stock Identification (GSI) methods.

These technologies could also complement a PBT system. GSI, in particular, would complement PBT readily, as the same set of genetic markers and data from them could be used for both types of analysis.

12. These alternative existing technologies cannot, by themselves, replace the CWT system, but they might be used jointly to achieve a similar purpose (e.g., GSI + otolith thermal marking). Although such combination of technologies may be theoretically possible, their combined use could have substantial increased costs and would require a degree of interagency coordination and collaboration that exceeds that which was necessary to develop the CWT system.

The costs and coordination requirements of alternative technologies, other than PBT, would also need careful consideration, but are beyond the scope of this report.

13. Modern GSI methods can be used to estimate the stock composition of the landed catch in a particular time/area fishery. However, the accuracy and precision of data required to estimate stock-age-fishery specific exploitation rates using GSI methods is

dependent upon a variety of factors. For example, microsatellite DNA-based GSI technology is not yet capable of generating consistent, replicable estimates due to the lack of a coast-wide genetic baseline, the history of stock transfers within and among watersheds, and differences in methodologies and mixture separation algorithms.

For Chinook salmon, a coastwide baseline based on microsatellites has since been developed and published (Seeb et al. 2007), as have single nucleotide polymorphism (SNP)-based baselines for stocks encountered in ocean fisheries in the California Current Ecosystem (Clemento et al. 2014) and in the Columbia River (Hess et al. 2014). However, different parties still use different baselines (e.g. in Satterthwaite et al. 2014b the microsatellite baseline was used to assign fish sampled off the coast of Oregon and the SNP one was used to assign fish sampled off California). The current SNP-based Chinook GSI baseline uses 96 SNPs and does not allow for full discrimination of northern stocks, but a coastwide PBT program would be based on 200+ SNPs and this same set of markers is also expected to prove adequate for construction of a coastwide GSI baseline.

For coho salmon, a microsatellite-based baseline covering 84 populations from southern British Columbia through northern California has been developed (Van Doornik et al. 2007) and SNP baselines for Cook Inlet, Alaska (DeCovich et al. 2013) and the California Current (Starks et al. in press) are being developed.

Coastwide coordination would also be crucial for PBT, and was one motivation for preparing this report.

14. Although GSI methods can provide estimates of stock composition in catches or spawning escapements, they cannot provide (with the exception of full parental genotyping, FPG, see Finding 18) information on age or brood year contribution from a particular stock. This information is, of course, required for estimation of age-fishery-specific exploitation rates. Theoretically, GSI data could be augmented by aging data, e.g. scale ages, to rectify this difficulty. Unfortunately, we do not believe that reliable ages of chinook salmon or coho salmon captured in mixed stock ocean fisheries can be obtained consistently by reading of scales. Based on a review of published and unpublished studies, it seems clear that aging errors can be substantial and that these errors are largely attributable to ambiguities in identification of freshwater annuli (juvenile life history).

Supplemental methods for aging, with associated uncertainty, remain necessary for the application of GSI, and other methods that only provide stock of origin, to estimation of age-fishery-specific exploitation rates. PBT would directly provide ages for tagged fish.

15. Large sample sizes will be necessary to use GSI methods to generate reliable estimates of fishery contributions for small (often natural) stocks, and results will be sensitive to small assignment errors for large stocks and ages.

Allen-Moran et al. (2013) provide some guidance on sample sizes for GSI sampling programs. Sample size requirements for CWT or PBT are also considerable, and were one motivation for preparing this report.

16. If sampling programs were sufficiently well designed, GSI methods could be employed to gather information on the incidence of particular stocks and identify opportunities for time-area management measures to reduce fishery mortalities of natural stocks of concern. However, stock-specific management approaches in the aggregate abundance based management fisheries (AABM) would need to be carefully evaluated and agreed upon by the PSC to ensure that the harvest rates on other stocks do not exceed the target levels appropriate for the AABM abundance index as established under the 1999 PST agreement.

This potential remains, but the evaluation and agreement has not occurred. A coastwide PBT system could facilitate collection of data suitable for GSI.

17. Over the past 20 years, first allozymes and more recently microsatellite markers have become the dominant tool for use in GSI. However, we believe that microsatellites will be replaced in the next several years by SNPs as the tool of choice for population genetic applications, as has already occurred in human genetics. The first step in the transition in marker type is the identification of appropriate SNP markers, a process that is already underway for chinook salmon through a multi-agency effort. SNP marker development and databases are also well underway for sockeye and chum salmon. Factors driving the replacement currently include the ease of data standardization, cost, and high throughput. Cost-effectiveness should rapidly improve as more SNPs are developed and multiplex processes drive the cost of analysis down.

A coastwide Chinook salmon baseline for GSI (Seeb et al. 2007) was developed based on microsatellites. GSI baselines using SNPs have since been developed for Chinook salmon, primarily for stocks originating in California through Washington (Clemento et al. 2014) and the Columbia River basin (Hess et al. 2014).

PBT depends on a parent database, distinct from the GSI baseline. PBT applications to date have used SNPs rather than microsatellites, for the reasons described above.

18. A novel genetic method, termed full parental genotyping (FPG), has been presented as an alternative to coded wire tagging. The method uses genetic typing of hatchery brood stock to "tag" all hatchery production. The tags are recovered through parentage analysis of samples collected in fisheries and in escapement. Because of the need for a low laboratory error rate, FPG would rely on SNP markers. FPG would provide the equivalent of CWT recovery data, and could be easily integrated with a GSI system to provide stock of origin for all fish and stock + cohort for fish from FPG hatcheries. However, further evaluation of the relative costs of FPG, GSI and CWT systems is needed. Moreover, an empirical demonstration is needed to validate theoretical results that suggest broad feasibility.

This report aims to further evaluate the potential, and costs, of using PBT (then FPG) as an alternative to CWT for coastwide fishery management. Relatively small-scale (not coastwide) empirical studies have demonstrated the ability to recover tags from Feather River Hatchery spring Chinook salmon via PBT from the California ocean fishery (Clemento 2013, see section I.B).

19. A number of existing or emerging electronic technologies could theoretically replace the CWT and may have substantial advantages over the CWT (e.g., tags can be read without killing the fish, unique tags for individual fish allow migration rates and patterns to be directly observed). Examples include at least Passive Induced Transponder (PIT) tags and Radio Frequency Identification (RFID) tags. PIT tags are currently too large to mark all sizes of juvenile chinook salmon released from hatcheries and are expensive relative to CWTs, but future technological improvements may reduce tag size and tag cost for these technologies.

Consideration of alternative electronic technologies is beyond the scope of this report.

I.A.1. Current status of mass marking.

The term "mass-marked" is used to describe those hatchery programs that target marking all of their released production with ADC, but tag only a subset of releases. This is distinct from 100% marking and tagging in which all fish receive both a mark and a tag (e.g. Sacramento River winter run Chinook salmon).

Coastwide, mass-marking of coho salmon has remained fairly constant from brood year 2005 through brood year 2012 at approximately 35 million fish per year, while mass-marked Chinook salmon have increased from approximately 80 million fish in brood year 2005 to approximately 115 million fish in brood years 2008-2012 (PSC 2015, SFEC (15)-1, their Figure 2-1). While these numbers have remained relatively stable of late, MM has not been implemented coastwide. Most MM occurs in hatcheries located in southern British Columbia, Washington, Idaho, and Oregon (PSC 2015, SFEC (15)-1). Although not marked with ADC, all coho salmon from Iron Gate and Trinity River hatcheries in California receive ventral fin clips and no CWT.

For a coastwide perspective on the numbers of fish currently marked and tagged, we queried the Regional Mark Information System (RMIS) database for "All Releases" of coho and Chinook salmon for the three most recent brood years, 2010–2012, for which the complete release data are available. We then parsed each release group into the numbers that were released unmarked+untagged, unmarked+tagged, marked+untagged, and marked+tagged, where mark=ADC and tag=CWT. These data were then stratified by brood year and location released (state/province), and aggregated within strata. The results, listed in Table I.A.1.1 for Chinook and Table I.A.1.2 for coho salmon, show that, for a given region, the relative composition of marks and tags was relatively stable over these brood years, but that the composition itself varied substantially across regions, as expected.

For each region, the average mark and tag numbers for brood years 2010–2012 are shown in the top left panel of Figure I.A.1.1 for Chinook and Figure I.A.1.2 for coho

salmon, and these numbers were used in computing the remaining panels in these figures, respectively. The remaining panels show the fraction of released fish that were ADC (top right panel), the fraction of released fish that were CWT (bottom left panel), and the fraction of released ADC'd fish that were CWT'd (bottom right panel).

Referring to Figure I.A.1.1 for Chinook salmon, the overall number of released fish varied significantly by region, ranging from about 10 million (Alaska) to about 115 million (Washington), as did the fractions of marked and tagged fish. The mark-rates and tag-rates were relatively low in Alaska and British Columbia (about 10%). The mark-rates in Washington, Idaho, and Oregon were very high (about 75-90%), reflecting the implementation of MM in those states. In California, the mark-rate was about 30%, a combination of 25% mark-rate for fall-run Chinook and 100% mark-rate for the smaller winter-run, spring-run, and late-fall-run Chinook salmon hatchery programs. The highest tag rates were in California and Idaho (about 30%), followed by Oregon and Washington (about 20%). The fraction of ADC'd fish released that were CWT'd (bottom right panel) was, as expected, low (15%-25%) for those regions that mass marked Chinook salmon (Washington, Idaho, Oregon), and high (> 85%) for those regions that did not (Alaska, British Columbia, California).

Similar patterns of release, marking, and tagging by region are observed for coho salmon (Figure I.A.1.2), with two notable exceptions: 1) Alaska released a relatively large number of coho salmon, while Idaho and California released relatively few , and 2) British Columbia mass marked about 40% of their coho salmon production.

The results reported above are also consistent with the MM that is planned for brood year 2013 (PSC 2015, SFEC (15)-1), except that Alaska is planning for the first time to mass mark a small number of Chinook salmon (0.3 million) for release into the Cook Inlet.

Table I.A.1.1. Numbers (millions) of Chinook salmon released coastwide for brood years (BY) 2010–2012, parsed into numbers released unmarked+untagged, unmarked+tagged, marked+untagged, and marked+tagged, where mark=ADC and tag=CWT, and stratified by location released (state/province). Data queried from RMIS, 17 Feb 2015.

			Region					
BY	ADC	CWT	AK	BC	WA	ID	OR	CA
2010	no	no	7.0	35.7	11.1	1.1	1.8	32.8
	no	yes	0.0	0.2	7.4	2.9	1.4	0.0
	yes	no	0.0	0.1	87.7	8.9	21.1	0.1
	yes	yes	1.1	4.7	14.4	2.0	7.2	14.8
2011	no	no	8.2	37.9	9.2	1.2	3.2	32.3
	no	yes	0.0	0.2	6.7	2.2	0.8	0.0
	yes	no	0.3	0.1	84.4	8.6	21.2	0.1
	yes	yes	0.9	4.7	14.3	2.3	6.7	15.7
2012	no	no	7.6	34.5	7.3	0.9	3.6	28.2
	no	yes	0.0	0.0	5.7	2.6	0.8	0.1
	yes	no	0.2	0.1	85.7	10.3	20.9	0.1
	yes	yes	0.9	4.8	13.2	1.8	5.6	14.0

Table I.A.1.2. Numbers (millions) of coho salmon released coastwide for brood years (BY) 2010–2012, parsed into numbers released unmarked+untagged, unmarked+tagged, marked+untagged, and marked+tagged, where mark=ADC and tag=CWT, and stratified by location released (state/province). Data queried from RMIS, 17 Feb 2015 (CA data for Iron Gate and Trinity River hatcheries provided by CDFW).

			Region					
BY	ADC	CWT	AK	BC	WA	ID	OR	CA
2010	no	no	24.5	7.7	3.2	0.3	0.0	0.6
	no	yes	0.0	0.1	2.4	0.1	0.1	0.0
	yes	no	0.0	6.0	23.3	0.0	5.5	0.0
	yes	yes	0.9	0.8	3.2	0.0	0.4	0.2
2011	no	no	28.6	5.4	3.5	0.7	0.1	0.6
	no	yes	0.0	0.2	2.5	0.2	0.2	0.0
	yes	no	0.0	5.3	23.4	0.0	5.5	0.0
	yes	yes	1.0	0.8	3.1	0.0	0.4	0.2
2012	no	no	25.6	4.3	2.4	0.0	0.1	0.6
	no	yes	0.0	0.2	2.4	0.0	0.1	0.2
	yes	no	0.0	5.4	23.1	0.0	6.1	0.0
	yes	yes	0.8	0.7	2.7	0.0	0.5	0.0

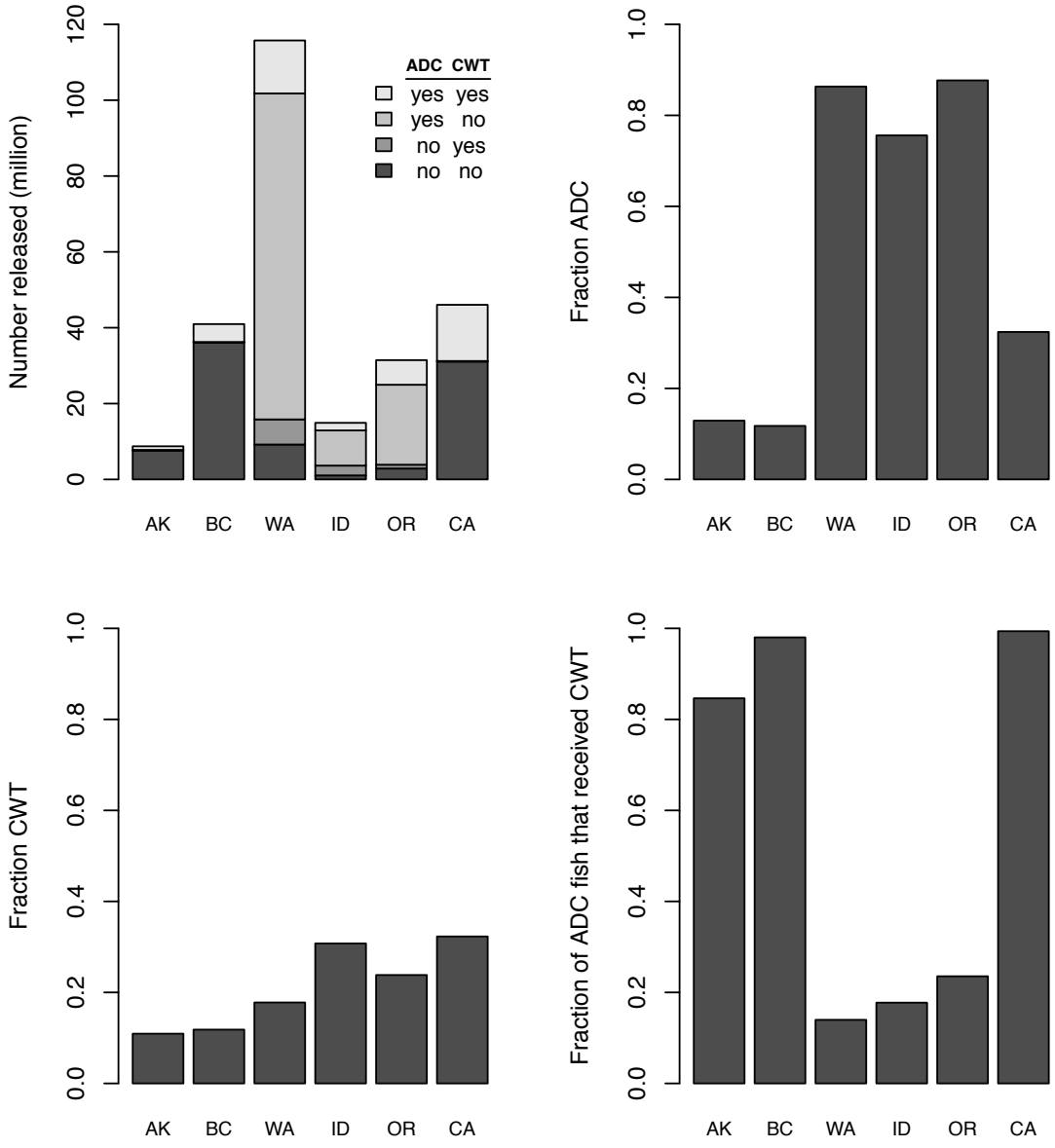


Figure I.A.1.1. Average numbers and fractions of marked (ADC) and tagged (CWT) Chinook salmon released by region, brood years 2010–2012 (Table I.A.1.1).

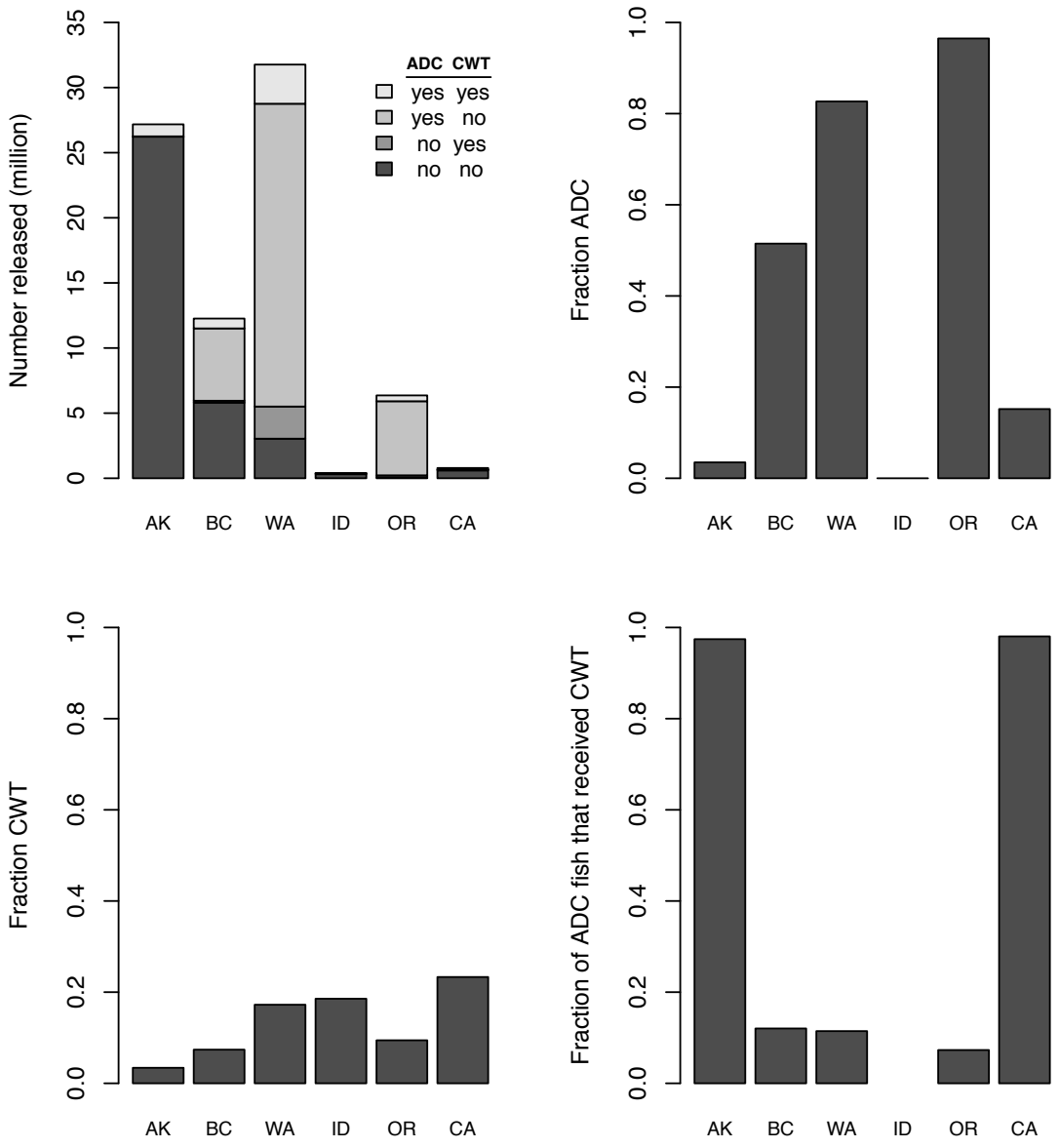


Figure I.A.1.2. Average numbers and fractions of marked (ADC) and tagged (CWT) coho salmon released by region, brood years 2010–2012 (Table I.A.1.2).

I.A.2. Current status of mark selective fisheries.

We provide below a general overview of MSFs for Chinook and coho salmon in the U.S. and Canada. We do not attempt to identify all MSF that have occurred over time, and do not present a detailed history of MSF for the west coast of North America, for which we refer readers to reports by the PSC's Selective Fishery Evaluation Committee (e.g., PSC 2015, SFEC (15)-1). Rather, we attempt to describe patterns of regional implementation of MSF.

At the time of this report, MSF have not been used in the northern and southern edges of salmon fisheries in North America; there have been no MSF in Alaska or California. However, British Columbia, Washington and Oregon frequently have MSF both off their coasts and in terminal areas.

In British Columbia, there have been limited commercial or aboriginal MSF for Chinook or coho salmon. However, beginning in 2008, localized recreational MSFs for Chinook salmon have occurred. For coho salmon, extensive recreational MSFs have been implemented throughout the Vancouver Island and southern British Columbia region's marine and freshwater fisheries since 2000.

There have been no commercial MSFs for Chinook salmon along the Oregon and Washington coasts, and no MSFs for Chinook or coho salmon in the Treaty troll fishery off Washington. Commercial MSFs for coho salmon occur north of Cape Falcon, Oregon, but none have been conducted further south. For recreational fisheries north of Cape Falcon, there have been spring quota MSFs for Chinook salmon since 2010, with non-selective Chinook salmon fishing during the summer quota period. Recreational coho fisheries in this area have been MSF since 1999. Between Cape Falcon and Humbug Mountain, there have not been recreational MSF for Chinook salmon except in small terminal area fisheries (near river mouths), though coho recreational MSFs have been common in recent years.

A variety of MSFs take place in Washington and Oregon inside fisheries (fisheries occurring in state internal waters). In Puget Sound, there are MSF restrictions on the commercial Reef Net fishery, though other commercial MSFs do not occur in this area. Recreational MSFs for Chinook salmon began in 2003 in the Strait of Juan de Fuca and are now common throughout Puget Sound marine waters. Recreational coho MSFs occur in the Strait of Juan de Fuca and portions of Puget Sound. No mark-selective tribal fisheries occur.

In Willapa Bay and Grays Harbor, there have been occasional MSFs in nontribal Chinook and coho fisheries.

In the lower Columbia River, the fall period nontribal commercial fishery has not implemented MSF regulations but some experimentation with alternative gears that allow for improved release survival have recently been tested. Spring run Chinook commercial net fisheries in the mainstem Columbia have been under mark-selective regulations since 2002. For lower Columbia River recreational fisheries, there are MSFs for coho (since 1998), spring run Chinook (since 2001), and limited MSFs (since 2012) for fall run Chinook salmon. Columbia River tribal fisheries are not mark-selective.

The information above is summarized in Table I.A.2.1.

Table I.A.2.1. Overview of west coast mark-selective fisheries for coho and Chinook salmon through 2014. Cape Falcon, Oregon, delineates boundary between Oregon and Washington. Abbreviations: AK=Alaska, BC=British Columbia, CA=California, GH=Grays Harbor, LC=Lower Columbia River, OR=Oregon, PS=Puget Sound, SJF=Strait of Juan de Fuca, VI=Vancouver Island, WA=Washington, WB=Willapa Bay.

			MSF		
Fishery	**Sector**	**Area**	**coho**	**Chinook**	**Notes**
ocean	Aboriginal	BC	No	No	
ocean	Treaty troll	WA	No	No	
ocean	Commercial	AK	No	No	
ocean	Commercial	BC	No	No	
ocean	Commercial	WA	Yes	No	
ocean	Commercial	OR	No	No	
ocean	Commercial	CA	No	No	
ocean	Recreational	AK	No	No	
ocean	Recreational	BC	Yes	Yes	Coho: since 2000, extensive MSF throughout VI and southern BC marine waters, some freshwater. Chinook: beginning 2008, localized MSFs.
ocean	Recreational	WA	Yes	Yes	Coho: since 1999. Chinook: since 2010, spring quota MSFs (non-selective summer quotas).
ocean	Recreational	OR	Yes	Yes	Coho: common in recent years. Chinook: occasionally in small terminal-area fisheries (near river mouths).
ocean	Recreational	CA	No	No	
inside	Tribal	PS	No	No	
inside	Commercial: reef net	PS	Yes	Yes	
inside	Commercial: other	PS	No	No	
inside	Recreational	PS	Yes	Yes	Coho: in SJF and portions of PS. Chinook: beginning 2003, in SJF and now common throughout PS marine waters.
inside	Recreational	WB/GH	Yes	Yes	Coho: occasional. Chinook: occasional.
inside	Tribal	LC	No	No	
inside	Commercial	LC	No	Yes	Chinook: since 2002, spring run.
inside	Recreational	LC	Yes	Yes	Coho: since 1998. Chinook: since 2001 spring run; first fall run MSF 2012.

I.B. Overview of the PBT concept and a review of recent applications of this concept, including both published applications and on-going implementations that have not yet generated published reports

Genetic methods have a long history in fisheries management, with some of the earliest applications of molecular methods involving the elucidation of population structure in salmonid fishes (Utter et al. 1966). The genetic method most commonly applied to fisheries has been GSI, which uses a reference "baseline" database of genotypes from known-origin fish to estimate stock proportions in mixed fisheries and to identify the stock of origin of individual fish. However, GSI requires genetic differences, manifested as significant differences in allele frequencies, between populations for fish originating from them to be distinguished. So, for example, hatchery stocks derived from the same broodstock source or natural populations that exchange, or have recently exchanged, a substantial number of migrants that successfully reproduce are typically not sufficiently distinct for successful discrimination with GSI. Moreover, GSI does not provide age for identified fish, nor allow identification back to specific release groups, which is necessary for the cohort reconstruction models currently used in much of salmon harvest management.

The coastwide CWT program has been successful in informing salmonid fishery management and biological investigation, but its ability to deliver certain information has been challenged by limitations of the CWT technology, as well as the demands placed on it by recent changes in the fisheries and associated regulations. As such, the PSC convened an expert panel more than a decade ago to evaluate the CWT program and explore ways to augment or modify it to ensure its continued utility. Some of the key challenges identified by the Expert Panel (PSC 2005, PSCTR 18) that are most relevant to the objectives of this report include:

1. Higher resolution stratification of fisheries (into time and area strata, for example) requires more tag recoveries (and hence higher tagging and/or sampling rates, when possible) to maintain statistical precision.

2. Higher fractional incidence of sport fishery interceptions and the straying of hatchery fish into natural spawning areas have increased the number of tags which end up in situations difficult for recovering samples, and recovery of CWTs may be particularly difficult in some cases since they require fishermen surrender the heads of fish.

3. Fish are now exposed to a variety of fisheries with more complex regulations than before. For example, any Chinook salmon caught in a species-specific coho salmon fishery will be thrown back, but they will still suffer some extra "non-landed" mortality that should be accounted for in the cohort analysis. The advent of MSFs has complicated the estimation of natural stock fishery exploitation rates from CWT recoveries in the associated hatchery indicator stocks. Because reading a CWT requires sacrificing the fish, CWTs are not helpful in determining which populations may be at risk of incidental mortality in these partial-retention fisheries. The lethality of CWT sampling may be one obstacle to sampling such non-landed catch.

4. CWTs in harvest may be underreported because of the burden (or economic cost) of having to remove the head from landed fish.

5. CWTs cannot be used to sample an individual multiple times throughout its life for the obvious reason that reading the tag requires killing the fish. This makes CWTs inappropriate for any estimation methods that can leverage mark-recapture techniques.
6. The entire production at many salmon hatcheries is now 100% ADC, without a commensurate increase in the tagging rate, eliminating the historical sequestration of the ADC to signify that a fish carries a CWT. This has required either processing heads of marked but untagged fish or the use of ETD to identify ADC fish that carry a CWT at additional expense. It has also interfered with the use of increased (typically 100%) marking and tagging rates of less abundant stocks to increase tag recoveries from them.

With the many challenges facing the CWT program, and the limitations of GSI and other genetic tagging methods, the idea arose of using large-scale parentage inference with genetic marker data, as an alternative to coded wire tags (PSC 2005, PSCTR 18; Anderson and Garza 2006; Garza and Anderson 2007). This method, now referred to as PBT, is theoretically capable of providing almost all of the same data as a CWT program, and since it proceeds by reconstructing pedigrees and provides individual-specific tags, also provides significant additional information. The underlying principle of PBT is that pedigree reconstruction using genetic markers allows one to establish kin relationships. Sampling and genotyping the broodstock at a hatchery, or the spawning adults in a natural population, will provide genetic "tags" for their offspring that can be recovered through statistical parentage analysis. So, sampling of fish in one generation, can allow the recovery of tags in another generation, so-called intergenerational tagging. Since this "tagging" process requires genotyping the parents only, and each female produces thousands of offspring, PBT is highly efficient, with one pair of genotypes providing thousands of tag releases. Juvenile fish need not be handled at all for the deployment of tags via PBT, although marking may still require it.

Anderson and Garza (2006) evaluated the analytical feasibility of performing PBT with SNP markers, which are now the standard for salmonid genetic analysis, and demonstrated that analysis with 100 SNP loci or less would allow parentage inference to be conducted with a false positive rate of only about four out of every 10^{13} parent/offspring trios examined. In a typical year of PBT on a scale relevant to the coastwide program, it is unlikely that more than 10^{13} trios would need to be examined, and consequently very few false positive (parental misassignments) errors in PBT would be expected with such data. There is a trade-off between the false positive and false negative (i.e. failure to assign parents with high confidence, then their genotypes are present in the parent database) rates (Anderson and Garza 2006), but with a target false positive rate of less than one parent pair-offspring rate per analysis, the false negative rate may be less than that of the rate of failure to recover a CWT from a group of tagged fish (Abadía-Cardoso et al. 2013; Clemento 2013).

Moreover, genetic differences between populations increase the realized power for PBT, since fish from such populations are less likely to carry genotypes that are compatible with Mendelian expectations by chance alone, than fish from the same population.

Parentage inference that relies simply on lack of Mendelian incompatibilities, so-called exclusion methods, are available and easy to implement, but they are considerably less powerful than likelihood-based methods. However, likelihood-based parentage inference on such a large scale, and in a mixed fishery context, was a novel concept when PBT was first proposed, and the necessity of dealing with such a large number of potential parent pairs required the development of additional mathematical tools (Anderson and Garza 2006).

The operational routine of conducting PBT and using parentage inference to identify the release group for hatchery-produced salmon consists of five primary phases:

1. Tissue samples are collected from parents of each release group, with the progeny reared separately from the time of spawning to release.
2. Parent tissue samples are genotyped and the genotypes are maintained in a coastwide digital *parent database*.
3. Tissue samples are taken from fish in the offspring generation caught in fisheries or at escapement. These are the samples for which release group-level identification of the hatchery origin fish is desired.
4. The offspring-generation samples are genotyped with the same set of genetic markers as the previous broodstock.
5. These genotypes are then compared to the coastwide parent database with statistical procedures that identify either single parent-offspring pairs, or two parent(mother&father)-offspring trios. When an individual's parents are identified, its release group of origin is known.

There are many ways to achieve these operational steps and details of them and other considerations are discussed below.

1. Tissue sampling

For genotyping, it is necessary to sample approximately 3-5 mm^2 of fin tissue from each spawner. These can be collected with no specialized tools—standard kitchen scissors work well—and, after clipping, the tissue samples can all be stored together in a jar of 95% ethanol until they are prepared for DNA extraction. The caudal fin usually provides good tissue and makes it easy to visually screen fish/carcasses to determine if they have been previously sampled.

It is not necessary to keep track of fish or crosses individually during spawning, although doing so and linking that information to the tissue samples allows the accuracy of the parentage inference for PBT to be maximized (see sections I.B and II.B) and provides an additional measure for quality control. It might be attractive to track samples from a modest number of matings in some hatchery programs for such purposes, but it is generally sufficient to combine the tissues of fish that are parents of a particular release group in a common jar of ethanol. It is also advantageous, if possible, to store the tissues from male and female fish separately, as this reduces the number of trios that need to be examined by a factor of 4. With sufficient SNP data, however, this is also not necessary, and use of genetic sexing assays, available for both Chinook and coho salmon, also renders sample separation by sex unnecessary.

While it is generally not desirable to sample only a fraction of the broodstock for a particular release group, instead of all the spawners, this too can be accommodated in a PBT program, with the corresponding methodology for estimating the realized tagging rate described in section II.B. However, PBT tag recoveries decrease if *both* parents of an individual are not in the parent database. If single parent-offspring analysis is used, this is because either parent can provide a tag to its offspring and genotypes from both parents of a cross would have to be unavailable for tag information from that cross to be lost. When parent-offspring trio analysis is used, both parents of an offspring are required to be in the database for a tag recovery to occur. Therefore, sampling of the parents of release groups should ensure that if a spawner is sampled for the parent database, all of the fish with which it is crossed are also sampled for genotyping.

2. Broodstock Genotyping and the Parent Data Base

PBT requires that many fish be genotyped, so it is critical that genotypes be collected with a cost-effective, rapid method that is amenable to automation. These criteria are met by SNPs, which are now the marker of choice for most applications in genetics and have seen dramatic declines in genotyping costs and increases in throughput capability in the last two decades (Morin et al., 2004; Campbell et al. 2015). Emerging technologies are continuing to reduce SNP genotyping costs while providing higher throughput (i.e. faster) genotyping.

A coastwide PBT system requires that all participating entities use the same set of genetic markers that are reported in the same way. We expect that a set of 200-500 markers will be required for coastwide application of an analytical system that can utilize both single parent-offspring pair and two parent-offspring trio approaches. SNPs are ideal genetic markers for such multi-agency collaboration, because SNP assays provide consistent genotype information in different laboratories and on different instrumentation, in contrast with previously used genetic markers, such as microsatellites (Moran et al. 2006; Seeb *et al*. 2007), for which allele identity is estimated by electrophoretic mobility. With SNPs, it is only necessary to adopt a set of standardized reporting requirements and most of this work has already been completed (PSC GSI workshop reports and FishGen).

Just as the centralized CWT data base, the RMIS of the Pacific States Marine Fisheries Commission's (PSMFC) Regional Mark Processing Center (RMPC) has been pivotal in the success of the coastwide CWT program, a centralized data base, along with the above-mentioned reporting standards, will be necessary for a coastwide PBT system. Such databases exist for GSI (Moran et al. 2013; Blankenship et al. 2011), and one has recently been developed for PBT, which is initially intended for Columbia Basin applications (FishGen). These efforts have utilized the current RMIS system as a model, in direct cooperation with the PSMFC staff, and incorporating much of conceptual infrastructure and standards. Such a centralized PBT database would need to be integrated or interoperable with the RMPC's catch and effort databases. We outline one such possibility in section II.A.5.

3. Sampling in Fishery, Escapement, and other Life Stages

The tissue sampling methods needed for PBT are identical to those needed for GSI, and it has been shown in a variety of contexts that such sampling can be done quickly and easily. This tissue may then be stored in ethanol or, preferably, stored in a

small piece of blotter paper and placed in a small, paper, coin envelope to dry. It is easy to incorporate this procedure into any program for sampling aboard boats, at ports, at hatcheries, or during carcass surveys. The need for just a small piece of tissue also simplifies recreational fishery sampling, as anglers will be much more amenable to taking of a small fin clip than an entire head from their catch.

Genetic sampling for PBT is done non-lethally, which potentially allows many more fish to be sampled in screw traps and when traversing fish ladders while returning to spawn. This makes possible the monitoring of individual salmon throughout their life cycle. Because fish may be sampled multiple times in their life cycle, they can be included in mark-recapture analyses, which is impossible at the individual level with CWTs. Samples can also be obtained from fish that are caught and released as bycatch in fisheries that are not targeting them, at sublegal size, or in non-retention sport fisheries. This information, used in a mark-recapture analysis framework potentially could improve estimates of the mortality due to non-retention fisheries, a prominent estimation challenge in salmon fisheries management.

4. Offspring Generation Genotyping

The same genetic technologies employed to genotype the broodstock are applied without modification to genotyping the offspring. As was noted above, it is important to genotype the offspring generation with the same markers used to create the parent database. Genotypes of returning spawners that are also used as broodstock can be used twice—first as samples for tag recoveries from the offspring of a previous generation returning to spawn (i.e. at escapement) and second as genotypes for the parent database for the following generation.

5. Parentage Inference

The parentage inference portion of a PBT system involves searching through the parent database and comparing every offspring sample to all the fish in the parent data base that could feasibly be its parents. The scale of this problem—searching for parents from amongst possibly billions of parent pairs—required the development of novel computational methods (Anderson and Garza 2006) and a new software program, SNPPIT (Anderson 2010, 2012). SNPPIT can handle problems on the scale necessary for implementation of a coastwide PBT system in no more than a few hours. This is partially because, in the interest of computational speed, SNPPIT takes a "categorical assignment" approach to parentage inference (Marshall et al. 1998)—each individual is assigned to either a single pair of parents, or none at all. Other software exists (e.g. Franz, CERVUS) that provide both two parent and single parent assignments, but are considerably slower and inadequate for analyses at the scale that would be necessary for a coastwide PBT system. For some uses (like estimating fractions of fish of different ages from different populations in certain fisheries) a fractional parentage approach (Devlin et al., 1988; Nielsen et al. 2001; Tringali 2006) might be more appropriate; however, existing fractional parentage approaches are incapable of accommodating the large amounts of data generated by PBT. We expect that software extending SNPPIT's capability to include identification of single parent/offspring pairs in the context of PBT at a coastwide scale could be developed within a year.

While PBT-related projects have been underway in several locations for nearly 10 years, and in many others for nearly 5 years, complete results require the passage of at least one generation, and the first two validation studies have only recently been published in peer-reviewed journals. Abadía-Cardoso et al. (2013) analyzed broodstock from a moderate sized steelhead program in the Russian River, California over a five year period (2007-11) and reconstructed pedigrees over as many as three generations. Complete age distributions for two cohorts were derived and patterns of reproductive success and family structure were elucidated. In addition, they were able to estimate the heritability of spawning time and identify deviations of hatchery practice from established policy.

Steele et al. (2013a) analyzed hatchery steelhead from multiple programs in the Snake River basin and further confirmed the ability to PBT conducted with less than 100 SNPs to accurately identify parent pairs. They also elaborate a framework for estimating tagging rate in situations when not all spawners are sampled. There is also a series of reports (Steele et al. 2011, 2012, 2013b, 2014) that describe the development of this project and a parallel project for Chinook salmon in Idaho (see Appendix 1).

Rawding et al. (2013) describe an approach for using PBT to estimate a quantity similar to the number of spawners in a naturally spawning population, with an "intergenerational mark/recapture" approach. Such an approach has many analytical and operational caveats, but holds promise for increasing the number of escapement estimates available for use in status reviews and stock assessment.

Clemento et al. (2011, 2014) also point out how the same SNP data used for PBT, can also be used with an analogous GSI baseline to provide stock of origin for fish who are sampled and genotyped, but whose parents are not in the parent database. This could allows the implementation of a "hybrid" system that uses PBT for the identification of release group origin and age of fish from stocks or populations where spawners are sampled and GSI for identification of origin of fish from stocks or populations where spawners are not sampled, but for which they are represented in the baseline reference database. Abadía-Cardoso et al. (2013) demonstrate how the same genotypes used for PBT can also be used as DNA or genetic "fingerprints", or uniquely identifying tags that allow an individual fish to be re-identified if it is re-sampled any number of times during its life. This allows the implementation of a "hybrid" system that uses PBT as above, and genetic "fingerprinting" to tag individuals for which parents are not readily sampled (e.g. natural-origin smolts), but that may be encountered again in fisheries or at escapement, such as juveniles in natural areas (as outlined in section II.A, system 5).

Clemento (2013) reports on a PBT study of the spring-run Chinook salmon program at the Feather River Hatchery (FRH) in California with results from 2006 through 2012 described (the study is ongoing). Over 12,000 broodstock from this stock, for which 100% of production is intended to be marked with an ADC and receive a CWT, were genotyped. PBT tag recovery rates for FRH spawners in the offspring generation were consistent with expectations, given the rates at which genotypes for parent broodstock were available and un-tagged fish were incorporated into the broodstock. Clemento (2013) also reports on the recovery of genetic tags from this stock in the 2010 ocean salmon fishery off California. The rate of tag recovery was again consistent with expectations and all tag recoveries consisted of assignment to parent pairs

for whom matings had been recorded at the time of spawning, even though this information was not used in the assignment process, further validating their accuracy.

Part II. Structure, Feasibility and Cost of a Coordinated Coast-Wide PBT Tag Recovery System

II.A. Detailed description of the structure of and requirements for a coordinated coast-wide PBT tag recovery system that could allow the same kind of tag group-specific run reconstruction analyses that are currently performed based on recoveries of CWTs. The description must include locations and requirements for tagging and sampling for tag recoveries; address the timeliness of sample analysis for both in-season and post-season applications; quantify the required laboratory capacities (throughput, precision/accuracy of genotyping and assignments, and resolution); identify the computing resources required to perform and store data related to parental assignments; and address coast-wide coordination, data sharing, and analytical verification of parental assignments and QA/QC. Requirements should be given separately for a system that would generate information from unmarked (adipose fin intact) fish belonging to paired groups designed to assess impacts of mark-selective fisheries, and for a system that does not attempt to generate this information.

It is important to realize that there is an interdependence between the decisions made about the marking/tagging process and the sampling process, and there are multiple combinations of marking/tagging and sampling schemes which, through different tradeoffs, can provide most or all of the information currently provided by the CWT-based system, at varying costs and with different amounts of additional useful information. We reserve a detailed discussion of the tradeoffs and considerations at each stage to the later part of this section. We start instead with a specific proposed marking/tagging/sampling system that would yield what we interpret as a direct replacement of the current CWT-based system, and then describe the benefits and drawbacks associated with various alternatives.

Note that we describe marking/tagging at hatcheries and sampling of returning adults to hatcheries as two discrete stages, however there is some overlap between sampling returning spawners and genotyping parent pairs. In this section, references to genotyping parent pairs for marking/tagging purposes refers to those spawners/spawning pairs that would be included in the parent database. Throughout, we refer to "spawning pairs" since assignment is more efficient and more confident when mating pairs are known, but it should be understood that assignments are frequently made on the basis on the basis of unknown crosses, and at least in theory, assignments can also be made on the basis of single parents. "Hatchery program" is used as shorthand for hatchery programs producing a particular species, life history, and release type with its own marking/tagging goal.

For each system, we describe separate procedures for hatchery programs which do not mass-mark (meaning they either target <100% of production to receive an ADC, or all ADC'd fish also receive a tag [CWT or PBT]) in contrast to hatchery programs that do mass-mark (target 100% ADC rate) but do not tag all ADC'd fish.

We describe five potential PBT systems and variants thereof. System 1 uses a combination of PBT, ADC, AWT, and ETD to essentially replicate the current CWT-based system. System 2 drops AWT and ETD and thus requires sampling some unmarked

fish, but could yield additional information on untagged stocks through GSI and potentially broadens the pool of potential natural-origin indicator stocks since there is no need to handle natural-origin smolts. System 3 builds on System 2 by using AWT as a secondary "mark" indicating ADC fish which would not be genotyped, increasing sampling efficiency in the face of mass-marking and reducing over-sampling of large release groups. System 4 combines PBT, ADC, AWT, and ETD along with a new at-sea sampling program to provide substantially improved information on the impacts of MSFs and natural-origin stocks. System 5 is a hybrid PBT/CWT system that uses PBT on hatchery stocks and CWT for natural-origin stocks, DIT, and potentially low production hatcheries or individual small or unplanned release groups.

Numerous other systems and variants are of course also possible, and the set of examples provided here is not exhaustive nor intended to preclude the consideration of other options. The order in which systems is presented and the resultant numbers are not intended to reflect rankings or preferences.

In particular, although fractional tagging (i.e., only genotyping some fraction of spawners) in System 1 would most closely match the current CWT system, we decided on full parental genotyping (but only fractional AWT deployment) as the default due to concerns about the practicality of maintaining separation between those fish in a particular rearing/release group whose parents had or hard not been genotyped and realizing that the additional cost of genotyping all parents is relatively small compared to total system costs (section II.I) but carries numerous benefits (section II.D). However, if there were accompanying changes in mark rates, they would carry significant implications for sampling.

In all cases parent pairs can contribute to one, and only one, release group.

System 1: PBT+AWT (or alternative mark)
Marking/tagging at hatchery programs that do not mass-mark: Genotype all of the release group parent pairs. A fraction of their offspring equivalent to the current mark/tag rate receive an ADC+AWT.
Marking/tagging at hatchery programs that do mass-mark: Genotype all of the release group parent pairs. All of their offspring receive an ADC (except in the case of DIT groups, see below), and a fraction equivalent to the current tag rate receive an AWT.
Marking/tagging of natural-origin stocks: Trap out-migrating smolts, genotype them, and give them an ADC+AWT. If natural-origin stocks need to be excluded from MSF, mark with an AWT but not an ADC; and areas where they could be recovered should be sampled as if DIT-present.
Marking/tagging of DIT groups: A distinct set of genotyped parent pairs would be used to produce fish for the unmarked component of DIT release groups. Their offspring receives AWTs only.
Sampling of ocean harvest: Inspect the current sampling fraction of fish for ADCs. In areas where the contribution of mass-marked stocks was deemed important, use ETD to screen for ADC+AWT'd fish. Genotype those fish which pass all applicable screenings, and compare them to the parent (+genotyped smolts) database.

Sampling of freshwater harvest: Inspect the current sampling fraction of fish for ADCs. In areas where the contribution of mass-marked stocks was deemed important, use ETD to screen for ADC+AWT'd fish. Genotype those fish which pass all applicable screenings, and compare them to the parent (+genotyped smolts) database.
Sampling of natural area spawners: Inspect the current sampling fraction of fish for ADCs. In areas where the contribution of mass-marked stocks was deemed important, use ETD to screen for ADC+AWT'd fish. Genotype those fish which pass all applicable screenings, and compare them to the parent (+genotyped smolts) database.
Sampling at hatcheries: Inspect the current sampling fraction of fish for ADCs. In areas where the contribution of mass-marked stocks was deemed important, use ETD to screen for ADC+AWT'd fish. Genotype those fish which pass all applicable screenings, and compare them to the parent (+genotyped smolts) database.
Sampling considerations for DIT: In DIT-present areas (and/or where unmarked but AWT'd natural-origin stocks would be present), some fraction of non-ADC'd fish would be screened for AWTs using ETD technology, and genotyped. Assuming the marked and unmarked components of DIT groups were of similar size, the fraction of fish to be genotyped for ADC'd fish would be similar to the fraction of fish to be genotyped for non-ADC'd fish. Thus, all of the fish in the overall sample, ADC'd and non-ADC'd, would be screened for AWTs, and the AWT'd fish would be genotyped.
Benefits (cost aside) relative to status quo: Heads can be left on fish, and provides information for pedigree reconstruction and heritability estimation.
Drawbacks (cost aside) relative to status quo: Some uncertainty in realized tag rates due to genotyping failures, but this is similar to current uncertainty about CWT-shedding. Presumes existence, and stable availability, of sufficient genotyping facilities.
Variants:
1a) Genotype all the release group parent pairs, and ADC all of their offspring. AWTs would not be required in ADC'd fish since all ADC'd fish are genetically tagged, but AWTs would still be required for unmarked DIT fish. Sample at the same rates as before. This will increase the information available on large stocks, at increased cost (costs will increase for marking, tagging and sampling). Potential negative consequences for fishery impacts assessment if MSFs expand into new areas as a result of 100% ADC at all hatcheries (see section I.A).
1b) Genotype only a fraction of each release group's parent pairs, equivalent to the current tagging fraction, and ADC+AWT the offspring of just the genotyped parent pairs. Sample the harvest and escapement as before. Tagging costs will decrease, but hatchery practices will need to accommodate separate genotyped and ungenotyped fish in each release group and this will sacrifice some additional information available when all broodstock is genotyped, as described in section II.D.
1c) Use an alternative mark (e.g. a ventral fin clip) in place of AWTs. The cost of AWTs would be replaced by the cost of the alternative marking, and the cost of ETD would be replaced by the cost of additional visual screening. It would need to be established that the mortality induced by the alternative mark was acceptable and acceptably constant across deployment scenarios (PSC 2005, PSCTR 18) and the alternative mark would need to have verifiably high detectability even in decomposing spawners. Estimating the impacts of MSFs would depend on the assumption that fully-unmarked and alternative-mark-only fish were treated equivalently by all fishermen.

1d) Genotype all the release group parent pairs, and ADC+AWT all their offspring. Sample at reduced rates such that overall tag recoveries are similar to status quo. This will result in more information on abundant stocks but less information on low-abundance stocks already marked+tagged at near 100% (or at the highest rate practical for natural-origin stocks). In addition, the minimum size required for confident inference about each release group will increase, potentially limiting how many separate release groups can be produced by small hatchery programs. Tagging costs will increase, sampling costs will stay the same (or rather sample genotyping costs will stay the same, a lower sample rate might slightly lower overall sampling costs). Potential negative consequences for fishery impacts assessment if MSFs expand into new areas as a result of 100% ADCing at all hatcheries (see section I.A).
1e) In hatcheries that produce a large number of tagged fish and for which recovery targets have been consistently exceeded, only give AWTs to as many fish as would be needed to meet recovery targets. This will reduce genotyping costs in later sampling, with little cost in terms of useful information. Or, reduced AWTing of abundant stocks may allow for increased (cost neutral) sampling fractions so as to increase the recoveries of low abundance stocks.

System 2: PBT only
Marking/tagging at hatchery programs that do not mass-mark: There would no longer be a distinction between mass-marking and other program types as all programs would attempt to mark and tag all fish released. Marking and tagging would be conducted as described below for programs that currently mass-mark.
Marking/tagging at hatchery programs that do mass-mark: Genotype all of the release group parent pairs. All of their offspring receive ADCs (except in the case of DIT groups, see below).
Marking/tagging of natural-origin stocks: Trap out-migrating smolts, genotype them, and give them ADCs. If natural-origin stocks need to be excluded from MSF, do not give them ADCs; and areas where they could be recovered should be sampled as if DIT-present.
Marking/tagging of DIT groups: A distinct set of genotyped parent pairs would be used to produce fish for the unmarked component of DIT release groups. Their offspring would not receive ADCs.
Sampling of ocean harvest: Inspect the current sampling fraction of fish for ADCs. Genotype those fish with ADCs, and compare them to the parent (+genotyped smolts) database.
Sampling of freshwater harvest: Inspect the current sampling fraction of fish for ADCs. Genotype those fish with ADCs, and compare them to the parent (+genotyped smolts) database.
Sampling of natural area spawners: Inspect the current sampling fraction of fish for ADCs. Genotype those fish with ADCs, and compare them to the parent (+genotyped smolts) database.
Sampling at hatcheries: Inspect the current sampling fraction of fish for ADCs. Genotype those fish with ADCs, and compare them to the parent (+genotyped smolts) database.
Sampling considerations for DIT: In DIT-present areas (and/or where unmarked but tagged natural-origin stocks would be present), some fraction of non-ADC'd fish would

need to be genotyped. Assuming the marked and unmarked components of DIT groups were of similar size, the fraction of fish to be genotyped for ADC'd fish would be similar to the fraction of fish to be genotyped for non-ADC'd fish. Thus, all of the fish in the overall sample, ADC'd and non-ADC'd, would be genotyped.

Benefits relative to System 1: No cost for AWTs or ETD. Increased information about high production stocks. Sampling non-ADC'd fish in DIT-present areas would provide GSI information on untagged stocks.

Drawbacks relative to System 1: Increased sampling costs (handling and genotyping), with diminishing returns in the value of additional information about abundant stocks from which a large number of tags are already recovered. Increased marking costs for programs that currently do not mass-mark. Potential negative consequences for fishery impacts assessment if MSFs expand into new areas as a result of 100% ADCing at all hatcheries (see section I.A).

Variants:

2a) Expand sampling of non-ADC'd fish into all areas, DIT-present or not. Then it would be possible to recover the unmarked, PBT tagged offspring of natural-origin parents. Thus, the tagging of natural-origin fish could be expanded to include stocks for which it would be difficult to handle an adequate number of smolts, but parents or parent carcasses could be genotyped. Although it could be difficult or impossible to estimate tagging fractions for such groups, it would still be possible to estimate exploitation and maturation rates for them. Thus, the potential pool of natural-origin indicator stocks could expand. Sampling costs would be increased.

2b) Do not sample non-ADC'd fish. Sample genotyping costs would be decreased, but DIT information would be lost, and natural-origin indicators could not be left unclipped to avoid MSF impacts.

2c) For hatchery programs that do not currently mass mark, mark with ADCs only a fraction of each release group that is equivalent to the current ADCs mark rate. This would reduce marking costs and sampling costs (effort and genotyping), would not facilitate the expansion of MSFs into new areas with their potential negative consequences for fishery impacts assessment (see section I.A), but would yield less information about high production stocks.

2d) Use an alternative mark (e.g. ventral fin clip) on "unmarked" (non-ADC'd) fish in DIT release groups, and then genotype alternatively marked fish recovered in samples from DIT-present areas. This would allow DIT information without the cost of genotyping a large number of unmarked fish. Also, use the alternative mark on the subset of ADC'd fish that would have formerly received CWT, and genotype only ADC'd+alternative mark fish. This would require the cost of the alternative marking and additional visual screening. It would need to be established that the mortality induced by the alternative mark was acceptable and acceptably constant across deployment scenarios and the alternative mark would need to have verifiably high detectability even in decomposing spawners. Estimating the impacts of MSFs would depend on the assumption that fully-unmarked and alternative-mark-only fish were treated equivalently by all fishermen.

2e) Genotype more parent pairs for the unmarked component of DIT release groups than for the marked component. Then, sampling rates on non-ADC'd fish could be lower, reducing overall sampling costs. For example, if an overall genotyping fraction of 20%

was adequate for ADC'd fish, and 4x as many fish were in the unmarked compared to marked components of paired DIT releases, an overall genotyping fraction of 5% would suffice for non-ADC'd fish. Thus, an overall sampling fraction of 20% would be taken with all ADC'd fish genotyped, but only one-fourth of the non-ADC'd fish genotyped.
2f) Decrease sample rates such that the number of fish genotyped would be comparable to the current number of CWTs read. This would reduce sample genotyping costs, but lose information on low abundance stocks and small release groups.

System 3: PBT, with AWT as a secondary "mark" to increase sampling efficiency
Marking/tagging at hatchery programs that do not mass-mark: Genotype all of the release group parent pairs. A fraction of their offspring equivalent to the current tag rate receive ADCs. Use recent recovery data to estimate the number of tagged fish necessary to yield adequate tag recoveries (per release group) in all relevant sampling strata for all but the "worst" years. If the number of ADC'd fish available for release significantly exceeds this level, "excess" ADC'd fish receive AWTs.
Marking/tagging at hatchery programs that do mass-mark: Genotype all of the release group parent pairs. All of their offspring receive ADCs (except in the case of DIT groups, see below). The fraction of production in excess of the current tag rate also receives AWTs, and additional AWTs would be added to "excess" offspring from the genotyped parents in very large (likely to be oversampled) tagged release groups as well.
Marking/tagging of natural-origin stocks: Trap out-migrating smolts, genotype them, and give them ADCs. If natural-origin stocks need to be excluded from MSF, do not give them ADCs; and areas where they could be recovered should be sampled as if DIT-present.
Marking/tagging of DIT groups: A distinct set of genotyped parent pairs would be used to produce fish for the unmarked component of DIT release groups. Their offspring would not receive AWTs.
Sampling of ocean harvest: Inspect the current sampling fraction of fish for ADCs. Use ETD to discard ADC'd fish with AWTs. Genotype only those fish with ADCs but without AWTs, and compare them to the parent (+genotyped smolts) database.
Sampling of freshwater harvest: Inspect the current sampling fraction of fish for ADCs. Use ETD to discard ADC'd fish with AWTs. Genotype only those fish with ADCs but without AWTs, and compare them to the parent (+genotyped smolts) database.
Sampling of natural area spawners: Inspect the current sampling fraction of fish for ADCs. Use ETD to discard ADC'd fish with AWTs. Genotype only those fish with ADCs but without AWTs, and compare them to the parent (+genotyped smolts) database.
Sampling at hatcheries: Inspect the current sampling fraction of fish for ADCs. Use ETD to discard ADC'd fish with AWTs. Genotype only those fish with ADCs but without AWTs, and compare them to the parent (+genotyped smolts) database.
Sampling considerations for DIT: In DIT-present areas (and/or where unmarked but tagged natural-origin stocks would be present), some fraction of non-ADC'd fish would need to be genotyped. Assuming the marked and unmarked components of DIT groups were of similar size, the fraction of fish to be genotyped for ADC'd fish would be similar to the fraction of fish to be genotyped for non-ADC'd fish. Thus, all of the non-ADC'd fish in the overall sample, and the ADC'd fish without AWTs, would be genotyped.

Benefits relative to System 1: Reduced genotyping cost for abundant stocks. Sampling non-ADC'd fish in DIT-present areas would provide GSI information on untagged stocks. Potentially, sampling rates (of non-AWT'd fish) could be increased for the same total sampling cost, providing more information on low abundance stocks.
Drawbacks relative to System 1: Increased number of non-ADC'd fish genotyped. Some genotyped fish would not have parents in the database, but could be identified to reporting group via GSI (although reporting groups may not match management stock boundaries, and wouldn't provide age).
Variants:
(as in 2a, sampling of non-ADC'd fish could be expanded to all areas, DIT-present or not.)
3a) The savings in reduced genotyping costs for abundant stocks could be used to boost the overall sampling fraction for ADC'd, non-AWT'd fish to increase the recoveries of low-abundance stocks.
3b) Do not sample non-ADC'd fish. Sampling costs would decrease, but DIT information would be lost.
3c) Genotype more parent pairs for the unmarked component of DIT release groups than for the marked component. Then, sampling rates on non-ADC'd fish could be lower, reducing overall sampling costs. For example, if an overall genotyping fraction of 20% was adequate for ADC'd fish, and 4x as many fish were in the unmarked compared to marked components of paired DIT releases, an overall genotyping fraction of 5% would suffice for non-ADC'd fish. Thus, an overall sampling fraction of 20% would be taken with all ADC'd, non-AWT'd, fish genotyped, but only one-fourth of the non-ADC'd fish genotyped.
3d) Use an alternative mark (e.g. a ventral fin clip) in place of AWTs. The cost of AWTs would be replaced by the cost of the alternative marking, and the cost of ETD would be replaced by the cost of additional visual screening. It would need to be established that the mortality induced by the alternative mark was acceptable and acceptably constant across deployment scenarios and the alternative mark would need to have verifiably high detectability even in decomposing spawners. Unlike the alternative mark variants of Systems 1 and 2, estimating the impacts of MSFs would not depend on the assumption that fully-unmarked and alternative-mark-only fish were treated equivalently by all fishermen, since only ADC'd fish would receive the alternative mark.

System 4: PBT, with at-sea sampling allowing a focus on MSF and natural-origin stocks
Marking/tagging at hatchery programs that do not mass-mark: There would no longer be a distinction between mass-marking and other program types as all programs would attempt to mark and tag all fish released. Marking and tagging would be conducted as described below for programs that currently mass-mark.
Marking/tagging at hatchery programs that do mass-mark: Genotype all of the release group spawning pairs. Some fraction of the production in each release group that would be expected to result in a minimum "safe" number of tagged fish per release group (based on past tag recovery rates) receive ADC+AWTs. Remaining production receives ADCs only.
Marking/tagging of natural-origin stocks: When possible, trap out-migrating smolts, genotype them, and estimate what fraction of the overall smolt production was

genotyped. These fish would not receive ADCs or AWTs. For other systems, genotype as many parents/carcasses as possible. If possible, estimate total escapement and thereby an estimate of the fraction of production tagged via PBT.
Marking/tagging of DIT groups: There is no need for DIT in this system.
Sampling of ocean harvest: Require observers on fishing vessels, similar to the current West Coast Groundfish Fishery. Observers would sample a fixed percentage of the catch, regardless of whether a fish is retained in a MSF and regardless of whether it is below the minimum size limit. Sampled non-ADC'd fish would be measured and have scales collected for aging and tissue collected for genetic analysis, as would sampled ADC+AWT'd fish (only ADC'd fish would be screened for AWTs using ETD technology). Resulting genotypes would be compared to the parent databases (hatchery and PBT tagged natural-origin stocks) and to the genotyped smolts database.
Sampling of freshwater harvest: Sample the current target fraction of all fish. Sampled non-ADC'd fish would be measured and have scales collected for aging and tissue collected for genetic analysis, as would sampled ADC+AWT'd fish (only ADC'd fish would be screened for AWTs using ETD technology. Freshwater MSF would also require observers.) Resulting genotypes would be compared to the parent databases (hatchery and PBT tagged natural-origin stocks) and to the genotyped smolts database.
Sampling of natural area spawners: Sample the current target fraction of all fish. Sampled non-ADC'd fish would be measured and have scales collected for aging and tissue collected for genetic analysis, as would sampled ADC+AWT'd fish (only ADC'd fish would be screened for AWTs using ETD technology). Resulting genotypes would be compared to the parent databases (hatchery and PBT tagged natural-origin stocks) and to the genotyped smolts database.
Sampling at hatcheries: Sample the current target fraction of all fish. Sampled non-ADC'd fish would be measured and have scales collected for aging and tissue collected for genetic analysis, as would sampled ADC+AWT'd fish (only ADC'd fish would be screened for AWTs using ETD technology). Resulting genotypes would be compared to the parent databases (hatchery and PBT tagged natural-origin stocks) and to the genotyped smolts database.
Sampling considerations for DIT: There would be no need for DIT in this system.
Benefits relative to all other systems (including status quo): Given an estimate of discard mortality, impacts of MSFs could be estimated directly. For those natural-origin stocks with a known (or estimable) tagging fraction and escapement, cohort reconstruction could be done directly without reliance on a hatchery proxy. (Age composition of the escapement could come from scale reading validated with known-age PBT tagged fish). Discards of sub-legal fish would be estimated rather than assumed. Stock composition of the untagged harvest would be estimated rather than assumed. MSF would no longer interfere with the estimation of impacts on unmarked stocks.
Drawbacks: Cost of, and political resistance to, observers. Difficulty of applying the observer sampling approach in recreational fisheries (outside of ocean commercial passenger fishing vessels), where many of the current MSFs are promulgated. Increased sampling costs for escapement (due to handling more unmarked fish), which may be balanced by processing fewer ADC'd fish (only those with AWTs, which could be fewer than the number of "excess" CWT'd fish processed in some systems currently). Need to validate natural-origin escapement and tagging fraction estimates.

4a) Use an alternative mark (e.g. a ventral fin clip) in place of AWTs. The cost of AWTs would be replaced by the cost of the alternative marking, and the cost of ETD would be replaced by the cost of additional visual screening. It would need to be established that the mortality induced by the alternative mark was acceptable and acceptably constant across deployment scenarios and the alternative mark would need to have verifiably high detectability even in decomposing spawners. Estimating the impacts of MSFs would depend on the assumption that fully-unmarked and alternative-mark-only fish were treated equivalently by all fishermen.

System 5: Hybrid PBT for hatcheries, CWT for natural-origin stocks and unmarked DIT
Marking/tagging at hatchery programs that do not mass-mark: Genotype all of the release group parent pairs. With the exception of unmarked DIT groups (see below), a fraction of their offspring equivalent to the current tag rate receive ADCs.
Marking/tagging at hatchery programs that do mass-mark: Genotype all of the release group parent pairs. All of their offspring receive ADCs (except in the case of DIT groups, see below).
Marking/tagging of natural-origin stocks: Trap out-migrating smolts and give them ADC+CWTs. If natural-origin stocks need to be excluded from MSF, do not give them ADCs; and areas where they could be recovered should be sampled as if DIT-present.
Marking/tagging of DIT groups: Fish for the unmarked component of DIT groups would receive CWTs but not ADCs.
Sampling of ocean harvest: Inspect the current sampling fraction of fish for ADCs, and screen all ADC'd fish in the sample using ETD. Genotype those fish with ADCs but not testing positive for CWTs, and compare them to the parent database. Process heads from all fish testing positive for CWTs.
Sampling of freshwater harvest: Inspect the current sampling fraction of fish for ADCs, and screen all ADC'd fish in the sample using ETD. Genotype those fish with ADCs but not testing positive for CWTs, and compare them to the parent database. Process heads from all fish testing positive for CWTs.
Sampling of natural area spawners: Inspect the current sampling fraction of fish for ADCs, and screen all ADC'd fish in the sample using ETD. Genotype those fish with ADCs but not testing positive for CWTs, and compare them to the parent database. Process heads from all fish testing positive for CWTs.
Sampling at hatcheries: Inspect the current sampling fraction of fish for ADCs, and screen all ADC'd fish in the sample using ETD. Genotype those fish with ADCs but not testing positive for CWTs, and compare them to the parent database. Process heads from all fish testing positive for CWTs.
Sampling considerations for DIT / unmarked CWT'd fish: In DIT-present areas (and/or where unmarked but CWT'd natural-origin stocks would be present), also perform ETD on non-ADC'd fish and process heads from all fish testing positive for CWTs.
Benefits relative to System 1: Not required to genotype a very large number of natural-origin smolts. Eliminates use of AWTs. Yields more information on currently mass-marked stocks.
Drawbacks relative to System 1: Redundancy in needing to maintain coastwide CWT database in tandem with coastwide PBT database, and maintain both CWT head lab facilities and genotyping facilities. Impaired ability to take advantage of economies of

scale for both systems. ETD would need to be expanded into all locations, even though very few tags would be recovered via ETD, since some ADC'd fish would be genotyped while others would have CWT extracted, and some non-ADC'd fish may be CWT'd. Natural-origin fish tags could not be recovered non-lethally.
Variants:
5a) Those hatchery fish currently receiving both ADCs and CWTs would receive both ADCs and alternative marks, while those receiving ADCs only would continue to receive only ADCs. Only fish with both ADCs and alternative marks, and not testing positive for CWTs, would be genotyped in later sampling. This would reduce sampling costs while providing an equivalent number of tag recoveries to System 1 (and thus less information on mass-marked stocks than System 5). This would require the cost of the alternative marking and additional visual screening. It would need to be established that the mortality induced by the alternative mark was acceptable and acceptably constant across deployment scenarios and the alternative mark would need to have verifiably high detectability even in decomposing spawners. Unlike the alternative mark variants of Systems 1, 2, and 4, estimating the impacts of MSFs would not depend on the assumption that fully-unmarked and alternative-mark-only fish were treated equivalently by all fishermen, since only ADC'd fish would receive the alternative mark.
5b) Use CWTs for rare-stock enrichment and unplanned release groups. For small hatchery programs and experimental release groups (i.e. stocks or release groups currently "enriched" with near 100% CWT deployment), 100% of their offspring receive ADC+CWTs. "Unplanned" release groups can also be marked/tagged with ADC+CWTs as necessary.
5c) In sampling areas where ADC'd fish predominate, the genotyping rate of ADC'd, non-CWT'd fish could potentially be reduced due to the high number of genetic tags deployed (i.e., 100% of ADC'd fish would be genetically tagged). However, any reduction of genotyping rates in ocean harvest sampling would be complicated by exchange of fish between areas where ADC'd fish predominate versus where they do not. For example, if California continued to mark 25% of hatchery production while areas to the north marked 100% of hatchery production, northern ocean sampling and genotyping would need to remain at the current level or recoveries of California tags would decrease relative to the status quo.
5d) Expand 100% marking+tagging (ADC+PBT) to all hatcheries while maintaining ADC+CWT for natural-origin stocks, DIT groups, and enrichment groups. Then the overall sampling (inspection) fraction could be increased relative to status quo, while only a subset of ADC'd, non-CWT'd fish in the sample were processed, effectively allowing for higher recovery rates of CWTs from low abundance stocks/small releases, and reducing the expense of genotyping large numbers of offspring from large production releases. This would result in increased marking costs for programs that currently do not mass-mark. There would be potential negative consequences for fishery impacts assessment if MSFs expand into new areas as a result of 100% ADCing at all hatcheries (see section I.A).

We now address the individual components of the tagging/marking/sampling schemes, and their inherent tradeoffs, in more detail:

II.A.1. Locations and requirements for tagging.

The tagging locations would be at the hatcheries and, for natural-origin stocks, field locations corresponding to whatever methods were chosen for tagging natural-area indicator stocks.

For tagging requirements at the hatchery, see section II.B, and the discussions earlier in this section regarding which fish parents would be included in the parent genotype database and which fish would receive ADCs or ADC+AWTs.

The most straightforward, albeit expensive, way to genetically tag natural-origin stocks would be to trap and genotype (and presumably mark with ADCs or ADC+AWTs) out-migrating smolts, as is currently done with CWTs. This would technically be genetic fingerprinting rather than PBT, but would have the advantage that the number of tagged out-migrants is known precisely. This number is needed in order to calculate "early life (pre-fishery) survival" in cohort reconstructions. Note that later exploitation and maturation rate calculations are not dependent on knowing the number of tags released. However, early marine survival is reported in PSC reports, may be important in diagnosing reasons for management shortfalls, and has numerous other management and scientific uses. Note that in addition, one might want to expand from the harvest of tagged fish to estimate total harvest of a stock or stock component (or tagged escapement to total escapement), for which one would need to know what fraction of the stock was tagged. This would require a separate estimate of total smolt production, counting "smolts" at an equivalent point of the lifecycle for both tagged and untagged production.

However, if straying into and out of the system was minimal (or could be fully accounted for) and ages could be assigned with high confidence to all fish in the escapement (tagged or not), the tagging rate for a cohort could be estimated retrospectively based on the fraction of age-specific, non-stray escapement to the basin marked with ADCs, discounted by the fraction of non-stray ADC'd fish not matching a genotype in the database. For cohorts returning over multiple run years, some system would need to be devised to account for variation in year-specific estimates made for the same cohort.

Alternatively, adults passing through constricted areas or carcasses could be genotyped to PBT tag a portion of the natural area production. This would require fewer tissue samples be analyzed at the tagging stage. However, there would be several drawbacks:

1. The tagged progeny would not be marked. Thus fisheries and escapement sampling would need to sample large numbers of unmarked fish to recover any tags deployed in this way.

2. Except for very constrained circumstances (i.e., sampling on a fish passage facility such that every parent passing above the facility was at least counted [if not all genotyped] and every smolt produced in the entire area in which these counted adults could have spawned was counted at out-migration) there would be no direct estimate of the number of fish tagged (which would have to be approximated, with unsupported assumptions about mating behavior and average reproductive output, if not all parents were successfully genotyped). Unless parent sampling was carried out at a fish passage

facility, there would be at best a coarse estimate of the total number of potential parents, such that there would be high uncertainty in the fraction (likely small) of parents genotyped. In all cases, patterns of matings would be unknown and sensitivity to variance in effective (number of smolts produced) family size would likely be higher than in a hatchery setting. Alternatively, the effective tagging rate might be estimated later based on the ratio of returning adults from a particular cohort (so, returning adults would need to be aged with high confidence) with parents in the database compared to the total number of returning adults of that age, taking proper measures to account for strays and aging error. Thus both the tagging fraction and the number of tagged fish would likely be estimated with high uncertainty if at all. As mentioned above, this would preclude (or cause high uncertainty in) estimation of "early mortality" and total harvest, but would still allow calculation of exploitation and maturation rates assuming adequate tag deployment and recovery. Note however that adequate recovery of tags would require genotyping a substantial percentage of unmarked fish in the harvest and escapement in any areas where the unmarked natural-origin stocks might be encountered.

3. Carcass sampling could be prone to high-grading, decomposition, or other non-representative sampling problems.

4. Parent pairs would not be known, and in some (perhaps many) cases only one parent from a spawning pair would be genotyped. Although this does not necessarily preclude accurate assignment given an adequate number of markers (see section I.B), it does reduce accuracy and might require a more cumbersome two-step assignment process where natural-origin fish would first be identified to stock of origin using the coastwide markers and then re-analyzed with more markers relative to the potential parents from the inferred stock of origin. This would increase cost of analyses and require increased record-keeping, coordination, and sample storage. In addition, new software would be needed to perform single-parent assignment at the coastwide scale.

II.A.2. Locations and requirements for sampling for tag recoveries.

Tag recoveries would need to be sampled for at all locations currently sampled for CWTs: ocean fisheries (commercial and sport), in-river fisheries, natural area escapement, and hatchery spawners. Cohort reconstructions require a knowledge of all the possible fates that might befall a tagged fish regardless of where that fate occurs – i.e. ocean harvest would need to be sampled consistently coastwide or at least over the entire range in which the stocks of interest could plausibly be encountered in the fishery. Likewise, some degree of sampling would be required in all of the areas a returning spawner might end up, including strays.

Sampling rates for a PBT-based system would depend on the system deployed and the correspondence between marks, tags, and potentially ETD. System 1 would provide similar information to the current CWT-based system if sampling occurred at similar rates if tagged release sizes were similar. Higher tagging rates resulting from full parental genotyping at hatcheries might allow for lower sampling rates while still obtaining sufficient recoveries of tags from abundant stocks, but currently some low production hatchery stocks (e.g. Sacramento winter run, spring run, late fall run, and San

Joaquin fall run) are already marked/tagged at essentially 100%, so information on these less abundant stocks would be reduced if sampling rates declined in response to high tagging rates of abundant stocks (i.e., the ability to "enrich" small stocks via higher mark/tag rates would be lost). Similarly, in this scenario, individual experimental release groups would need to be larger to get adequate recoveries per treatment even from large production hatcheries. It is possible that escapement sampling in basins including only well-tagged and well-represented stocks could be done at a lower rate than in basins that include smaller stocks and/or smaller release groups.

MM and MSFs would complicate sampling (for PBT or CWT, although some complications are more severe for PBT due to the lack of an ETD option without use of blank wire) as described below. In addition, if natural-origin stocks were tagged via carcasses or other methods that did not allow for marking juveniles, an appropriately high (depending on what fraction of carcasses were genotyped and thus what assumed fraction of offspring were tagged) recovery sampling/genotyping rate would need to be applied to unmarked fish to recover natural-origin, tagged fish.

II.A.3. Timeliness of sample analysis for both in-season and post-season applications.

The scenarios described for PBT generally plan for post-season genotyping on an annual basis, however in-season analyses could be accommodated as necessary with special considerations and coordination. While it is possible to provide genotypes in 24-72 hours from fishery samples, genotyping for in-season applications is expected to represent a limited and relatively small component of a broad PBT program.

II.A.4. Quantify the required laboratory capacities (throughput, precision/accuracy of genotyping and assignments, and resolution).

Laboratory capacity would need to be scaled to the necessary level of throughput (number of tissue samples to be genotyped). The current genotyping method that is best suited for PBT applications is known as GT-seq (genotyping-in-thousands by sequencing; Campbell et al. 2015) but various versions of amplicon sequencing are expected to provide similar results. Information on GT-seq in Campbell et al. (2015) provides the following estimates:

-Throughput: 4-6 weeks for one lab technician to genotype 2,000 samples (Campbell et al. 2015), extrapolated to an estimate of 17,000-24,000 samples genotyped annually.
-Precision/accuracy of genotyping: genotype data from GT-seq are 99.9% concordant with known genotypes and have call rates of 96.4% (Campbell et al. 2015) for 192 SNPs.
-Precision/accuracy of assignments: accuracy of parentage assignment for PBT was estimated with simulations in Anderson and Garza (2006) and validated empirically in Steele et al. (2013a). Empirical results demonstrate that PBT assignments are greater than 96% accurate when 96 or more SNP markers are genotyped.
-Resolution of assignments: Empirical results from Steele et al. (2013a) demonstrate that assignments to the level of parent pairs can be correctly identified for greater than 96-97% of fish, with all remaining mis-assignments as false negatives (3-4% unassigned fish) and zero false positives (incorrect parents).

While the error structure of two-parent-parent trio based assignments is well understood, the error structure of single-parent assignments is a topic of ongoing research.

II.A.5. Identify the computing resources required to perform and store data related to parental assignments; and address coastwide coordination, data sharing, and analytical verification of parental assignments and QA/QC.

Coastwide implementation of a PBT release and recovery program would require a clearly defined operational arrangement between the participating genetics labs and resource management agencies. We briefly outline below the key elements of one such arrangement, which we believe would be practical and would integrate well with the existing RMIS system. In this arrangement, the focus of the genetics labs is to perform the genotyping and check for tag recoveries, while the focus of the resource agencies is on the summary data compilation and reporting. Of course, other suitable arrangements may also be possible.

A resource agency would provide individually stored tissue samples to the genetics lab it is associated with, along with a unique identifier (UID) for each tissue sample, and its sample type: whether the tissue was from a hatchery release group parent, a fish to be tagged via genetic "fingerprinting" (see sections II.A and II.F), or a fish to be examined for possible tag recovery (PBT or fingerprint). For a hatchery release group parent, or a fish to be tagged via genetic fingerprinting, the agency would also provide the corresponding release group UID.

The genetics lab would be responsible for performing the genotyping, conducting the parentage and fingerprint-lookup analyses as necessary, and uploading the resulting data to a coastwide tag recovery database. This database would be publicly accessible to allow each genetics lab to conduct tag recovery analyses for its tissue samples onsite, and to allow for cross checking of the tag recovery results by other entities. The design of this database would be based on standards for the reporting, storage, and retrieval of the genotype and related information that was mutually agreed to by all of the participating genetics labs and resource agencies. The data to be uploaded to the database would include the tissue UID, the sample-type, whether successfully genotyped, the genotype, the sex, the release group UID (if known) and, for PBT tag recoveries, the parent UIDs. For accountability purposes, either the lab would provide these results directly to the agency that supplied the tissue sample or the agency would download them directly from the database.

The resource agency would continue to upload the same data to RMIS that it does currently (release group information, fraction marked and tagged, sample information, recoveries, locations, catch and effort data, etc.), except that the releases and recoveries would now be labeled according to the release group UID rather than by a unique CWT code.

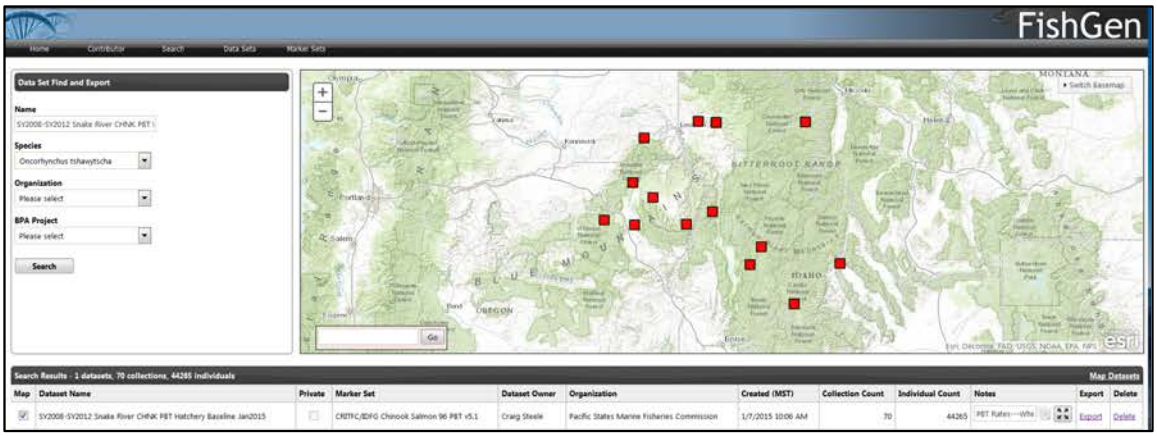
An example database does currently exist for the storage and serving of fish genetics data: "FishGen (http://www.fishgen.net/) is a final repository for salmon and steelhead genetic data as part of Genetic Stock Identification and Parentage Based Tagging projects in the Columbia River basin and throughout the Pacific Coast of North

America. FishGen was developed by Resource Data, Inc. for the Idaho Department of Fish and Game with funding from the Pacific Coast Salmon Recovery Fund." (FishGen 2014). It is an online database web application built using Microsoft technologies. The front-end uses ASP.Net with a combination of Web Forms and AJAX with jQuery hosted on Internet Information Server. The back-end, for processing uploads, is a Windows Service. Currently, the system uses SQL Server 2008 R2 and Windows Server 2008, but in the future it will use Amazon Web Services operated on Windows Server 2012 and SQL Server 2014.

The cost for housing the current FishGen database on Amazon Web Services, up to 50 GB of data, would be approximately $4,200/year. For context, the FishGen database currently houses the Snake River spring/summer Chinook PBT parental genotypes for brood years 2008–2012 (44,265 individuals, Figure II.A.5.1), which requires 36.7 MB of storage, or about 8.3×10^{-4} MB per fish. Coastwide, the number of PBT parents (broodstock) to be genotyped would be approximately 220,000/year (section II.I). In addition, scenarios other than System 5 would include approximately 1,100,000 juvenile fish that would be genetically fingerprinted to match current tagging rates of natural-origin stocks (Table II.I.5). Finally, the number of sampled fish to be genotyped for recovery purposes would range from, approximately, 180,000–1,900,000 / year depending on the system employed (section II.I, numbers of fish can be derived by dividing costs in Table II.I.3 by $7.00 or appropriate summation of sampled, processed, and ad-clipped fish in Table II.I.7). All together, the number of fish to be genotyped annually would range from 1,500,000-3,200,000, which would require storage on the order of 1.2–2.6 GB / year, and after 15 years would result in a modest 18–39 GB of genotype data. Restricting the database to only PSC indicator stocks would reduce the required numbers substantially, but it would no longer serve as an integrated coastwide database. Database requirements would also be substantially decreased if natural-origin stocks were not tagged by genetic fingerprinting of smolts.

One alternative to having separately managed genetics and RMIS databases, would be to directly link the genetics database to the RMIS database system, or vice-versa. This might provide increased assurance that the combined system would function "as one", with common reporting standards, etc.

Figure II.A.5.1. Screenshot of Snake River hatcheries stored in the FishGen repository. Currently, all meta- and genetic data for all Chinook salmon hatchery broodstock for spawn years 2008 through 2012 are stored in the database.



II.A.6. Requirements should be given separately for a system that would generate information from unmarked (adipose fin intact) fish belonging to paired groups designed to assess impacts of mark-selective fisheries, and for a system that does not attempt to generate this information.

For a system that does not attempt to generate this information, assuming that essentially all tagged hatchery-origin fish are marked and assuming that natural-origin tagged fish are also marked, simply sample marked fish at either a rate determined to be adequate (e.g. as described above), or at a rate comparable to the current CWT sampling scheme. Note that in the presence of mass marking, this will require more genotyping than the current amount of CWTs read, given that not all ad-clipped fish have CWTs but ETD can screen out most such fish before the tagless heads are processed. However, even though there is a point of diminishing returns in recovering very large numbers of tags from any one release group, if there is full parental genotyping/100% tagging at all mass-marking hatcheries, there is some benefit to the increased sampling as unlike processing an empty head for CWTs, some information is gained by genotyping any marked fish whose parents were genotyped.

A similar process of pre-screening is not easily achievable with PBT unless an additional mark or tag is used to designate stocks to either target or under-sample. The approximate increase in the number of fish to be genotyped can be inferred from Table II.I.7, comparing the Ad Clipped (D) column to the Processed (B) column. Coastwide, roughly 3x as many Chinook salmon and 5x as many coho salmon would need to be genotyped versus processed for CWT under the current system of partial ETD, although ratios vary widely by region. However, these ratios may change in the future given changes in the prevalence of mass marking and/or adoption of ETD.

Attempting to assess the impacts of MSFs is very difficult whether using CWT or PBT. It may well be impossible to reliably estimate the individual impacts of multiple MSFs in practice (PSC 2005, PSCTR18) with CWT, and PBT will not solve this problem.

However, while the impacts of individual MSFs may not be separately estimable, it appears possible to generate approximately unbiased estimates of total non-landed mortalities at age in all MSFs from a full age-structured cohort analysis of paired DIT releases of CWT groups. This would require sampling for both marked/tagged and unmarked/tagged (CWT or PBT) fish in fisheries, in the hatchery returns, and in spawning escapement. Without ETD or a new external mark, this would require sampling large numbers of unmarked fish and either attempting to extract CWTs or genotyping. The lack of ETD would probably result in more fish needing to be genotyped under PBT than would be processed under CWT. However, since decomposing carcasses may have unclear mark status regardless, it might make sense to sample a comparable number of marked and unmarked fish in the escapement. Thus the main additional challenge in attempting to apply PBT to cohort reconstruction of DIT fish would be the need to genotype a large number of unmarked fish from (non-mark-selective) fisheries. Assuming that an approximately equal number of marked and unmarked DIT fish would be released, sampling unmarked fish at the same rate as marked fish (i.e., 20% of all harvested fish, whether marked or not) would be expected to yield similar tag returns of marked and unmarked DIT fish (presumably unmarked recoveries would be slightly higher after the MSF due to lower exploitation of unmarked fish). Genotyping 20% of the catch would entail large sample sizes. However since tagging via PBT is relatively cheap, it might prove more efficient to construct DIT groups such that the marked component was of the typical target size established for CWT release groups, while the unmarked component was larger by a ratio f. Then, comparable recoveries from the unmarked component could be obtained by genotyping the unmarked portion of $20/f\%$ of the catch; i.e. given a particular overall sampling fraction, all ADC'd fish in the sample would be genotyped but only a subset ($1/f$) of the non-ADC'd fish in the sample would be genotyped.

Currently, it appears that cohort reconstruction of the unmarked component of DIT releases is not typically performed. Instead, the ratio of marked versus unmarked members of DIT release groups are compared in the escapement (PSC 2014, TCCHINOOK (14)-1 V.1). To continue this practice under PBT would require that a constant fraction of the escapement and hatchery returns be genotyped regardless of mark status. Whatever fraction is currently determined adequate for analyzing marked fish would suffice for unmarked fish as well, since the unmarked members of the DIT release pair should have had slightly higher cumulative survival to return. Similarly, if the unmarked component of DIT releases were larger relative to the marked component, unmarked fish could be genotyped at a correspondingly lower rate.

Alternatively, Anderson and Garza's section in (PSC 2005, PSCTR18) suggests:

> *"FPG would be most effectively applied in the DIT framework if another, sequestered, preferably externally visible, method of marking the non-adipose-clipped DIT release group was available. For example, if the non-adipose-clipped DIT release groups were clipped on the ventral or pelvic fins, the amount of genotyping necessary to fully implement DIT by FPG would be greatly reduced. Alternatively, both DIT groups could be tagged by FPG, but the marked[sic] group fish could have CWTs implanted in them so that they can be detected in electronic surveys of unmarked fish in*

the fisheries. This scenario could confer cost savings because it would be unnecessary to electronically survey adipose-clipped fish [assuming 100% genotyping of parents], and the electronic sampling effort for non-adipose-clipped fish could be focused on fisheries most relevant to the DIT study design. Depending on the sizes of two DIT release groups, and the recovery rate, it may be cost effective to implant inexpensive blank wire that can be electronically detected into the unmarked individuals, and recover their stock and cohort via FPG."

However, the potential drawbacks of alternative marks are not well characterized. Recommendation 10 of the Expert Panel Report (PSC 2005, PSCTR18) states:

"Additional experiments should be conducted to evaluate the use of alternative external marks (e.g., a ventral fin clip or some alternative fin clip) for identification of fish bearing CWTs. Existing published information suggests that application of other external marks (e.g., a ventral fin clip) will reduce the survival of hatchery fish from release to age 2, but there is little evidence of differences in survival or behavior of externally marked versus unmarked fish past age 2. We propose some experiments that would allow, among other things, testing of a null hypothesis that survival rates for (A) Ad+CWT+alternative external mark fish and (B) Ad+CWT fish are the same from age 2 on, i.e., that there is no lingering differential mortality due to, for example, ventral fin marking."

And page 124–125 of the same report (PSC 2005, PSCTR18) states:

"In our review of the effects of application of ventral fin clips, we found substantial evidence that overall apparent survival rates (e.g., total recoveries over a cohort's lifespan compared to numbers released) of hatchery Chinook and coho salmon can be reduced by application of ventral fin clips, sometimes dramatically (more than 50% reduction) but sometimes only modestly (< 5%). We suspect, but have not verified, that the observed wide range of survival effects due to application of a ventral fin clip in large part reflect differences in fish size at the time of mark application or fish release size. Generally, if fish are smaller at marking and release, then effects on survival will probably be greater than if fish are larger at marking and release.

If a ventral fin clip were used as a mass mark, instead of the adipose clip, then the adipose fin clip could once again serve as a unique identifier of CWT fish. However, use of a ventral fin clip as a mass mark might result in unacceptable loss of fishing opportunities on hatchery fish due to reduced survival. If the ventral fin clip were instead used as an identifier of CWT fish, then the effect on overall survival could presumably be compensated for by increased numbers at release. If the ventral fin clip reduced survival from release to age 2 only, but did not thereafter affect

ocean survival or migratory behavior of CWT fish, then the ventral clip would be an acceptable external mark from the standpoint of providing data necessary to estimate stock-age-fishery specific exploitation rates for unmarked fish.

...

Finally, we wish to emphasize that we fully recognize the potential disadvantages (primarily reduced survival rate to age 2) of applying some non-adipose fin clip to fish released as part of CWT experiments in terms of the number of marked fish available for harvest. But we believe that the mortality impacts of mark application must be weighed against the full costs of using electronic detection in all fisheries or of taking heads from all adipose clipped fish in all fisheries."

An additional concern with alternative visual marks such as a ventral fin clip is that fishermen might handle fish with alternative marks differently than fish with no marks whatsoever. This could complicate estimation of MSF impacts even further since the "unmarked" (alternative mark only) component of DIT groups would not experience the same fishery impacts as fully unmarked fish.

II.B. Description of the requirements for hatchery programs to implement a parental-based tagging program, to maintain tagged groups without mixing between different tagged groups, and to accurately assess the number of tagged individuals per tagged group at the time of release. This section would also determine the degree to which substantial hatchery infrastructure changes would be needed to implement PBT.

Managers currently use CWTs to address a number of different management and research needs at widely different scales.

- Large scale (Release groups of 1-5 million)
    - To brood year, hatchery, and stock. (e.g. stock specific abundance, age-distribution)
- Moderate scale (Release groups of 200,000-750,000)
    - To release group. (e.g. examine effects of size, timing, and location of release on ocean distribution, maturation, harvest and straying).
- Fine scale (Release groups of 30,000-100,000)
    - To experimental group. (e.g. Density and diet rearing experiments, experiments examining heritability of specific traits).

Whether PBT can serve as an efficient and accurate tag at moderate and fine scales, depends on the ability of the hatchery to separately rear and track groups of families through the culture phase of their life cycle. This is necessary because every unique release group must have a unique set of families. As an example, we will use the hatchery steelhead program in the Snake River basin to describe the transition from CWT to PBT systems. Approximately, 7.8 million steelhead are released annually, from 7 stocks (5 hatcheries) in Idaho. Up until recently, managers used CWTs to estimate stock specific abundance, age-distribution, and harvest. Approximately 1.9 million steelhead (~25%) were CWT'd annually, comprising ~30 separate release groups.

In order to transition the tagging program from CWT to PBT:

- Each hatchery had to devise a PBT tracking system that allowed groups of families to be separately reared and tracked from PBT sampled parents to egg tray incubators to vats to raceways and then to release sites (Figure II.B.1).

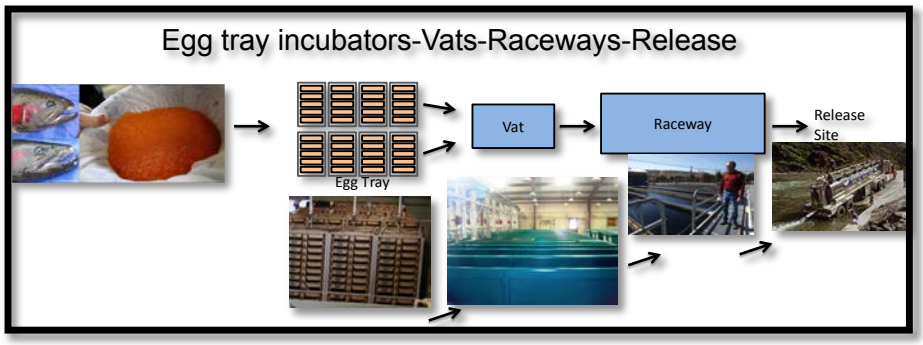


Figure II.B.1. Diagram showing the movement of steelhead through the culture phase of their life cycle from spawning to release.

Approaches to tracking family units to release group

- Set release group size in intervals of the largest group of intact family units.
- When not possible due to logistic or management needs:
    - Limit the number of split family units
    - Pool multiple release groups into a single release group.

Consideration for hatcheries regarding PBT

- Requires more detailed management than standard operating procedures
    - Must eliminate "topping off" to meet production goals, unless offspring come from a "slop" vat. A slop vat contains a group of families raised separately for the specific purpose of supplementing a release group. While this lowers the overall tagging rate, it does not compromise the integrity of the remaining tagged families (Figure II.B.5). Alternatively, fish from the "slop" vat can be differentially marked depending on which release group they are added to. This technique has been used periodically by managers in the Snake River basin to maintain high tagging rates. However, it is important to note that this technique requires that recovery sampling designs for the areas in which these fish will be encountered would need to be aware of, and account for, the differential mark.
    - In some cases, hatcheries may have the flexibility of keeping 2-3 groups of families separated to release (each group produced from unique sets of parents). This would provide the flexibility of supplementing a release group without lowering the tag rate. These groups could also be used in the event of a catastrophic loss of a raceway of fish.
    - Develop egg request goals for each release group
- Must maintain intact family units throughout the rearing process (from conception to release). In particular, during:
    - Egg enumeration
    - Marking
- Catastrophic events may impact ability to track family units.
- Coordination of information is essential for:
    - Tracking family groups
    - Relating release information to recovered samples

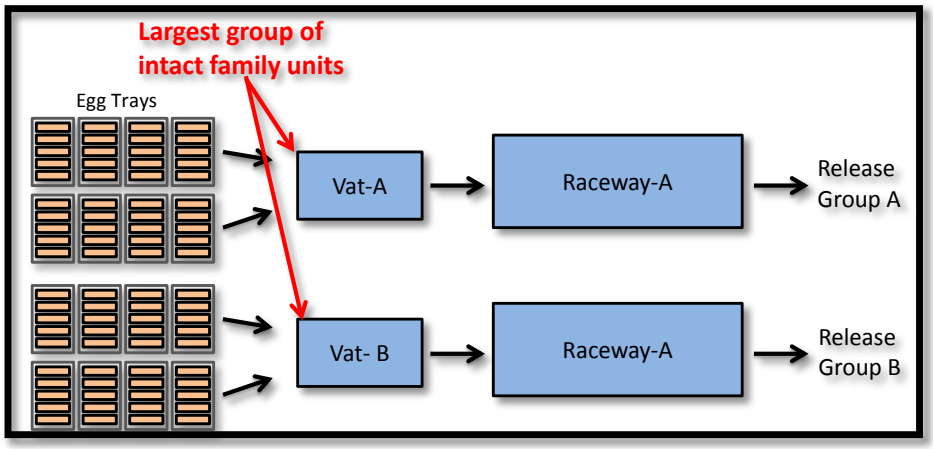


Figure II.B.2. Diagram showing best case scenario, where unique sets of families are grouped and tracked from egg to release. Release group A is 100% PBT tagged to a unique set of families that are distinct from those of Release group B.

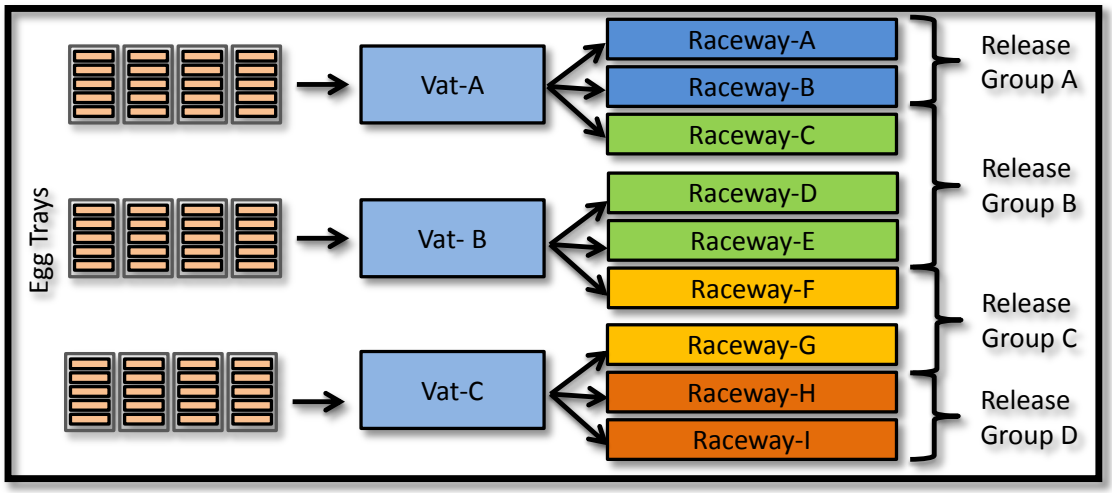


Figure II.B.3. Diagram showing worst case scenario where families are split across different release groups. Individuals could not be unambiguously assigned back to a single release group through PBT under this scenario.

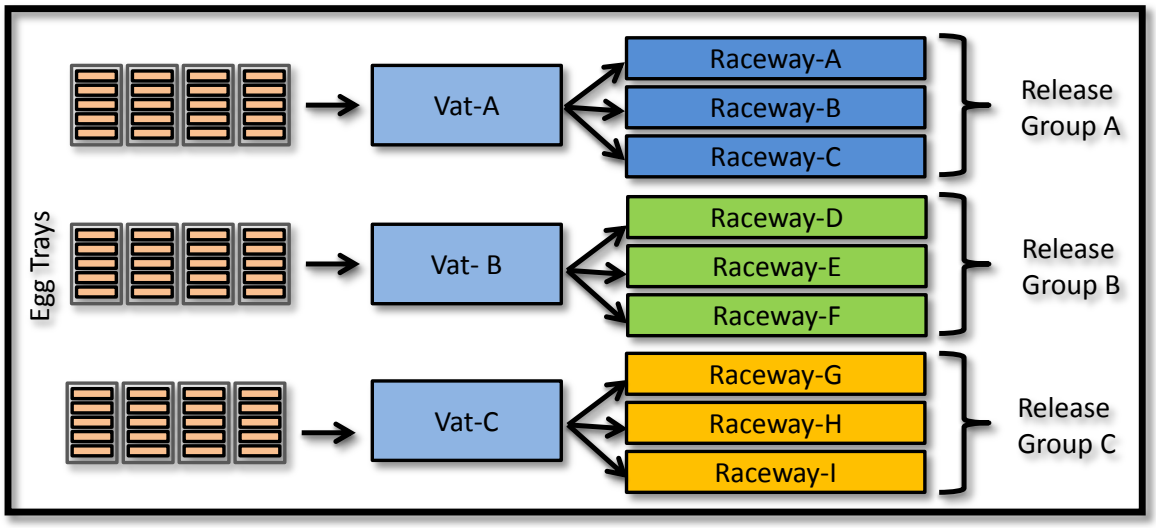


Figure II.B.4. Diagram showing maximized PBT scenario. All individuals can be assigned back to a single release group through PBT under this scenario.

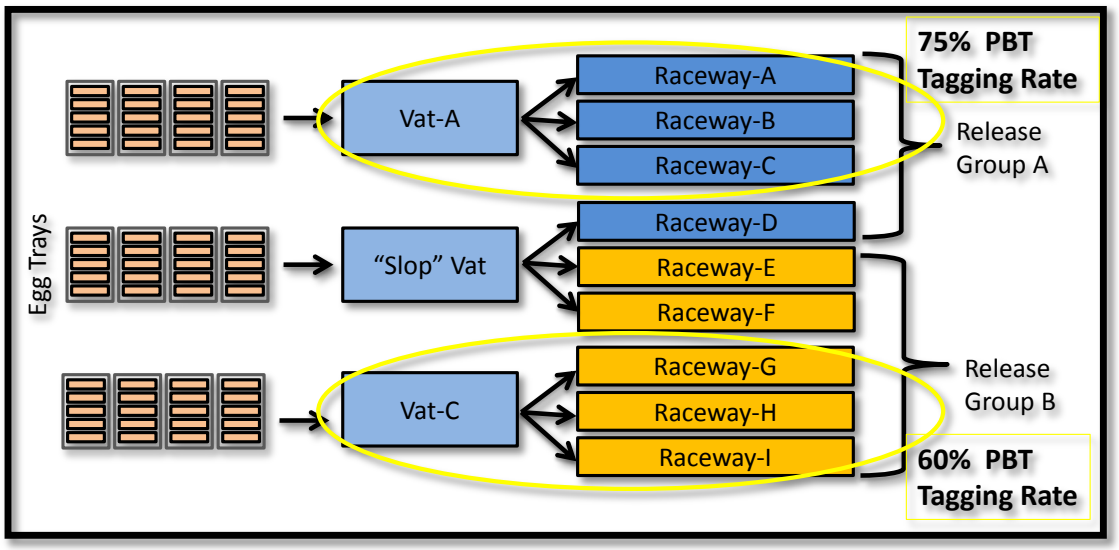


Figure II.B.5. Diagram showing managed splitting and the use of a "slop" vat which maintains integrity of release groups and but reduces overall PBT tag rate.

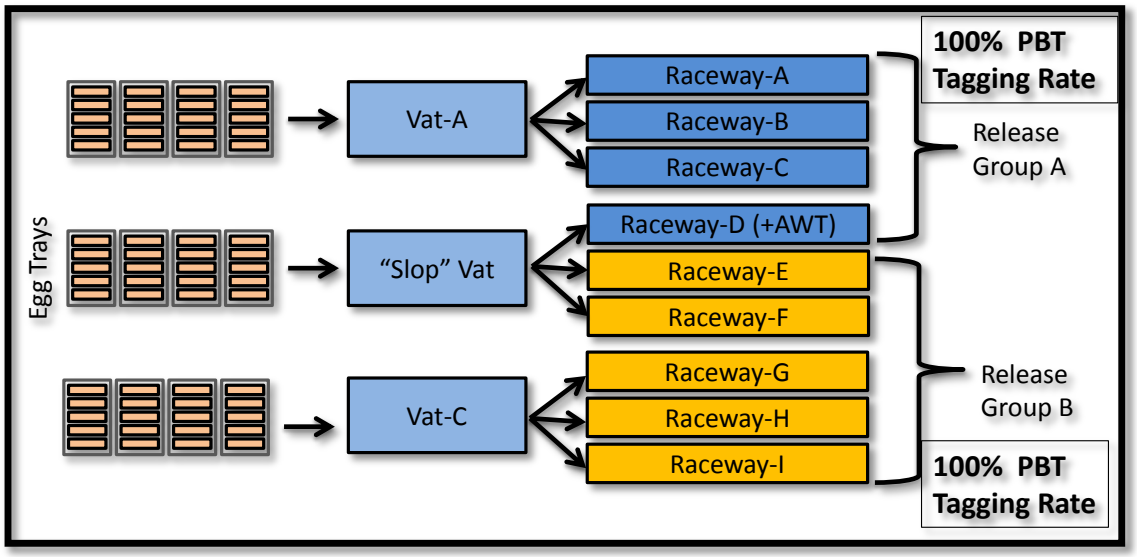


Figure II.B.6. Diagram showing managed splitting and the use of a "slop" vat. In this case, fish from the "slop" vat are differentially marked depending on which release group they are used for. This has enabled Snake River basin managers to maintain a 100% tagging rate.

Estimated PBT tagging rates for Idaho hatchery steelhead

PBT tagging rates for Idaho hatchery steelhead can be estimated at the hatchery stock, rearing stock, and release group level. For brood years 2012 and 2013, average PBT tagging rates at the hatchery stock level were 98.0% and 96.9%, respectively. At the rearing stock level, they were 98.0% and 96.9%, respectively. At the release group level, they were 89.1% and 91.8%, respectively (Tables II.B.1 and II.B.2). It should be noted that if single parentage assignment was possible, all of these tag rates would approach 100%.

Table II.B.1. Estimated PBT tagging rates at the release group level for Snake River basin steelhead brood year 2012. For each release group, the total unique families genotyped, total females spawned and genotyped, total males spawned and genotyped, number of smolts released and number tracked via PBT, and the estimated PBT tagging rate are shown. AD = adipose-clip, ADint = adipose intact, ADintCWT = adipose intact with CWT.

Hatchery Stock	Rearing Stock	Release Site	Mark/Tag	Total Unique Families Genotyped	Total Females Genotyped	Total Females Spawned	Total Males Genotyped	Total Males Spawned	Number of smolts Released	Number of PBT tagged smolts tracked to release group	Estimated PBT tagging Rate
DWOR	Clearwater	Meadow Cr.	AD	65	65	65	53	53	258,967	258,967	1.000
DWOR	Clearwater	Meadow Cr.	ADint	21	21	21	21	21	71,484	71,484	1.000
DWOR	Clearwater	Newsome Cr.	ADint	27	27	29	29	29	121,648	113,258	0.931
DWOR	Clearwater	Red House Hole	AD	50	51	51	50	51	208,673	204,581	0.980
SFCLW	Clearwater	Meadow Cr.	AD	68	68	69	64	64	144,286	142,195	0.985
SFCLW	Clearwater	Meadow Cr.	ADint	33	33	33	27	27	152,761	152,761	1.000
DWOR	Dworshak	Dworshak NFH	AD	357	371	380	328	338	1,245,219	682,236	0.547
DWOR	Dworshak	Clear Cr.	AD	99	104	107	94	97	307,057	-	0.000
DWOR	Dworshak	Red House Hole	AD	151	153	155	132	134	399,753	-	0.000
DWOR	Dworshak	Lolo Cr.	Adint	36	36	36	34	34	208,761	208,761	1.000
EFNAT	Hagerman	Up. E.F. Salmon R.	ADint	32	33	34	36	37	155,612	146,462	0.941
SAW	Hagerman	McNabb Pt.	AD	26	26	26	26	26	127,179	127,179	1.000
SAW	Hagerman	Sawtooth	AD	194	194	194	194	194	842,034	842,034	1.000
SAW	Hagerman	Yankee Fk.	AD	48	48	48	48	48	214,860	214,860	1.000
DWOR	Magic Valley	Little Salmon R.	AD	48	49	49	48	49	220,162	215,669	0.979
DWOR	Magic Valley	Pahsimeroi	ADintCWT	97	98	98	83	84	75,786	75,013	0.989
DWOR	Magic Valley	Squaw Cr.	AD	44	44	44	34	34	188,535	188,535	1.000
DWOR	Magic Valley	Yankee Fk.	AD	56	57	57	47	48	250,965	246,573	0.982
DWOR	Magic Valley	Yankee Fk.	ADint	65	65	66	56	56	214,865	211,599	0.984
PAH	Magic Valley	Colston Cnr.	AD	20	20	20	20	20	94,360	94,360	1.000
PAH	Magic Valley	Little Salmon R.	AD	54	54	54	54	54	219,155	219,155	1.000
PAH	Magic Valley	Red Rock	AD	19	19	20	20	20	93,908	89,213	0.950
PAH	Magic Valley	Shoup Brd.	AD	20	20	20	20	20	93,563	93,563	1.000
USAL	Magic Valley	Pahsimeroi	ADint	27	27	27	15	15	112,571	112,571	1.000
OX	Niagara	Hells Canyon Dam	AD	62	63	63	62	63	252,613	248,603	0.984
PAH	Niagara	Hells Canyon Dam	AD	77	80	80	77	80	319,252	259,298	0.812
PAH	Niagara	Little Salmon R.	AD	111	114	114	111	114	451,040	439,171	0.973
PAH	Niagara	Pahsimeroi	AD	172	174	174	172	174	782,532	559,658	0.715
PAH	SBT Egg Box	Panther Cr.	Adint	116	118	120	118	120	N/A	N/A	0.966
SAW	SBT Egg Box	Yankee Fk.	Adint	104	104	104	104	104	N/A	N/A	1.000
Total				2299	2358	2336	2208	2177	7,827,603	6,217,762	0.891

Table II.B.2. Estimated PBT tagging rates at the release group level for Snake River basin steelhead brood year 2013. For each release group, the total unique families genotyped, total females spawned and genotyped, total males spawned and genotyped, number of smolts released and number tracked via PBT, and the estimated PBT tagging rate are shown. AD = adipose-clip, ADint = adipose intact, ADintCWT = adipose intact with CWT.

Hatchery Stock	Rearing Stock	Release Site	Mark/Tag	Total Unique Families Genotyped	Total Females Genotyped	Total Females Spawned	Total Males Genotyped	Total Males Spawned	Number of smolts Released	Number of PBT tagged smolts tracked to release group	Estimated PBT tagging Rate
DWOR	Clearwater	Meadow Cr.	AD	44	48	48	44	48	159,547	146,251	0.917
DWOR	Clearwater	Meadow Cr.	Adint	27	28	29	28	29	69,403	64,617	0.931
DWOR	Clearwater	Newsome	Adint	34	35	35	34	35	134,353	130,514	0.971
DWOR	Clearwater	Red House	AD	44	50	50	35	39	224,416	197,486	0.880
SFCLW	Clearwater	Meadow Cr.	AD	31	31	31	31	31	107,394	107,394	1.000
SFCLW	Clearwater	Meadow Cr.	Adint	47	47	47	40	40	151,280	151,280	1.000
DWOR	Dworshak	Clear Creek	AD	102	107	109	90	92	360,430	134,555	0.373
DWOR	Dworshak	Dworshak	AD	305	313	314	202	203	1,201,895	918,867	0.765
DWOR	Dworshak	Lolo	Adint	63	63	63	58	58	247,629	247,629	1.000
DWOR	Dworshak	Red House	AD	127	127	127	94	94	418,067	284,500	0.681
EFNAT	Hagerman	East Fork	Adint	11	11	12	13	13	59,209	54,275	0.917
SAW	Hagerman	McNabb Pt	AD	31	32	32	31	32	118,874	115,159	0.969
SAW	Hagerman	Sawtooth	AD	254	254	254	254	254	1,263,084	1,263,084	1.000
DWOR	Magic Valley	Pahsimeroi	Adint	46	47	47	41	42	138,195	135,255	0.979
PAH	Magic Valley	Colston	AD	16	16	16	16	16	93,986	93,986	1.000
PAH	Magic Valley	Little Salmon	AD	41	41	41	41	41	198,548	198,548	1.000
PAH	Magic Valley	Red Rock	AD	16	16	16	16	16	94,415	94,415	1.000
PAH	Magic Valley	Shoup	AD	20	20	20	20	20	93,544	93,544	1.000
USAL	Magic Valley	Little Salmon	AD	79	82	82	52	54	237,997	229,290	0.963
USAL	Magic Valley	Squaw Creek	AD	38	39	39	26	27	186,763	181,974	0.974
USAL	Magic Valley	YFK 3rd	AD	57	60	60	34	36	241,504	225,762	0.935
USAL	Magic Valley	YFK Ponds	ADint	75	80	80	55	59	265,258	210,296	0.793
OX	Niagara	Hells Canyon	AD	100	103	103	100	102	578,380	561,534	0.971
PAH	Niagara	Hells Canyon	AD	8	8	8	8	8	N/A	N/A	1.000
PAH	Niagara	Little Salmon	AD	102	102	104	104	104	441,206	384,012	0.870
PAH	Niagara	Pahsimeroi	AD	152	152	153	162	162	818,653	767,869	0.938
PAH	Pahsimeroi	Egg-Box	ADint	137	143	144	138	144	N/A	N/A	0.951
				2007	2055	2064	1767	1799	7,904,032	6,992,098	0.918

Hatchery infrastructure changes

The extent to which hatcheries will have to make organizational and infrastructure changes to accommodate PBT obviously depends on the existing infrastructure and the numbers of uniquely identifiable release groups required. In Idaho, a query of hatchery managers indicated that few infrastructure changes (purchase and/or use of additional egg trays, rearing vats, or raceways) were needed to meet the management required PBT tagging rate goals (Gary Byrne, IDFG Fish Production Manager, gary.byrne@idfg.idaho.gov; personal communication). For context, we asked hatchery managers at IDFG's Magic Valley Steelhead Hatchery (MVSH) to review the hatchery's transition to PBT tagging and tracking. We chose MVSH because it maintains the highest number of unique release groups in the Snake River basin (N = 10) and includes both fine-scale (<100,000) and moderate scale (>200,000) releases.

Magic Valley Steelhead Hatchery Transition to a PBT program

MVSH currently produces 1.6 million steelhead smolts to a target average release size of 4.5 fish per pound (fpp), or approximately 344,500 pounds annually. Both A-run and B-run steelhead are represented in the three stocks currently reared for release at ten specific release sites. PBT has been incorporated into the MVSH rearing program since the 2010 brood year production cycle.

No significant increases in rearing costs were incurred by MVSH as the facility transitioned to a PBT-based rearing and release production model. Through careful planning, hatchery staff have been able to manage discrete PBT tagged populations from eyed egg through smolt release within existing hatchery incubation and rearing infrastructure. Therefore, for the MVSH, the addition of incubators, vats, or raceways have not been needed in order to realize the number of distinct release groups required for management.

From a hatchery perspective, establishing a "PBT tracking chain" follows a similar methodology to existing protocols for tracking parent-specific pathology samples (e.g. bacterial kidney disease, infectious hematopoietic necrosis virus) from egg take through incubation to allow culling of high-risk eggs. Where 100% pathology sampling protocols are already established, the additional PBT sample collection and data management costs are minimal. At facilities that need to increase sampling and data tracking to represent 100% of brood stock for PBT, spawning operations are slower, and labor costs are moderately increased over the duration of the egg take.

In order for hatchery operations to proceed smoothly while maintaining PBT segregation, staff develops early and thorough plans for each stage of the production cycle, working back from the release of individual release groups, through fish marking, raceway loading, vat loading, and eyed egg transfer to egg take. The complexity of incubator and rearing unit loading plans is directly correlated to the number of fish stocks and release sites included in the facility production plan.

At MVSH, eyed eggs received from brood stock facilities are seeded in upwelling incubation jars, each of which is situated in one of the facility's twenty early rearing vats. Therefore, MVSH can accommodate no more than twenty distinct release groups. Prior to shipment of eyed eggs to MVSH, database inventory of PBT genetic samples from parent fish must be complete, and release group specific rearing and release plans must be developed, so that incubator jars/vats can be loaded with appropriate inventories and PBT groups. At brood stations, egg picking apparatus must be cleared between processing of each tray of eggs so that the segregation and integrity of release groups are maintained.

Maintaining segregated, release group inventories of PBT tagged fish has resulted in a reduction of flexibility relative to MVSH operations in the past. For example, a potential inventory shortfall in one rearing unit may not be covered by adding fish from other rearing units. Therefore, to ensure that inventories across all PBT release groups will be adequate to meet release goals, higher "buffers" have been incorporated into egg requests. This approach has the potential to produce an inventory of parr that is in excess of the facility's release goals (and smolt rearing capacity), but culling of the excess inventory at this early stage limits any increased feed costs associated with these fish to relatively inconsequential levels. It should be noted, that under a PBT program, managers do not have the same flexibility of providing fish for "unplanned" release groups in response to unanticipated needs encountered during later rearing, and the only way to provide this type of flexibility would be to separately rear family groups specifically for this purpose.

Estimating PBT Tagging Rates

Under a PBT program, managers will need to become more familiar with genetic tagging rates, understand how they are estimated for PBT, and be aware of how various sampling scenarios of broodstock can affect the estimation of tagging rate.

The tagging rate is estimated using one of several mathematical formulas presented below, depending on the information available, however we also present graphical illustrations to reveal the concepts behind each of the formulas.

Managers are likely already familiar with tagging rates as they apply to physical tagging technologies, such as CWTs. Estimating the tagging rate is usually straightforward for physical tags because the total number of hatchery-produced fish is enumerated, and a subset of the total is then counted and tagged allowing a direct measure of tagging rate to be calculated. With genetic tagging the approach is inherently different because the parental broodstock, not the progeny, are used to estimate the tagging rate. Using current technology both the male and female broodstock in any particular spawning cross must be tissue sampled and successfully genotyped in order for their progeny to be PBT tagged.

If all parents are successfully genotyped, then the stock is 100% tagged (e.g. all offspring can be assigned back to their parents). Currently, PBT in the Snake River basin is performed with 96 SNP markers and genotypes from these 96 SNP markers are required from both parents to perform parentage analyses. The failure to sample or genotype either parent from a spawning cross decreases the overall tagging rate for a release group. However, if larger numbers of SNP markers are genotyped than currently used (i.e. increasing from 96 SNPs to 200-500 SNPs), it is expected that it will be possible to perform single parent assignments accurately. With this advancement, the failure of sampling or successfully genotyping one parent does not reduce the tagging rate since the other parent can still be assigned with confidence. The use of single parent assignments as a backup to two parent assignments should help reach the goal of 100% PBT tag rates. When PBT tagging rates are <100%, they must be estimated. There are several simple methods used to estimate tagging rates, each with its own assumptions.

Below we review the various ways PBT tagging rates can be estimated and illustrate that some methods provide a more accurate estimate of the tagging rate under certain scenarios of incomplete broodstock sampling/genotyping. The methods described below do not incorporate variation in family size and its influence on the realized tagging rate (see section II.C).

Method 1: The 'cross information' method

This method is the most direct and accurate method of estimating the tagging rate. However, this method requires the most information about broodstock and their spawning crosses.

For this method, the identity of each individual must be known and recorded correctly for every cross. This allows the total number of crosses to be enumerated and allows crosses to be identified in which a parent was not sampled or genotyped.

In the example depicted in Figure II.B7, there are 25 crosses. Each cross is 1:1, (one male spawned with one female). We know which male is spawned with each female and we know which samples failed to genotype, allowing us to identify if failed sample was male or female.

In this example, both parents were successfully genotyped in only 21 crosses, resulting in 4 crosses that produce un-tagged offspring.

This translates to an estimated PBT tagging rate of 84%.

$$\frac{number\ genotyped\ crosses}{total\ crosses} = \frac{21}{25} = 0.84$$

If single parent assignments were possible, the estimated tagging rate would be higher (24/25 = 0.96) and only offspring from cross #10 would not be tagged.

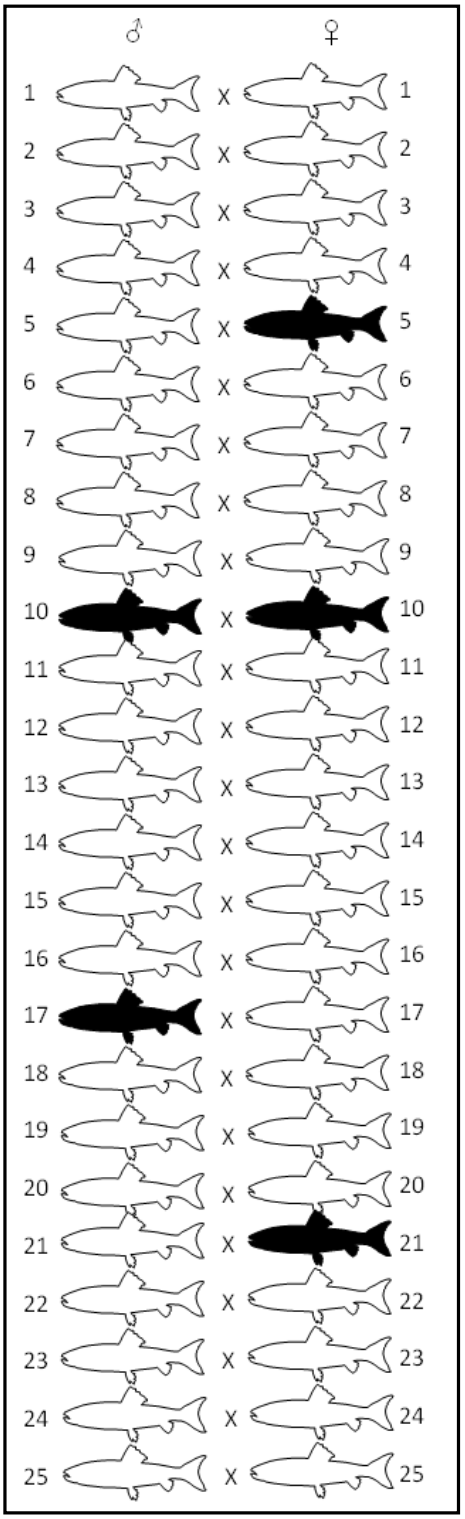


Figure II.B.7. Cross individual method. Unshaded fish = successfully genotyped parent. Shaded fish = unsuccessfully genotyped parent.

Method 1 continued – another example

In this next example (Figure II.B.8), there are still 25 crosses. Each cross is 1:1, but most males have been used twice. The identity of each spawner in each cross is known along with which samples failed to genotype.

In this case, because one of the failed samples was a male (#9) which was spawned with two females, progeny from both of these crosses are untagged.

Even though there are fewer un-genotyped broodstock than in the previous example, the estimated tagging rate is the same because of the multiple crosses with the ungenotyped male:

$$\frac{number\ genotyped\ crosses}{total\ crosses} = \frac{21}{25} = 0.84$$

Under this scenario, the ability to additionally perform single parent assignments would increase the tagging rate to 100%. For example, family 3M X 6F would be identified by dual parent assignment, but family 3M X 5F could also be identified by exclusion.

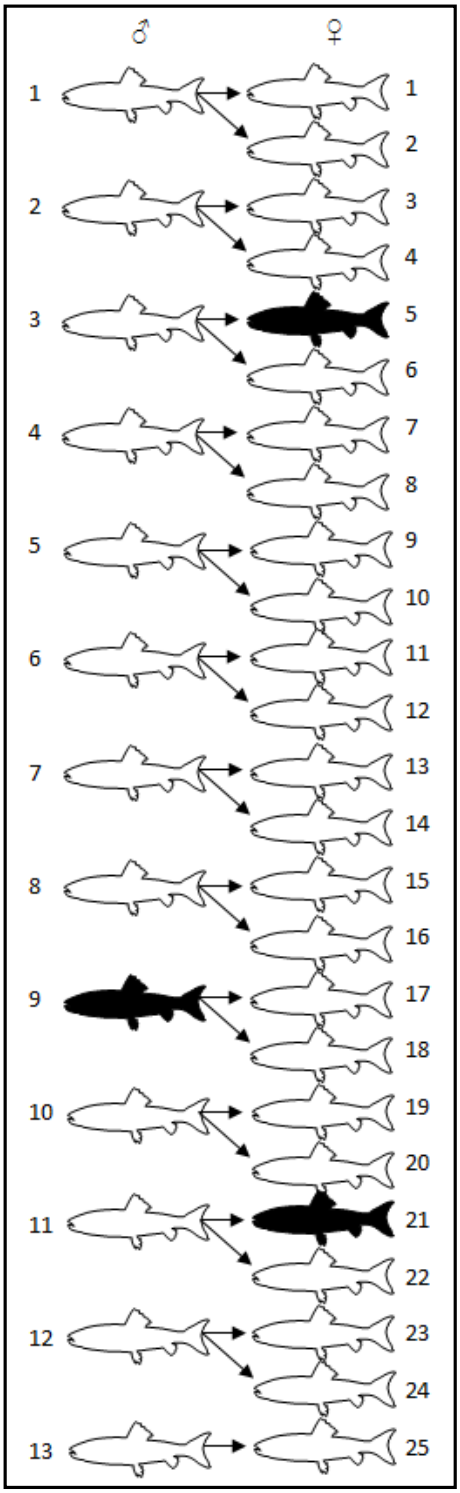


Figure II.B.8. Cross individual method with males used twice. Unshaded fish = successfully genotyped parent. Shaded fish = unsuccessfully genotyped parent.

Method 2: the '(m)*(f)' method

This second method can be used in the absence of cross information to estimate the tagging rate. For this method, only the proportions of unsampled/ ungenotyped males and female broodstock are needed. This method assumes that each male and each female is expected to participate in the same number of matings and that the probability of successfully sampling and genotyping any male or female is independent of whether the individual it was crossed with was successfully sampled and genotyped.

In the example depicted in Figure II.B.9, it is assumed that 25 crosses were spawned 1:1. Cross information is not known but we know that of the 25 males and 25 females spawned, 2 and 3 failed to be sampled or genotyped, respectively.

This translates to an estimated PBT tagging rate of 81%.

$$(prop.\,genotyped\ males) * (prop.\,genotyped\ females) = \left(\frac{23}{25}\right) * \left(\frac{22}{25}\right) = 0.92 * 0.88 = 0.809$$

This value is different from that previously calculated in Example 1 even though it is the same spawning scenario. This is because Method 1 accounts directly for the observed proportion of crosses with an ungenotyped parent.

If single parent assignments were possible, then the probability of both parents in a specific cross failing to genotype would need to be estimated (assuming random and independent failures for each sex) and subtracted from 100%. In the example, the probability of both parents of a cross failing would be 2/25 * 3/25 = 0.0096, and the tagging rate would be estimated as 1-0.0096 = 0.9904 or 99.04%.

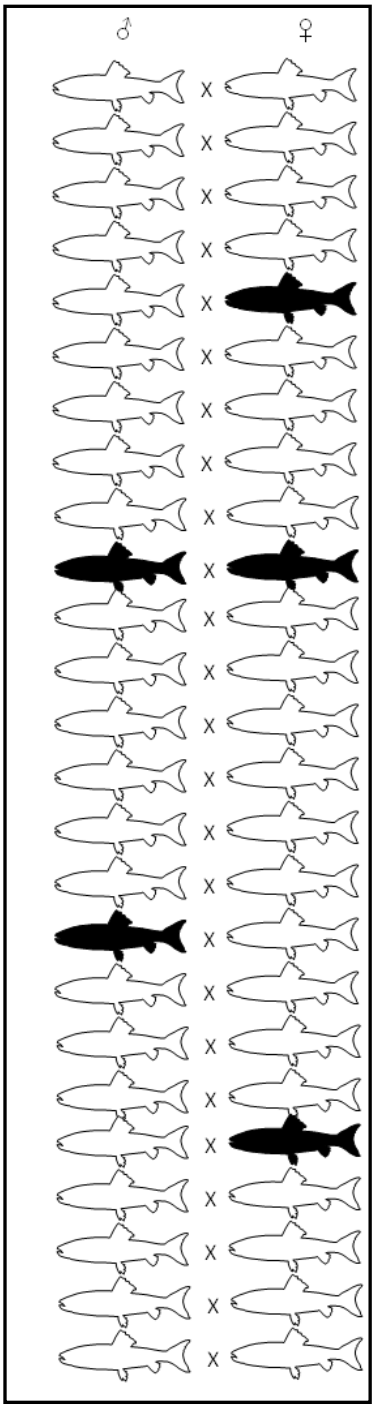


Figure II.B.9. Male * female method. Unshaded fish = successfully genotyped parent. Shaded fish = unsuccessfully genotyped parent.

Method 2 continued: another example of the male * female method.

In this example, a complicated multi-day factorial spawning protocol is used (Figure II.B.10). Cross information is available in this example and it is clear that the failure to sample/genotype female #2 results in three crosses producing untagged offspring, and an estimated tagging rate of:

$$\frac{number\ genotyped\ crosses}{total\ crosses} = 24/27 = 0.889$$

Assume now that cross information is not available and that only the proportion of successfully genotyped males and females is known. Using this information the tag rate can be estimated with the second method as:

$$(prop.\ genotyped\ males) * (prop.\ genotyped\ females) = \left(\frac{9}{9}\right) * \left(\frac{8}{9}\right) = 1.0 * 0.889 = 0.889$$

Again, this method only provides an accurate estimate of the tagging rate if the corresponding assumptions are met.

Under these scenarios, if single parent assignments were possible, the tagging rate would be 100% since all families would be identifiable by the male parent.

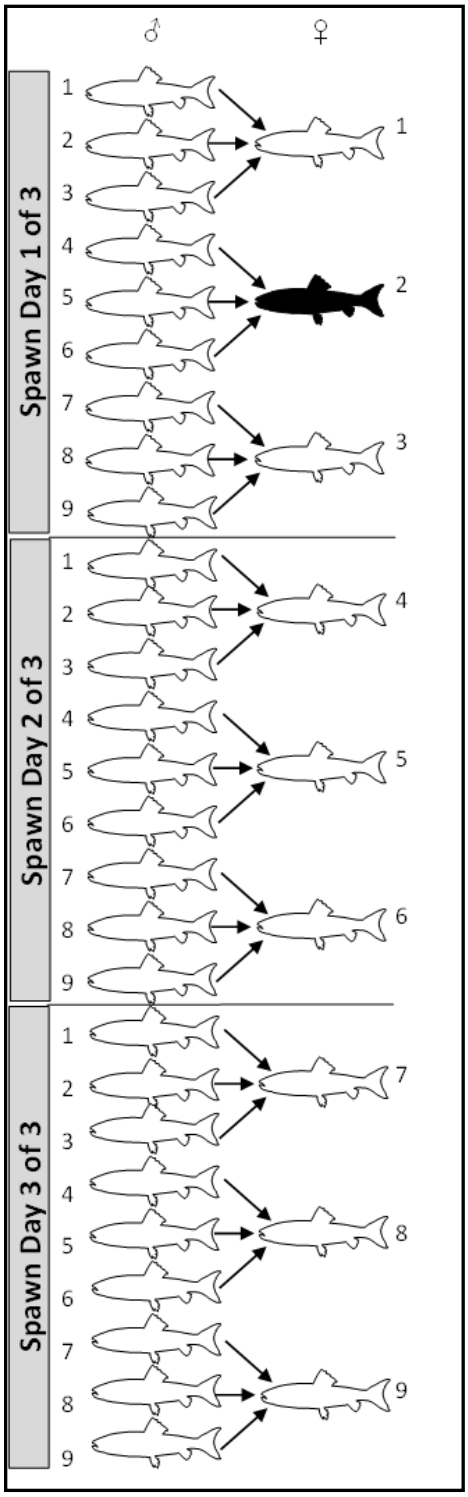


Figure II.B.10. Another example of the male * female method. Unshaded fish = successfully genotyped parent. Shaded fish = unsuccessfully genotyped parent.

Method 3: the 'squared' method

For this final method, neither cross information nor sex of the sample is available to estimate the tagging rate. In this example we will again assume 25 crosses made 1:1; however, in this case we know only that of the 50 samples, 5 failed to genotype (Figure II.B.11).

This yields an estimated PBT tagging rate of 81%:

$$(prop.genotyped\ samples)^2 = \left(\frac{45}{50}\right)^2 = (0.9)^2 = 0.810$$

Because we don't know which of the failed samples are male and which are female, we assume that the proportion of successfully genotyped samples is equal for each sex. These proportions must then be multiplied together as they were in the previous example. In this case, because the proportions are equal, we can simply take the square of this value.

This value is similar to that calculated in Method 2, example 1, and should be similar if the assumptions are not violated. This method also assumes that the probability of an individual fish being unsampled or failing to genotype is the same regardless of whether the fish that it is crossed with is unsampled or fails to genotype. It also assumes that within each sex, each individual participates in the same number of matings.

In this example, if single parent assignments were possible, the probability of both parents of a cross failing to be genotyped would be 2/25 * 3/25 = 0.0096, and the tagging rate would be estimated as 1-0.0096 = 0.9904 or 99.04%.

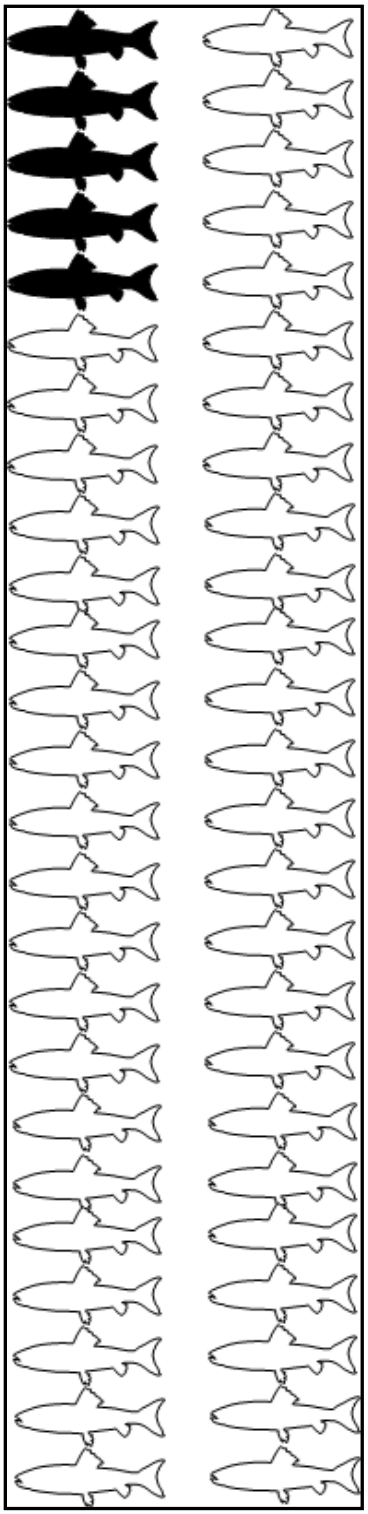


Figure II.B.11. The "squared" method. Unshaded fish = successfully genotyped parent. Shaded fish = unsuccessfully genotyped parent.

Case Study 1: incomplete broodstock sampling

Invariably, cases will arise that violate the assumptions of Methods 2 and 3 leading to an inaccurate estimate of the PBT tagging rate.

Consider the example depicted in Figure II.B.12, in which the last five crosses were not sampled.

Using Method 1, the tagging rate is correctly estimated with either dual or single parent assignment as:

$$\frac{number\ genotyped\ crosses}{total\ crosses} = \frac{20}{25} = .80$$

Using Method 2, the tagging rate is incorrectly estimated as 64%.

$$(prop.genotyped\ ♂) * (prop.genotyped\ ♀)) = \left(\frac{20}{25}\right) * \left(\frac{20}{25}\right) = 0.8 * 0.8 = 0.64$$

Why the difference? It is because of the assumption made by Method 2, which assumes the probability of an individual fish being un-sampled or failing to genotype is the same regardless of whether the fish that it is crossed with is un-sampled or fails to genotype.

This scenario highlights the importance of using Method 1 whenever genotyping failures are not equally distributed among individuals and sexes, as assumed by the alternative methods.

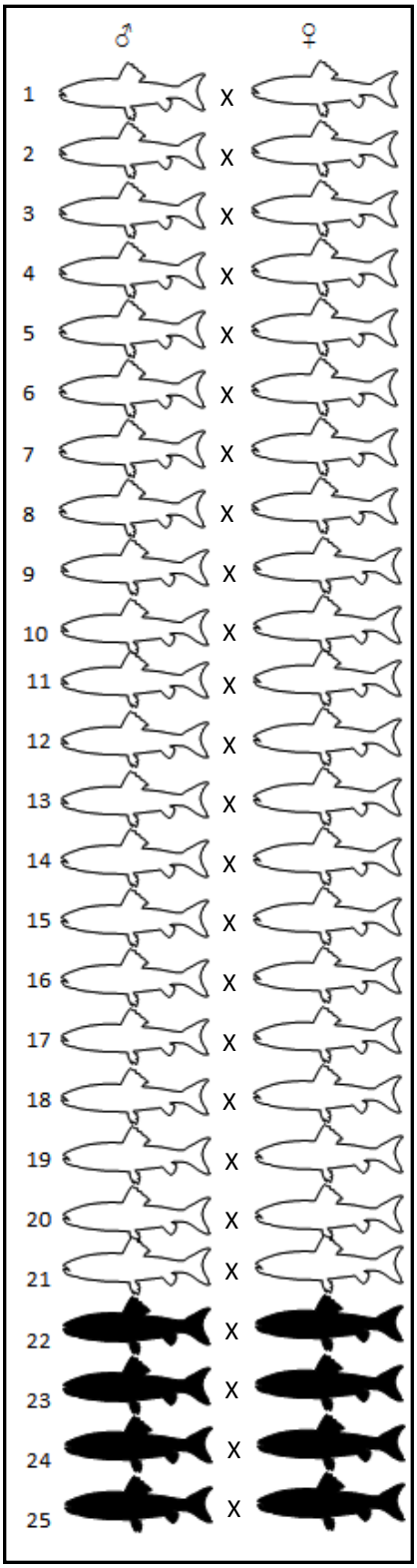


Figure II.B.12. Incomplete broodstock sampling. Unshaded fish = successfully genotyped parent. Shaded fish = unsuccessfully genotyped parent.

Conclusion

Enumerating the total number of independent crosses, and then identifying crosses that produce 'un-tagged' offspring provides most direct estimate of the PBT tagging rate. However, cross information many not always be available and other methods must be used to estimate the tagging rate. Many hatcheries already collect cross information as part of their spawning protocols. Its use in estimating the PBT tagging rate is a good argument for continuing to collect this information.

If cross information is not available, the tag rate can be estimated using Method 2 or Method 3. However, these methods will yield accurate estimates only if their assumptions are met. In large spawning programs, we expect the success/failure of sampling and genotyping are independent between fish, and that within each sex each individual participates in the same number of matings. If these assumptions are violated, then Method 1 (using cross information) must be used to estimate the PBT tagging rate. Please see section II.C for a discussion on the influence of genotyping rates and variance in family size on the realized tagging rate.

Estimating the total number of fish and marked proportion in PBT release groups

In the Snake River basin, most PBT tagged release groups are enumerated during application of ADC+CWT in the automated marking trailers. Release groups are kept segregated during this operation, and precise numbers are obtained for the total number of fish comprising each release group at that time. This operation occurs in the fall. Following that, mortality is recorded daily for every raceway/release group prior to their release in the spring. These release group-specific mortalities are then subtracted from the initial release group size to obtain an estimate of the total number of fish at the time of release for each release group. An estimate of the proportion of these fish that are marked can be similarly derived (fraction clipped in auto-trailer, corrected for later mark-specific mortalities occurring between the times of clipping and release).
There are instances when PBT release groups are not ad-clipped and not run through the automated marking trailers. For these groups, fish may be trucked off-site for release, or released on-site at the hatchery. For fish that are transported off-site, their release size is estimated from the hatchery inventory (fish-per-pound counts) and volumetrically from the transport tank just prior to release. For groups released on-site, the total number of fish is estimated based on egg enumeration, mortality records, and fish-per-pound counts.

Estimating the number of PBT tagged, and PBT tagged and marked, fish released

To estimate the number of PBT tagged fish released, the estimated release group total number at release is multiplied by the estimated tagging rate (calculated as described earlier in this section).

In the Snake River basin, individual release groups are not fractionally marked (ad-clipped). Release groups are either 100% marked or not marked at all. Outside the Snake River basin, hatcheries that fractionally mark rely on visual inspection of sampled fish for the presence/absence of an ADC. Under a fractional marking program, an estimate of the total and proportion of fish released that are tagged and marked is needed. All release group fish that are PBT tagged will not necessarily be marked (even if intended), and all release group fish marked will not necessarily be tagged (parents may not have been successfully genotyped). Having an estimate of the fraction

marked+tagged at release enables expansion of marked+tagged fish recovered later into an estimate of the total number of release group fish (regardless of mark/tag status) represented by those recoveries in the overall sample. This is required, for example, to estimate release group total harvest and escapement, as well as the proportion of hatchery origin fish in any particular sampling sector. To estimate the fraction of marked+tagged fish in a release group, the marking and tagging processes are assumed to be independent (i.e., whether an offspring's parents were successfully genotyped has no bearing on whether that fish was successfully marked). In that case, we can estimate the fraction of marked+tagged fish in the release group at the time of release as the product of the estimated fraction marked and the estimated fraction tagged. To estimate the number of tagged and marked fish released, the marked+tagged estimated fraction can be multiplied by the estimated release group total number at release.

II.C. Assessment of the degree to which this system could or could not deliver estimates of the key life history and fishery parameters that are currently delivered from the CWT program and do so with similar or better accuracy (i.e., consider errors of estimation). Identify areas or issues where implementation of PBT on a coast-wide basis seems most problematic.

The accuracy of estimates of life-history and fishery parameters derived from CWT or PBT data depend on two principal factors: 1) the number of tags that are actually recovered, and 2) the accuracy with which those tags can be expanded into estimates of the total number of release group fish represented by those recoveries. We address the question of errors of estimation by evaluating each of these factors.

Number of Tag Recoveries

Ultimately, errors of estimation for a specific stock or release group are reduced as tag recoveries from that stock or release group are increased under either a CWT or PBT system. Tag recovery rates in turn are a function of the number of tags deployed and the sampling intensity. As per-fish tagging costs using PBT are lower than for CWTs, implementation of PBT would likely allow increased tagging rates for many stocks, which would reduce errors of estimation if sampling rates were held constant and the additional fish tagged were also marked such that they would be recovered in later sampling.

However, alterations in marking, tagging, and sampling rates may interact in complicated ways and have consequences that are not always immediately obvious. For example, increasing the tagging and marking rates on stocks that are only rarely encountered in subsequent sampling would increase the information available on such stocks with little increase in the overall burden on the sampling system, but increasing the tagging and marking rates on stocks that already make up a large number of recoveries could lead to substantially increased sampling costs with marginal information benefits for these larger stocks, and this might create financial pressure to reduce the overall sampling intensity and thus the tag recovery rate for small and rare stocks.

Optimization of the marking, tagging, and sampling rates would need to be done with careful consideration of individual stocks and the constraints on tagging, marking, and sampling each in the context of a larger coastwide sampling scheme. Fully addressing this issue is beyond the scope of this report. Exploration of the effects on recovery rates from changes to the current levels of marking, tagging, and sampling rates could be done using existing systems such as PlanIt! (Morishima et al. 2012).

Uncertainty in Expansion Factors

We note that the PBT + AWT "System 1" in section II.A would essentially duplicate the number of stock-specific tag recoveries of the current CWT system. This is a good reference point from which to consider the additional factors affecting errors of estimation between PBT and CWT. The qualitative differences between CWT and PBT as they affect errors of estimation are as follows:

1) PBT tagged fish from which tissues are sampled in fishery or escapement areas may not yield successful (scorable) genotypes, and thus render their release group of origin

unknown. However the rate of genotyping failures can be quantified, and so long as the probability of such failure is not stock-dependent, can be dealt with using expansion factors the same way as is done for unreadable CWTs or cases where CWTs are lost while attempting to extract them from the fish.

2) Assignment of offspring to parents may be subject to either false positive errors (a sampled fish is assigned to a parent that is not its true parent) or false negative errors (a sampled fish has parents in the database, but is not assigned to them). With a sufficient number of genetic markers, such rates are typically low; however, there is a tradeoff: setting stringent assignment criteria in order to reduce the occurrence of false-positive errors will necessarily lead to a higher rate of false negative errors (Anderson & Garza 2006). Since there is no obvious way of correcting sample recovery estimates for false positive errors (since they are difficult to detect), it will usually be best to minimize them, and account for false negatives using expansion factors in the same manner that CWT loss is accounted for. Currently there is not a standardized way of estimating false negative rates in a particular fishery, though they can be predicted for a particular population using estimated genotyping error rates, the allele frequencies in the population, and knowledge of the degree of relatedness within the population. For PBT, it will be necessary to develop and agree upon a defensible method of estimating the false negative rate.

3) There is some uncertainty in the tagging rate achieved through PBT, due to the variance in family size, if scorable genotypes were not obtained from all the parents of the release group. If mating pairs are unknown, there is additional uncertainty in the tagging rate because it is unknown how many matings the ungenotyped parent(s) participated in. Appendix 2 addresses the first of these two issues in detail, but the main findings are summarized below.

When considering the uncertainty in the PBT tagging rate, it is important to realize that even in the case of a CWT release group, where the tag rate is estimated based on a count of tagged versus untagged individuals, the tagging fraction of adults may differ slightly from this tagging rate due to the stochastic nature of individual survival and the fact that it is unlikely that the exact proportions of tagged versus untagged individuals will survive to adulthood, even if there is no tagging effect on the probability of survival. With CWTs (and with PBT), the variance in the adult tagging rate varies inversely with the size of the release group. However, with PBT, the situation is more complex because survival to adulthood typically is correlated with family. Were the number of surviving adults per family to be Poisson distributed with constant mean, then this correlation between family and survival would not be an important factor. However, salmon populations tend to exhibit overdispersion in family size, making the effect of family-specific survival relevant to the question of uncertainty in the tagging rate. Accordingly the use of PBT for tagging has the potential to increase the variance in realized tagging rates.

The practical implications of this increased variance, however, must be evaluated in the context of the total variance of estimates obtained by sampling expansions. In

many cases, the variance due to sampling only a small fraction of each release group far outweighs the additional variance from the PBT tagging rate variance.

Appendix 2 describes simulations exploring the role of three main factors influencing the variance in the realized PBT tagging rate: 1) the variance in family size (which can be expressed in terms of the ratio of the effective number of spawners to the actual number) amongst the spawners contributing to a release group; 2) the number of families (distinct parent pairs) used to produce the juveniles in the release group; and 3) the fraction of parent pairs contributing to the release group whose offspring are successfully tagged, which, in turn, is a function of the fraction of the parents of the release group for which successful genotypes were obtained. However, the influence of the variance of the realized PBT tagging rate on expanded estimates of the total number of release group fish represented by tag recoveries is dependent on the rate of sampling in the recovery stratum. When the fraction of fish recovered from a release group recovered is expected to be less than 25 or 50% of the total present in the sample stratum, there are many cases in which the extra uncertainty due to variance in the realized PBT tagging rate is negligible compared to the large amount of uncertainty due to the sampling rate itself.

The simulations indicate that all four of the following conditions must be met in order for the variance in the realized PBT adult tagging rates to create noticeable decreases in accuracy of PBT-based estimates of sample-expanded tag recoveries:

1. Release groups are composed of offspring of fewer than 30 parent pairs.
2. The ratio of the effective number of spawners to the actual number of spawners contributing to a release group is lower than 0.88.
3. More than 50% of all the fish from a release group present in a recovery area are expected to be marked and sampled.
4. Fewer than 96% of families contributing to a release group are successfully tagged via PBT.

Appendix 2 should be consulted for the magnitude of the effects of changes in each of these conditions.

In connection with condition #4, it is important to recognize that, if parent pairs are required for parentage assignment, the expected fraction of families with both parents successfully genotyped decreases with the square of the fraction of successfully genotyped parents. Thus, if 95% of male parents and 95% of female parents are successfully genotyped, one expects only 90.2% of the families to have both parents genotyped and hence have offspring that are tagged. With a sufficient number of markers to perform single parent assignments, however, the fraction of tagged families would decrease much more slowly with the fraction of successfully genotyped parents, because both parents of a family must be unsuccessfully genotyped for the offspring to remain untagged. For example, even if only 86% of male and female spawners are successfully genotyped, the tagging rate of their offspring would still be expected to be higher than 98% (see also section II.B).

Appendix 2 describes the data and analyses used to determine how frequently the above four conditions are likely to be encountered given the practices in current tagging and sampling programs. In summary:

- Condition 1 may be encountered frequently. More than 50% of release groups in the last decade were small enough that they could have been produced by fewer than 25 parent pairs.
- Condition 2 is usually encountered. Data from hatchery programs suggest that the ratio of effective to actual number of spawners can be expected to be between 0.3 and 0.7 in salmon hatcheries.
- Condition 3 is infrequently encountered in ocean fisheries. Only a small fraction of CWTs are recovered in situations where the product of the sampling rate and the mark rate on tagged fish from the release group exceeds 50%. However, a much higher fraction of fish may be sampled in terminal recovery areas.
- Empirical data from a five-year old PBT program in Idaho (Appendix 1) indicates that the rate of individual genotyping success can be maintained at a level near 98%. With such rates, using parent-pair assignments for parentage yields a family tagging rate of near 96%. However, were single parent assignments possible, this genotyping success rate would yield family tagging rates of effectively 1.0, at which point the variance in the realized PBT tagging rate is effectively 0.

In conclusion, the additional variance in estimates of fishery and life history parameters incurred due to variance in realized the PBT tagging rates is likely to be negligible in a PBT program if it is capable of making accurate single-parent assignments.

Other Considerations

Related to the increase in the PBT tagging rate variance due to the variance in family size is the additional complication that PBT tagging might place upon studies designed to identify survival rate differences between a set of release groups. Under PBT, a test for a significant difference in survival rates will have to parse out the contributions of the random family effects and the fixed treatment effects on the observed difference. Consequently, PBT-based studies will likely require larger release groups within each treatment to ensure that each treatment is represented by an adequate number of distinct families. Furthermore, it would likely be useful to adopt new statistical analysis methods that use the observed distribution of recovered family sizes to account for the additional family-based variance in these treatment effect studies. On the other hand, such PBT-based study designs might make it possible to detect treatments that increase the variance in family size, thus approaching the question of whether there may be treatment-by-family interactions.

II.D. Identification of additional information that could be generated from a coast-wide PBT system, over and above the kind of information that is currently generated from CWTs.

This section provides examples of how PBT could provide additional information beyond what CWTs currently provide for fisheries applications. Genetic tagging with PBT is inherently different than the typical application of CWTs because fish can be tracked over multiple generations with PBT. All fish have DNA in cells of every tissue so each fish inherits a potential internal genetic tag that can be genotyped and matched to a parent or offspring, and can be sampled non-lethally. Therefore in a PBT system, molecular markers can be used to reconstruct multi-generational pedigrees of parents and offspring which contain useful information. The following section includes examples that capitalize upon genetic information from PBT systems to address various fisheries applications. This is not a comprehensive list of potential applications for PBT, but highlights some of the key additional utilities for this technology.

Estimate fitness and reproductive success of family groups

Hatchery origin fish can have lower reproductive success than natural-origin origin fish when they spawn in the wild, but relative reproductive success can vary greatly depending on hatchery practices (e.g., segregated vs. integrated broodstock programs). Reproductive success has been shown to be highly variable among family groups but pedigrees of parents and offspring can be reconstructed with molecular markers such as those used in PBT programs. If parents are genotyped as hatchery broodstock, their offspring can be identified non-lethally at multiple life stages to estimate survival and track their reproductive success. These pedigree or parentage studies have become an effective approach to determine fitness of hatchery origin fish spawning in nature along with natural-origin fish (Araki et al. 2007; Williamson et al. 2010; Hess et al. 2012). These mating events and multi-generational pedigrees are only possible to track with molecular markers such as those used for PBT and this application is not possible with CWTs since these tags are not inherited.

Estimate heritability of biological traits over generations

Many biological traits of salmonids such as run-timing, age and size at maturity, growth rate, smoltification, and thermal tolerance have an underlying genetic component that is heritable from parents to offspring. Since PBT programs provide extensive pedigree information for parents and offspring, this information could be coupled with measurements of fish characteristics (phenotypes) to estimate heritability of these traits. For example, this approach has been used to estimate heritability of "jacking" in Chinook salmon hatchery programs in the Columbia River Basin (e.g., Ford et al. 2012). Hatcheries with PBT programs provide pedigreed stocks to investigate traits such as jacking and estimate heritability rates across multiple stocks. Results such as these contribute to an understanding of the genetic basis of biological traits and will likely lead to improved hatchery practices. While it is possible in a hatchery to apply a CWT code that is unique to each family that could be used to estimate heritability of traits, it is not common to do this and requires facilities that can accommodate the separate rearing of

individual families of offspring (e.g., Livingston Stone National Fish Hatchery winter-run Chinook).

Estimate stock of origin from non-lethally sampled stocks

Recovery of genetic tags can be done non-lethally by sampling a small piece of fin tissue and releasing the fish. There are several applications where non-lethal recoveries from PBT tagged fish would provide useful information for fisheries management and conservation. This includes estimates of stock specific abundance and run-reconstruction within major river basins (Schrader et al. 2013; Rawding et al. 2014), and selective fisheries with sublegal-sized discards and/or non-retained unmarked fish. Non-lethal sampling is possible by collecting relatively small tissues (e.g., portion of a fin or opercle) that are stored in ethanol or by desiccation, which preserves the tissue for DNA to be extracted and genotyped in a laboratory. Genotypes can then be matched to broodstock parents in order to identify hatchery of origin. Non-lethal tag recoveries are not possible with CWTs that require head-removal to recover the tag, but some other tag types, such as PIT tags, are also capable of providing non-lethal tag recoveries.

Broodstock management to maximize and maintain genetic diversity

Since all broodstock would be genotyped in a PBT-based system, genetic diversity of hatchery stocks could be monitored annually, and over multiple years. Annual monitoring could include procedures to estimate relatedness of broodstock and determine breeding matrices that would decrease inbreeding and maximize genetic diversity of hatchery stocks, particularly in programs used for supplementing wild populations at risk of extirpation. Estimates of genetic diversity and effective population size could also be monitored over several years to examine relative genetic diversity among hatchery stocks and to compare with local natural-origin stocks. While it is possible in a hatchery to apply a CWT code that is unique to each family that could be used to minimize inbreeding for hatchery broodstock, it is not common to do this and requires facilities that can accommodate the separate rearing of individual families of offspring.

Efficiently Achieve High Tagging Rates

High tagging rates (90-100%) are possible with any type of tag in a hatchery setting, but this can be achieved very efficiently with PBT since adult broodstock are genotyped in order to tag large numbers of offspring. For example, for each pair of Chinook salmon broodstock that are genotyped in a hatchery, approximately 3800 released smolts are PBT tagged. In scenarios where stocks are PBT tagged at a high rate and PBT tags can be recovered non-lethally or cost efficiently relative to CWTs, this may be an effective solution to enhance applications such as:

-Identify hatchery of origin for stray fish among river drainages.

While dispersal among natural populations of salmonids is a vital component to maintain genetic diversity and recolonize habitats, excessive levels of straying are expected to reduce advantages of locally adapted stocks. Thus, identifying hatchery strays and estimating stray rates among drainages are important to monitor. Since PBT programs are expected to provide approximately 90-100% tagging rate of offspring from hatchery broodstock fish, strays among drainages may be identified if tissue samples are

genotyped and assigned to parental broodstock fish. Non-lethal sampling to recover PBT tags such as at weirs or other trapping facilities allows identification of the origin of hatchery strays and would allow for estimation of straying rates if adequate sampling designs are in place to recover tags (e.g., Ford et al. 2015). While strays can also be identified using CWTs, PBT enables non-lethal sampling and expected higher tagging rates for many stocks that are not already tagged at a high rate with CWTs.

-Estimate proportion of hatchery origin fish on spawning grounds

Reliable estimates of the proportion of hatchery-origin adults in spawning areas are needed to assess population status and potential for interbreeding with natural-origin adults. Estimates of these proportions should be hatchery- or release-specific so that it is possible to identify the sources of the hatchery-origin fish on the spawning grounds. To identify hatchery-origin fish on spawning grounds, some hatchery releases are given visible marks, some are tagged with CWT, or PBT, or all three. Since approximately 90-100% of hatchery origin fish from PBT programs are expected to be tagged, large numbers of hatchery origin fish can be identified. Estimating the proportion of hatchery origin Chinook salmon on spawning grounds with PBT has been demonstrated (e.g., Rawding et al. 2014). Precision in the estimate of hatchery spawners is expected to increase as tag recoveries become higher in a carcass survey, regardless of tag type. Thus, in a particular setting, if stocks that are tagged at a higher rate in a PBT-based system than current CWT programs, estimates may be more precise than CWT-based estimates because of a higher number of PBT tag recoveries. In scenarios where stocks are PBT tagged at a high rate and PBT tags can be recovered non-lethally or cost efficiently relative to CWTs, this may enhance this application.

II.E. Identification of any qualitative benefits that might be realized if PBT were adopted (e.g., no need to remove heads on fish destined for "whole fish" market; no issues re cooperation of fishermen with recovery of heads).

Adoption of a PBT program in place of the CWT program would confer several additional benefits. These are due to the need to take only a small sample of tissue (e.g., fin clip) for tag recovery with PBT, whereas it is necessary to remove the entire head for tag recovery with CWT. Removal of a fin clip only would increase value of these "head-on" tagged fish in the "whole fish" market, and would not remove the head weight from the total weight used by wholesalers when purchasing such fish from commercial fisherman, as is current practice. It may also increase tag recoveries in recreational fisheries from anglers who are sometimes reluctant to allow the heads of their fish to be removed by survey samplers and decrease the burden of sampling programs on commercial fishermen compared to the current CWT program.

The segregation of egg lots for PBT at the release group level, and careful attention necessary to their fate from fertilization to release, could improve record keeping and data collection procedures at many hatcheries and facilitate implementation of elements lacking from monitoring and evaluation programs (e.g. California Hatchery Scientific Review Group 2012).

II.F. Assessment of whether or not the PBT concept could be applied to tagging of wild stocks, specifically when access to parent spawners is impossible or impractical.

The principles of Mendelian transmission of genetic markers apply to all fish, regardless of origin (i.e. hatchery or natural), so PBT can be used with natural-origin fish in the same way that it can with hatchery-origin ones. However, the application of PBT is constrained by the ability to sample fish that will spawn or have spawned, and applying an ancillary mark (e.g., ADC) to the PBT tagged offspring may not be an option. When natural-origin fish pass through a weir or over a fish ladder with a fish trap while returning to spawning grounds, it is straightforward to take tissue samples and apply parentage analysis to derive tagging information, although estimating the tagging rate when <100% of potential parents are trapped, sampled, and successfully genotyped is not always straightforward. In the same way, natural-origin fish that enter traps in hatcheries that are at the termini of anadromy, but are released so that they may spawn in natural areas, can also be tissue sampled and PBT applied to derive tag information. In the absence of any such trapping capacity, it is also feasible to sample carcasses and apply PBT to derive tag information (Rawding et al. 2014). However, the analysis of carcasses does typically have a higher genotyping error rate than the analysis of live or freshly dead fish.

Another option for applying genetic tagging to natural-origin stocks is to capture, and mark juvenile fish in the same way that coded wire tagging is conducted with such stocks, but instead of inserting a CWT a tissue sample is taken and genotyped. Some of these fish would then be recaptured at later life stages in fisheries or at escapement and identified (i.e. tag recovered) through a "matching genotypes" analysis. Elsewhere in this document we have referred to this approach as "genetic fingerprinting". While this application would serve the purpose of tagging natural-origin stocks with a genetic technique and using the same genetic data as PBT, it does not employ parentage analysis and does not have the tagging efficiency of PBT, but would allow marking of all genotyped smolts as they were handled and thus facilitate recovery of the tagged fish in harvest and escapement sampling. However, the genotypes collected from juvenile fish could serve as the basis for PBT with fish from the next generation, although this would require additional information and several assumptions regarding sampling fraction, mortality, straying rate, age-at-spawning, and the distribution of reproductive success.

With the exception of applications involving 100% passage trapping efficiency (i.e. ideal weirs), these other applications would not be expected to sample both parents for all juveniles produced in a given area, so would involve a combination of parent-offspring trio and single parent-offspring pair analysis to recover tags from progeny of all sampled individuals. Unless 100% trapping efficiency of both all upstream-migrating spawners and all of their downstream-migrating offspring could be achieved at the same point in space, it would not be possible to apply a mark (e.g. an ADC) unambiguously indicating the presence of genetic tags in all tagged natural-origin offspring, and thus sampling schemes for tag recovery would need to be modified to sample sufficient numbers of unmarked fish.

For additional information on this topic, including aspects of efficiency, see sections II.A.1 and II.H.1.

II.G. Assess more limited and targeted applications of the PBT technology that could cost-effectively supplement or replace "parts" of the existing CWT system.

Section II.D identifies numerous kinds of supplemental information that a PBT-based system would provide. System 5 in section II.A describes a hybrid system using PBT on most hatchery production and CWT for natural-origin stocks, DIT groups, other special cases where fish are tagged but not marked, and possibly also small hatchery release groups and unplanned releases. Sections II.A and II.F describe how PBT could be used to tag, and recover tags from, natural-origin indicator stocks from which it is impractical to handle and mark/tag a large number of smolts, but fish could still be genetically tagged through parent genotyping. In theory, a hybrid system could be developed where hatchery stocks (and natural-origin stocks with adequate smolt access) would still be marked, tagged, and sampled as in the current CWT-based system while additional genetic sampling of unmarked fish would provide information on natural-area indicator stocks tagged through PBT.

II.H. Assess the degree to which additional specific issues (see Appendix A) might rule out feasible or cost-effective application of PBT (for fisheries management purposes) on a coast-wide basis.

1. Is there any way to efficiently apply the PBT concept to wild stocks? For example, some wild AK populations that have no hatchery indicators are currently CWT'd (wild smolts), but access to adults for PBT is essentially impossible?

No. The efficiency of PBT lies in its ability to genetically "tag" thousands of offspring through the sampling and genotyping of only one or both of their parents. Without near 100% trapping efficiency when sampling natural-origin adults returning to a stream, PBT technology could not be adopted for natural-origin stocks without high uncertainty in tagging rates, and marking of tagged juveniles would also be problematic (See also section II.F, and the description of natural-origin tagging and sampling schemes in section II.A, for further discussion of alternatives when adult spawners are accessible.)

However, it would be possible to genetically tag natural-origin juveniles in a manner that allowed them to be recovered in fisheries being sampled for PBT tags. For example, in any system where smolts can be handled, they could simply be genotyped rather than CWT'd, and then when fish are sampled later, their genotypes would be compared against the smolt database as well as the parent database (i.e. genetic fingerprinting). This would be a genetic tagging alternative that could be compatible with a coastwide PBT-based system.

Replicating the current coastwide CWT system for tagging of natural-origin smolts would require genetically fingerprinting approximately 1,100,000 smolts each year (Table II.I.6). Not all of these stocks are currently used as indicator stocks in PSC models (Table II.H.1). According to PSC documents there are currently 14 natural-origin stocks used in PSC models (PSC 2008, PSCTR 25 Tables 4-2, 4-3, and D-2; PSC 2014, TCCHINOOK(14)-1). There are 9 coho stocks totaling ~160,000 tagged fish per year, and 2 Chinook stocks totaling ~240,000 tagged fish per year for a total of ~400,000 fish that would need genetic fingerprinting. Even this smaller figure represents more than the estimated hatchery broodstock genotyping required coastwide (see section II.I). It would likely be more economical to employ a hybrid system where natural-origin stocks are CWT'd, such as system 5 in section II.A.

Table II.H.1. Summary of natural-origin indicator stocks currently used in PSC models (PSC 2008, PSCTR 25).

Species	MU (Code)	MU	Stock	Recent Tagging Level	Indicator Needed?
	SEAK			10,000	
Coho	NOASKA	Wild	Ford Arm Lake		Possibly
	SEAK			11,000	
Coho	NOASKA	Wild	Nakwasina River		Possibly
	SEAK			28,000	
Coho	SIASKA	Wild	Hugh Smith Lk		Possibly
	SEAK			13,000	
Coho	SOASKA	Wild	Chuck Creek		Possibly
Coho	QUEENC	Wild	Deena Cr	21,000	Yes
Coho	JNSTRT	Wild	Keogh	17,000	Possibly
Coho	FRSUPP	Wild	Eagle River	14,000	Yes
Coho	SKAGIT	Wild	Baker River	17,000	Possibly
Coho	HOODCL	Wild	Big Beef	28,000	Possibly
Chinook	LCOL	Wild	Lewis (fall Bright)	155,120	Yes
Chinook	UCOL	Wild	Hanford (fall Bright)	85,027	Yes
			Total	399,147	

2. How could PBT be used for mark-selective fisheries evaluation? Is there any possible DIT analogue for PBT and, if so, what would the sampling requirements be to achieve the equivalent of DIT groups?

Several options for this are described in section II.A. The most straightforward analogy to the current system would be to use AWT ("blank" wire) and ETD such that unmarked fish could be appropriately targeted for sampling, or an alternative mark such as a ventral fin clip if mortality associated with the alternative mark was acceptable and predictable, and assuming fishermen did not treat fish with alternative marks differently from fish with no mark at all. Alternatively, unmarked fish could be genotyped. Since PBT is relatively inexpensive to deploy on a per-tag basis compared to costs of genotyping individual tagged fish upon recovery, the number of genetically tagged fish in the unmarked components of DIT paired releases could be increased, allowing a lower sampling fraction of unmarked fish (assuming current recovery rates are adequate).

3. Coast-wide coordination of PBT databases and analyses would be required to implement a useful scheme. What genetic data would be reported and to whom? (e.g., just summaries of assignments of sampled fish to PBT parental groups, or genotypes for individual sampled fish)?

See section II.A.5.

4. Achieving the equivalent of CWT release groups (where hatchery fish are released at different times/location/sizes/methods) using PBT would appear to require that all progeny from a particular set of genotyped and spawned parents are held separately from others throughout their rearing prior to release. Would significant new hatchery infrastructure be needed to support such separation of progeny from different sets of genotyped parents? Also important is ensuring that tagged fish are "representative" of all hatchery releases of the same type/time of release. How could this be accomplished? Finally, how could PBT be used to achieve the equivalent of "unanticipated" CWT groups that might need to be released in response to events (e.g., drought or unusually low flows) that could not have been foreseen at the time when parents were spawned?

Section II.B describes a system for tagging multiple release groups using PBT. The need for new hatchery infrastructure would be hatchery-specific and we did not attempt a comprehensive survey of every Chinook and coho hatchery program coastwide.

"Representativeness" of untagged fish by tagged fish could most easily be ensured by attempting to genotype all broodstock, such that a large fraction of fish would be tagged. For Snake River steelhead hatcheries using PBT, tag rates have typically been 90%+.

"Unanticipated" release groups would be difficult to accommodate in a PBT framework, but keeping family groups separate for as long as possible would maximize flexibility. With sufficient facilities available, one or more groups of fish derived from distinct parent pools could be kept in reserve in case they were needed for unplanned release groups, otherwise they could be merged with an existing release group and information in the parent database adjusted to reflect this. It might also be possible, at substantial cost, to genetically fingerprint all individuals going into an unplanned release group, and cross-reference this set of genetic fingerprints when attempting to assign parents from sampling strata where such releases might be recovered. Finally, in a "hybrid" system such as System 5b of section II.A, CWTs could be deployed to tag unplanned releases.

5. How feasible would it be to develop a consistent and effective coast-wide set of SNPs that could be used at all laboratories, along with a consistent and mutually agreed upon procedures for tissue handling, genotyping, QA/QC, data management, and algorithms for generating assignments to PBT groups?

Previous efforts to standardize genotype data among genetics labs demonstrate that it is feasible to develop a consistent and effective panel of markers that could be used for coastwide PBT purposes. We estimate that 200-500 SNP markers would be adequate for parentage assignment for various stocks of Pacific salmon throughout their range. Recent efforts to develop PBT in the Snake River basin have shown that it is relatively easy to standardize SNP markers and lab procedures (tissues, quality control measures, databases) in order to obtain genotypes that are transferrable and allow for accurate parentage assignment (Appendix 1), and we expect that agreement on molecular genetic technical details and the set of markers could be reached within a year of initiating a serious effort.

The program SNPPIT can perform assignment of offspring to parent pairs with

high efficiency and at the scale required for a coastwide program. Software capable of single-parent assignment at the required scale is not currently available, but we anticipate the SNPPIT framework could be extended to include this capability within a similar timeframe to that required for reaching agreement on a coastwide panel of 200-500 SNP markers.

6. Would detection of PBT-tagged groups occurring at very low proportions in fisheries be a more serious problem for PBT than for CWT?

Not if the PBT-based system is designed carefully. Small numbers of tags are always a problem, regardless of tag-type. System 1 proposed in section II.A would be expected to match the current CWT-based system in recovering tags from small release groups because sampling based on visual detection of ADC, plus ETD in some areas to detect AWT (blank wire) which would be deployed the same way CWT are currently, could proceed as in the current system. Some of the alternative sampling schemes described in section II.A might actually allow for increased overall sampling fractions, allowing higher recovery rates of low abundance/proportion tag groups at similar overall sampling costs. Similar adjustments to avoid "oversampling" of large release groups might also be possible in the CWT-based system, although using AWTs as a secondary "mark" of fish that should not be given a high sampling priority would not be possible (a secondary mark such as a ventral fin clip might be used, but carries complications). Of course, if mark rates for large production hatcheries were to increase to accompany higher tagging rates, that would cause either sampling to be more expensive (because more fish would be genotyped) or some loss of information on small releases (if sample rates decreased in response to increasing sample genotyping costs), but this is not a necessary component of PBT.

7. The ability to use electronic detection to locate fish (heads) with CWTs provides an efficient way to screen out 'untagged' fish from fishery or escapement samples. This reduces costs associated with shipping, storing and dissection. Could there be a PBT analogue for this capability?

Yes. Most directly, AWTs ("blank" wire) and ETD could be used to identify which fish should have tissues collected for genotyping. It might also be possible to use a secondary mark such as a ventral fin clip.

8. *The California Hatchery Scientific Review Group has recently recommended that all hatchery Chinook salmon should be released from CA hatcheries with CWT, but that only a fraction (about 25%) should also be released with externally visible adipose fin clips. Would this marking scheme pose special problems for implementation of PBT?*

No. This system could be replicated by genotyping all hatchery spawners, with a fraction of the offspring released with ADC+AWTs and the remaining offspring released with AWTs ("blank wire") only. This would allow for real-time identification of all hatchery-origin fish via ETD, and would provide the hatchery/release-group of origin via

tissue genotyping and parentage analysis. This design is similar to System 1 (described in section II.A), except that all of the offspring would receive AWTs rather than a fraction.

II.I. Quantify the probable range of costs for implementation of a coast-wide tag recovery system based on PBT and compare the cost of this system against the costs of supporting the existing CWT tag recovery system.

II.I.1 Introduction

We estimate the costs of aspects of the current CWT-based system and several of the proposed alternative PBT-based systems described in section II.A. Our approach is to parse each system into discrete steps and multiply the number of fish handled at each step by an estimated unit cost. The alternative systems vary in the types of action required at each step (and the associated unit cost) and the number of fish handled at each step. The cost estimates presented and described in sections II.I.2, II.I.3, II.I.4, and II.I.5 represent annual operating costs only and do not include capital costs. Section II.I.6 presents a brief description of some capital costs considerations of CWT- and PBT-based systems.

The steps for which costs are estimated include 1) marking and tagging and 2) tag decoding. Marking and tagging costs for the CWT-based system include the costs of inserting a tag into juvenile fish and clipping the adipose fin of juvenile fish prior to release. Marking and tagging costs for PBT-based systems include clipping the adipose fin of juvenile fish prior to release, genotyping the hatchery broodstock, and (in some systems) the costs of inserting a blank wire tag (AWT) into juvenile fish.

To estimate hatchery broodstock, we assume that each Chinook female produces 3,800 offspring released and each coho female produces 1,800 offspring released. We further assume that the sex ratio among spawners is one male to one female in the hatcheries. Total broodstock is calculated as the total number of fish released divided by half the number of released offspring per female. The number of broodstock genotyped is therefore total releases/1,900 for Chinook and total releases/900 for coho salmon. Based on historical data from 2010-2012, this results in 135,709 Chinook broodstock genotypes per year and 87,489 coho broodstock genotypes per year if 100 percent of hatchery releases are tagged via PBT.

We acknowledge two caveats to our broodstock estimates. First, our cost calculations include some double-counting of adult fish genotypes because some fish genotyped as part of escapement sampling are also broodstock. We are unable to quantify the extent of this double-counting and sampling crews and hatchery managers will need to determine how best to track sampled and broodstock fish. As a result, our analysis overstates the number genotypes required each year by an unknown and possibly substantial amount. Second, currently some hatcheries use more spawning pairs than strictly necessary to meet production needs, in the interest of genetic diversity. Eggs are then culled to match production goals, resulting in more and smaller families among released fish. This means that our calculation of the number of broodstock may understate the number of genotypes required per year, relative to current practices.

Tag decoding costs include head lab costs (excising and reading a CWT in a laboratory) for the CWT-based system. Tag decoding costs in PBT-based systems consist of genotyping tissue from sampled adult fish. We use two genotyping unit cost estimates, one for genotyping-by-sequencing (GBS, $7 per genotype) and one for exonuclease-based techniques (ExN, $22.50 per genotype).

There are two important assumptions to be aware of. First, we do not estimate the cost of survey sampling – that is, the cost to survey catch and escapement leading up to

tag recovery. This assumes equivalent costs to screen the same proportion of the catch (typically about 20%) or escapement for ADC and to remove heads or take genetic samples as needed. This assumption is consistent with instructions in the RFP. Second, we do not include costs of performing parentage analysis on the raw genotype data. Thus, genotyping costs only include lab supplies and lab technician labor to generate and upload genotype data to a database.

Section II.I is organized as follows.

- II.I.1 Introduction
- II.I.2 Numbers of Fish and Unit Costs Assumed by Proposed Systems. Outlines the source of the numbers of fish and unit costs used in each step of the process for each proposed alternative systems.
- II.I.3 Summary of Estimated Total Operating Costs. Estimated total annual operating costs are reported for each proposed system and further broken down by species and steps.
- II.I.4 Estimation of Numbers of Fish Processed. Provides additional details on how the numbers of fish used in each system are estimated including sources of historical data.
- II.I.5 Estimation of Unit Costs. Provides additional details on how the unit costs for each step are estimated including sources of historical data.
- II.I.6 Capital Costs Discussion

II.I.2 Numbers of Fish and Unit Costs Assumed by Proposed Systems

Below, we outline the source for the number of fish and state the unit costs for each step in the process. We estimate costs for 11 alternative tagging systems. The first system is the current CWT-based system, while the remaining 10 are a subset of the PBT-based systems first defined in section II.A.

The historical numbers and unit costs referred to below are presented/derived in sections II.I.4 and II.I.5, respectively.

System 0: Current CWT-based system

1. Mark and Tag Hatchery Fish
   - ADC+CWT: Historical average ADC+CWT number of fish. Cost = $0.236 per fish in AK, BC; $0.154 per fish in WA, ID, OR, CA
   - ADC only: Historical average ADC only number of fish. Cost = $0.1095 per fish in AK, BC; $0.048 per fish in WA, ID, OR, CA
   - CWT only: Historical average CWT only number of fish. Cost = $0.236 per fish in AK, BC; $0.154 per fish in WA, ID, OR, CA

2. Mark and Tag Natural-Origin Stocks: Historical average number of natural-origin fish CWT (with or without ADC). $.236 per fish for ADC+CWT

3. Decode Tags at Head Lab: Historical average heads processed; $5 per head

System 1: PBT+AWT. 100% PBT tag rate, fractional ADC+AWT marking/tagging

1. Mark and Tag Hatchery Fish
    - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
    - ADC+AWT: Historical average ADC+CWT number of fish. Cost = $0.186 per fish (by hand), $0.104 per fish (auto-trailer)
    - ADC only: Historical average ADC only number of fish. Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)
    - AWT only: Historical average CWT number of fish. Cost = $0.186 per fish (by hand), $0.104 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $.186 per fish to handle, mark, and tag fish (ADC+AWT) plus $7 and $22.50 per fish (for genotyping).

3. Decode tags by genotyping: Historical number of sampled heads "Processed". Cost = $7 or $22.50 per genotype.

System 1a: PBT+AWT, 100% PBT tag rate 100% marking (all releases except DIT given ADC), AWT for DIT

1. Mark and Tag Hatchery Fish
    - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
    - ADC only: Historical average number of fish released less DIT fish. Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)
    - AWT only: historical number of fish CWT only. Cost = $0.186 per fish (by hand), $0.104 (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $.186 per fish (for ADC+AWT) plus $7 and $22.50 per fish (for genotyping).

3. Decode Tags by Genotyping: Historical number of ad-clipped fish sampled expanded to account for 100% ADC mark rate and maintaining DIT. Cost = $7 or $22.50 per genotype.

System 1b: Fractional PBT. Only genotype parents with marked (ADC+AWT) offspring

1. Mark and Tag Hatchery Fish
    - Genotype broodstock: Chinook broodstock = historical number of fish ADC+CWT /1,900; coho broodstock = historical number of fish ADC+CWT /900. Cost = $7 or $22.50 per genotype
    - ADC+AWT: historical number of fish ADC+CWT. Cost = $0.186 per fish (by hand), $0.104 per fish (auto-trailer)

- ADC only: historical number of fish ADC only. Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)
- AWT only: historical number of fish CWT only. Cost = $0.186 per fish (by hand), $0.104 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $.186 per fish to handle, mark, and tag fish (ADC+AWT) plus $7 or $22.50 per fish (for genotyping).

3. Decode tags by genotyping: Historical number of sampled heads "Processed". Cost = $7 or $22.50 per genotype.

System 1c: PBT+alternative mark, 100 percent tag rate and fractional marking

1. Mark and Tag Hatchery Fish
   - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
   - ADC+alternative mark: historical number of fish ADC+CWT. Cost = $0.146 per fish (by hand), $0.064 per fish (auto-trailer)
   - ADC only: historical number of fish ADC only. Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)
   - alternative mark only: historical number of fish CWT only. Cost = $0.1095 per fish (by hand), $0.064 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $.186 per fish to handle, mark, and tag fish (ADC+AWT) plus $7 or $22.50 per fish (for genotyping).

3. Decode tags by genotyping: Historical number of sampled heads "Processed". Cost = $7 or $22.50 per genotype.

System 2: PBT only; 100% PBT tag rate, 100% ADC mark, sample for recoveries according to DIT areas (ADC fish genotyped everywhere, also unmarked fish also genotyped in DIT-present areas)

1. Mark and Tag Hatchery Fish
   - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
   - ADC only = total releases (historical average) less DIT. Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $.146 per fish to handle and mark plus $7 or $22.50 per fish (for genotyping).

3. Decode tags by genotyping: In areas with DIT, historical number of fish "Sampled"; in other areas number of fish = historical ad clipped fish sampled expanded to account for 100% ADC mark rate. Cost = $7 or $22.50 per genotype.

System 2a: PBT only; 100% PBT tag rate, 100% ADC mark, genotype ALL sampled adult fish

1. Mark and Tag Hatchery Fish
    - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
    - ADC only: total releases (historical average) less DIT (historical average CWT only). Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $0.146 per fish to handle and mark plus $7 or $22.50 per fish (for genotyping).

3. Decode tags by genotyping: Historical number of fish "Sampled." Cost = $7 or $22.50 per genotype.

System 2b: PBT only; 100% PBT tag rate, 100% ADC mark, tissue from only ADC'd adult fish (no accommodation for DIT)

1. Mark and Tag Hatchery Fish
    - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
    - ADC only: total releases (historical average). Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $0.146 per fish to handle and mark plus $7 or $22.50 per fish (for genotyping).

3. Decode tags by genotyping: Historical ad-clipped fish sampled expanded to account for 100% ADC mark rate. Cost = $7 or $22.50 per genotype.

System 2c: PBT only; 100% PBT tag rate, fractional ADC mark

1. Mark and Tag Hatchery Fish
    - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
    - ADC only: Historical number of fish with ADC (with or without CWT). Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $0.146 per fish to handle and mark plus $7 or $22.50 per fish (for genotyping).

3. Decode tags by genotyping: Historical ad-clipped fish sampled. Cost = $7 or $22.50 per genotype.

System 2d: PBT only; 100% PBT tag rate, 100% ADC mark, alternative mark for sampling efficiency and DIT

1. Mark and Tag Hatchery Fish
    - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
    - ADC+alternative mark: Historical average ADC+CWT number of fish. Cost = $0.146 per fish (by hand), $0.064 per fish (auto-trailer)
    - ADC only: Total releases (historical average) = Historical values for total releases – less {historical values for ADC+CWT, historical values for DIT (CWT only)}. Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)
    - Alternative mark only: Historical average CWT only. Cost = $0.1095 per fish (by hand), $0.064 (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $.146 per fish to handle and mark plus $7 or $22.50 per fish (for genotyping).

3. Decode tags by genotyping: Historical "Processed" fish sampled. Cost = $7 or $22.50 per genotype.

System 5: Hybrid; 100% PBT tag rate hatchery releases, fractional ADC hatchery releases, CWT natural-origin stocks and unmarked DIT fish

1. Mark and Tag Hatchery Fish
    - Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
    - ADC only: Historical number of fish with ADC (including historical ADC+CWT). Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)
    - CWT only: Historical number of fish CWT only. Cost = $0.236 per fish (by hand), $0.154 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $.236 per fish to handle, mark, and tag fish (ADC+CWT).

3. Decode tags by genotyping: Historical ad-clipped fish sampled. Cost = $7 or $22.50 per genotype.

System 5a: Hybrid; 100% PBT tag rate hatchery releases, fractional ADC and alternative mark hatchery releases, CWT natural-origin stocks and unmarked DIT fish
1. Mark and Tag Hatchery Fish

- Genotype broodstock: Chinook broodstock = total releases (historical average)/1,900; coho broodstock = total releases (historical average)/900. Cost = $7 or $22.50 per genotype
- ADC only: Historical number of fish with ADC only. Cost = $0.1095 per fish (by hand), $0.048 per fish (auto-trailer)
- ADC+alternative mark: Historical number of fish with ADC+CWT. Cost = $0.146 per fish (by hand), $0.064 per fish (auto-trailer)
- CWT+alternative mark only: Historical number of fish CWT only. Cost = $0.236 per fish (by hand), $0.154 per fish (auto-trailer)

2. Mark and Tag Natural-Origin Stocks: Number of fish handled (marked or tagged) = Historical number of CWT fish (with or without ADC). Cost = $.236 per fish to handle, mark, and tag fish (ADC+CWT).

3. Decode tags by genotyping: Historical "Processed" fish sampled. Cost = $7 or $22.50 per genotype.

II.I.3. Summary of Estimated Costs

Table II.I.1 contains a list and brief description of the 11 alternative tagging systems evaluated for cost. Tables II.I.2 summarizes total costs. Tables II.I.3 and II.I.4 decompose system costs by step (hatchery fish marking and tagging, hatchery broodstock genotyping, natural-origin fish marking and tagging, natural-origin fish genotyping, and tag reading).

Table II.I.1 List of alternative systems evaluated in the cost analysis

System Number	Description
0	Current CWT-based system. Fractional CWT, fractional ADC mark that incorporates MM fisheries and DIT
1	PBT+AWT. 100 percent PBT tag rate, fractional ADC/AWT marking, and AWT in unmarked DIT fish
1a	PBT with AWT for DIT. 100 percent PBT tag rate, 100 percent ADC marking, AWT for DIT
1b	Fractional PBT+AWT. Fractional PBT tag rate (only tagged parents of marked offspring), fractional ADC marking/AWT rate
1c	PBT+AWT. 100 percent PBT tag rate, fractional ADC/alternative mark rate
2	PBT only. 100 percent PBT tag rate, 100 percent ADC mark, genotype sampled adult fish according to DIT areas (DIT information maintained by genotyping all sampled adult in DIT-present areas)
2a	PBT only. 100 percent PBT tag rate, 100 percent ADC mark, genotype ALL sampled adult fish (DIT information maintained)
2b	PBT only. 100 percent PBT tag rate, 100 percent ADC mark, genotype ADC-marked sampled adult fish (DIT information lost)
2c	PBT only. 100 percent PBT tag rate, fractional ADC mark, genotype sampled adult fish according to DIT areas (DIT information maintained by genotyping all sampled adult in DIT-present areas)
2d	PBT only. 100 percent PBT tag rate, 100 percent ADC mark (non-DIT fish), alternative mark for DIT and sampling efficiency
5	Hybrid. Hatchery releases: 100 percent PBT tag rate, fractional ADC mark. Natural-origin stocks: CWT (ADC+CWT unless exclusion from MSF is required)
5a	Hybrid. Hatchery releases: 100 percent PBT tag rate, fractional ADC mark hatchery releases, alternative mark to target sampling of ADC fish. CWT only DIT fish and other unmarked but tagged fish. Natural-origin stocks: CWT (ADC+CWT unless exclusion from MSF is required)

Table II.I.2 Total Cost Summary using Costs for genotyping are provided for two methods, genotyping-by-sequencing (GBS) at $7.00/sample and Exonuclease (ExN) at $22.50/sample. All values are millions of U.S. dollars.

	Chinook		Coho		Total	
System	Total Cost GBS	Total Cost ExN	Total Cost GBS	Total Cost ExN	Total Cost GBS	Total Cost ExN
0 CWT	14.96	14.96	3.91	3.91	18.87	18.87
1	18.46	33.28	6.74	14.88	25.20	48.16
1a	25.03	44.69	13.01	27.56	38.04	72.25
1b	17.71	30.86	6.19	13.12	23.90	43.97
1c	16.30	31.12	6.40	14.54	22.70	45.66
2	24.88	46.69	13.26	29.10	38.14	75.79
2a	25.63	49.10	17.61	43.08	43.24	92.19
2b	24.50	44.35	12.96	27.79	37.46	72.14
2c	16.12	33.81	8.27	21.25	24.39	55.06
2d	23.20	38.01	10.10	18.24	33.30	56.25
5	12.73	19.21	6.29	13.79	19.02	33.00
5a	12.24	15.84	4.22	6.88	16.46	22.73

Table II.I.3 Detailed summary of costs by step assuming genotypes performed using GBS ($7 per genotype). At 100% PBT tag rate, Chinook hatchery broodstock = 135,709 and coho hatchery broodstock = 87,489. All values are millions of U.S. dollars.

System	Mark and Tag	Genotype Parents	Handle Natural-origin Fish	Genotype Natural-origin Fish	Decode Tag	Total
0 CWT	17.71	-	0.25	-	0.91	18.87
1	14.64	1.56	0.20	7.54	1.27	25.20
1a	22.39	1.56	0.20	7.54	6.35	38.04
1b	14.64	0.26	0.20	7.54	1.27	23.90
1c	12.18	1.56	0.16	7.54	1.27	22.70
2	20.98	1.56	0.16	7.54	7.90	38.14
2a	20.98	1.56	0.16	7.54	13.01	43.24
2b	21.64	1.56	0.16	7.54	6.57	37.46
2c	10.38	1.56	0.16	7.54	4.75	24.39
2d	22.78	1.56	0.16	7.54	1.27	33.30
5	12.45	1.56	0.25	-	4.75	19.02
5a	13.37	1.56	0.25	-	1.27	16.46

Table II.I.4 Detailed summary of costs by step assuming genotypes performed using ExN ($22.50 per genotype). At 100% PBT tag rate, Chinook hatchery broodstock = 135,709 and coho hatchery broodstock = 87,489. All values are millions of U.S. dollars.

System	Mark and Tag	Genotype Parents	Handle Natural-origin Fish	Genotype Natural-origin Fish	Decode Tag	Total
0	17.71	-	0.25	-	0.91	18.87
1	14.64	5.02	0.20	24.22	4.08	48.16
1a	22.39	5.02	0.20	24.22	20.42	72.25
1b	14.64	0.84	0.20	24.22	4.08	43.97
1c	12.18	5.02	0.16	24.22	4.08	45.66
2	20.98	5.02	0.16	24.22	25.40	75.79
2a	20.98	5.02	0.16	24.22	41.80	92.19
2b	21.64	5.02	0.16	24.22	21.10	72.14
2c	10.38	5.02	0.16	24.22	15.28	55.06
2d	22.78	5.02	0.16	24.22	4.08	56.25
5	12.45	5.02	0.25	-	15.28	33.00
5a	13.37	5.02	0.25	-	4.08	22.73

Some of the key results from the cost analysis are:

- Total estimated costs for the current CWT system = $ 18.87 million
- Total estimated cost for the lowest cost PBT-only system with GBS (1c, 100% PBT with fractional alternative mark): $22.70 million
- Total estimated cost for the highest cost PBT-only system with GBS (2a, 100% PBT, 100% ADC, recover tags from all sampled adult fish providing coastwide GSI information as well): $43.24 million
- Total estimated cost for the lowest cost PBT-only system with ExN (1b, fractional PBT): $43.97 million
- Total estimated cost for the highest cost PBT-only system with ExN (2a, 100% PBT, 100% ADC, genotype (recover tags) from all sampled adult fish): $92.19 million

The estimated costs for the current CWT based system are lower than all of the proposed fully PBT-based systems. A great deal ($7.54 million or $24.22 million) of the cost disadvantage of PBT comes from the cost of genotyping natural-origin smolts. The proposed hybrid systems (5 and 5a) do not genotype natural-origin fish and System 5a has an estimated cost that is lower than the estimated cost of the System 0 under the $7/fish genotyping cost scenario.

Costs for 1a, 2, 2a, and 2b are high because each requires a 100% ADC mark rate and because each requires genotyping of a large number of adult fish to decode tags.

II.I.4. Estimation of Scenario Fish Numbers

Each proposed alternative system varies in the number of fish that must be handled at various steps in the tagging and recovery process and in the cost of performing each step. To estimate costs, we multiply the number of fish handled by the unit cost of each step. The derivation of the number of fish handled at each step of the marking,

tagging, and tag decoding process is described in this section. We use historical numbers from the RMIS database.

II.I.4.a Historical Marking and Tagging Numbers

II.I.4.a.1 Hatchery Releases

We extract historical totals (by species, state or province, and year) for the following numbers of fish handled in the marking and tagging process: total releases, hatchery fish receiving both ADC and CWT, hatchery fish ADC only, and hatchery fish receiving CWT only. These totals for brood years 2010-2012 are given in Table I.A.1.1for Chinook salmon and Table I.A.1.2 for coho salmon in this report. We use the annual average from 2010-2012 to estimate costs. The annual averages used in the cost estimation are reported in Table II.I.5 for both Chinook and coho.

Table II.I.5 Annual average (2010-2012) of total juvenile Chinook and coho salmon released, marked, and tagged by state or province. Source: RMIS database query

State/Province	Total Releases (A)	ADC+CWT (B)	ADC only (C)	CWT only (D)
Chinook				
Alaska	8,733,799	954,632	173,282	0
British Columbia	40,956,206	4,720,789	95,251	121,505
Washington	115,752,460	13,964,150	85,967,225	6,624,108
Idaho	14,916,114	2,001,648	9,274,477	2,586,519
Oregon	31,449,600	6,492,490	21,083,507	1,000,907
California	46,038,662	14,824,064	92,388	42,803
Coastwide Total – Chinook	257,846,840	42,957,774	116,686,130	10,375,841
Coho				
Alaska	27,175,400	923,726	24,324	411
British Columbia	12,264,848	759,501	5,554,653	146,457
Washington	31,763,644	3,006,772	23,250,324	2,469,814
Idaho	384,940	0	0	71,438
Oregon	6,361,877	447,986	5,690,097	152,478
California	788,427	117,391	2,348	66,441
Coastwide Total - Coho	78,739,136	5,255,375	34,521,746	2,907,040

Each scenario varies in the number of fish handled at each step and the treatment each fish receives. The number of fish handled is derived from the numbers in Table II.I.4. Note that in that column D "CWT only" is the number of fish that are tagged, but not marked. In many cases, these fish are part of DIT release groups. However, this is not always the case. In Idaho, for example, some fish are CWT'd in order to obtain information for broodstock management, and not marked in order to exclude them from MSF.

II.I.4.a.2 Natural-Origin Stock Tagging

We extracted historical totals (by species, state or province, and year) from the RMIS database for the following numbers of fish handled in the marking and tagging process: total releases, hatchery fish receiving both ADC and CWT, hatchery fish ADC

only, and hatchery fish receiving CWT only. We use the annual average from 2005-2012 to estimate costs. The annual averages used in the cost estimation are reported in Table II.I.5 for both Chinook and coho.

In PBT-based proposed alternative systems 1, 1a, and 1b some proportion of hatchery fish are marked with ADC and AWT. In each of these scenarios, natural-origin out-migrating smolts are trapped and all captured fish are genotyped and marked/tagged with ADC+AWT. If natural-origin stocks need to be excluded from MSF, these are marked with AWT but not ADC; and areas where they could be recovered should be sampled as if DIT-present (i.e. with ETD). In these systems the number of natural-origin fish genotyped is equal to the historical number of natural-origin fish receiving a CWT. The total number of natural-origin fish given AWT+ADC is equal to the historical number of fish receiving ADC+CWT. The total number of natural-origin fish given AWT only (to exclude them from MSFs) is equal to the historical number of fish receiving CWT only. In system 1e, the total number of natural-origin fish given ADC+alternative mark is equal to the historical number of fish receiving ADC+CWT. The total number of natural-origin fish given the alternative mark only (to protect them from MSFs) is equal to the historical number of fish receiving CWT only.

In PBT-based proposed alternative system 2, 2a, 2b, and 2c fish are either ADC and genotyped or genotyped only (when needed to exclude them from MSFs). Areas where genotyped but unmarked natural-origin fish might be recovered need to be sampled as if DIT-present (i.e., genotyping unmarked fish) We assume that the primary cost per fish is related to handling in this case, the number of fish handled is the historical number of fish receiving ADC+CWT plus the number receiving CWT only. In system 2d, the total number of natural-origin fish given an alternative mark+ADC is equal to the historical number of fish receiving ADC+CWT. The total number of natural-origin fish given the alternative mark only (to exclude them from MSFs) is equal to the historical number of fish receiving CWT only.

Table II.I.6 Annual average (2005-2012) of total sampled out-migrating natural-origin juvenile Chinook and coho salmon marked and tagged by state or province. Source: RMIS database query

	ADC+CWT	CWT only	ADC only	Neither CWT or ADC
Chinook				
Alaska	112,089	-	110	-
British Columbia	995	-	-	95,131
Washington	212,262	42,285	875	94,487
Idaho	-	-	-	-
Oregon	29,578	-	404	-
California	324,960	1,014	3,534	931
Coho				
Alaska	117,583	-	266	10
British Columbia	52,396	22,597	975	224,012
Washington	4,693	155,994	-	0
Idaho	-	-	-	-
Oregon	-	-	-	-
California	-	-	-	-

II.I.4.b Historical Sampling, Recovery, and Tag Decoding Numbers

As instructed in the RFP, we use average annual sample statistics detailed in Table 1, Morishima and Alexanderdottir (2013) to estimate costs due to tag recovery in each scenario. These numbers represent the annual averages from 2008-2011.

In some systems, the adipose fin clip rate on released fish is increased to a target rate of 100%, and tissue is taken from all sampled ADC fish for genotyping. For these systems, we estimate the expected number of sample-encountered ADC fish as follows. First, the current number of hatchery non-ADC fish sampled is estimated as the current number of ADC fish sampled (Morishima and Alexandersdottir, 2013, their Table 1) times the number of hatchery non-ADC fish released (Table II.I.5) divided by the number of hatchery ADC fish released (Table II.I.5), and this is done on a state-by-state basis (details discussed below). For these systems, these non-ADC sampled fish would now be ADC fish, thus representing the additional number of sample-encountered ADC fish to be expected under these systems. Second, adding this to the current number of sample-encountered ADC fish provides an estimate of the total number of sample-encountered ADC expected under these systems.

The estimate just described is calculated on a state-by-state basis because current, observed mark rates vary across states. For each state, the number of observed unmarked and marked fish used in the calculation is from that state and each of its neighbors. This is because fish sampled from the ocean fisheries can are from a mixture of stocks with different mark rates. If we do not do this (and simply apply state-specific mark rates) we come up with an estimated number of ADC'd fish sampled that is greater than the total number of sampled fish in states that currently have low mark rates where ocean harvest is influenced by hatchery production in neighboring states with high mark rates. This applies particularly to British Columbia and California.

The number of ADC'd fish sampled at a 100 percent mark rate is estimated for two cases: where DIT is maintained (i.e., there are a number of unmarked fish in DIT release groups) and when fully 100 percent of fish are marked.

We summarize the sampling and recovery numbers by state in Table II.I.7.

Table II.I.7 Annual average (2008-2011) of total sampled adult Chinook and coho salmon sampled and recovered by state or province of sampling. Source for Columns A-D: Table 1, Morishima and Alexandersdottir (2013). U.S. Fed includes fish sampled by USFWS, NMFS, and NMFSNWR. Source for Columns E-F: Authors' calculations as described in section above.

State or Province	Sampled (A)	Processed (B)	Tags Decoded (C)	Ad Clipped, current mark rate (D)	Estimated Ad Clipped 100% mark with DIT (E)	Estimated Ad Clipped 100% mark without DIT (F)
Chinook						
AK	116,369	10,198	6,040	10,198	83,545	85,253
BC	44,049	7,282	2,958	7,785	11,436	12,165
WA	270,612	34,529	30,623	169,366	191,208	197,843
ID	0	0	0	0	0	0
OR	90,415	13,484	12,093	27,088	35,731	36,685
CA	52,161	15,362	14,590	15,360	28,215	28,215
U.S. Fed.	81,852	15,971	14,115	52,540	59,316	61,374
Coastwide Chinook	655,458	96,826	80,419	282,337	409,451	421,535
Coho						
AK	635,861	10,329	7,789	10,291	54,781	55,889
BC	42,119	588	221	636	1,253	1,351
WA	438,469	62,144	32,107	339,317	388,546	403,371
ID	0	0	0	0	0	0
OR	67,406	6,989	6,366	31,168	36,220	37,671
CA	16	8	1	8	10	9
U.S. Fed.	18,695	4,324	4,033	15,199	17,404	18,068
Coastwide Coho	1,202,566	84,382	50,517	396,619	498,214	516,359

The columns in Table II.I.7 are defined as follows:

A. "Sampled" is the number of fish encountered by sampling crews. The sample mode (e.g. ocean fishery port sampling, in-river recreational creel survey, carcass survey, hatchery survey) does not matter in our cost calculations since the cost to screen the same proportion of the catch for marks and to remove heads/take genetic samples is assumed equivalent. The numbers also assume sampling rates that occurred from 2008-2011. This column is observed historical data and is summarized from Morishima and Alexandersdottir 2013, Table 1.

B. "Processed" is the number of sampled fish for which head were removed. This column is observed historical data and is summarized from Morishima and Alexandersdottir 2013, Table 1.

C. "Tags Decoded" is the number of CWT's recovered from fish heads and submitted to RMIS. This column is observed historical data and is summarized from Morishima and Alexandersdottir 2013, Table 1.

D. "Ad Clipped" is the number of sampled fish for which an adipose fin clip was detected. This column is observed historical data and is summarized from Morishima and Alexandersdottir 2013, Table 1.

E. “Ad Clipped 100% mark with DIT” is the estimated number of sampled for which an adipose fin clip would be detected if the ad clip mark rate were 100% for hatchery releases (except DIT releases) in the state being sampled and adjacent states. In this case, we assume that DIT occurs and PBT tagged fish are released with no mark.
F. “Ad Clipped 100% mark without DIT” is the estimated number of sampled for which an adipose fin clip would be detected if the ad clip mark rate were 100 percent. In this case, it is assumed that fully all hatchery fish are PBT tagged and marked.

II.I.5 Estimation of Unit Costs

The systems vary in the number of fish that must be handled at various steps in the tagging and recovery process and in the cost of performing each step. To estimate costs, we multiply the number of fish handled by the unit cost of each step. The estimation of the unit costs each step of the marking, tagging, and tag decoding process is described in this section. These estimated unit costs come from interviews of program managers and published information on historical costs. In all cases, historical costs are adjusted to 2014 US dollars using the “Government consumption expenditures and gross investment: Nondefense” series for the GDP price deflator.

II.5.a Estimated Unit Costs of Marking and Tagging

Table II.I.8 reports the unit costs estimated for each step involved with mark and tagging juvenile fish.

Table II.I.8 Estimated Unit Costs – Marking and Tagging

Step	Estimated Unit Cost (2014 US Dollars)
CWT-based proposed alternative systems	
ADC+CWT • Auto-tagging trailer	0.154
ADC+CWT • Hand tag	0.236
CWT only • Auto-tagging trailer	0.154
CWT only • Hand tag	0.236
ADC only • Auto-tagging trailer	$ 0.048
ADC only • Hand tag	$ 0.1095
PBT-based proposed alternative systems	
ADC+AWT • Auto-tagging trailer	$ 0.104
ADC+AWT • Hand tag	$ 0.186
ADC only • Auto-tagging trailer	$ 0.048
ADC only • Hand tag	$ 0.1095
AWT only • Auto-tagging trailer	$ 0.104
AWT only • Hand tag	$ 0.186
ADC+ alternative mark • Auto-tagging trailer	$ 0.064
ADC+ alternative mark • Hand tag	$ 0.146
Alternative mark only • Auto-tagging trailer	$ 0.064
Alternative mark only • Hand tag	$ 0.146
Natural-origin Stock Tagging	
ADC+CWT • Hand tag	$ 0.236
ADC only (with tissue sample) • Hand tag	$ 0.146
ADC+ alternative mark • Hand tag	$ 0.146
ADC+AWT • Hand tag	$ 0.186
AWT only • Hand tag	$ 0.186

Further details on how each unit cost for marking and tagging is estimated are given below.

Apply ADC and CWT to juvenile fish released at the hatchery by hand: $0.236 per fish tagged

This step occurs under the current CWT-based system. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Alaska and Canada are tagged by hand. For hand marking and tagging we rely on data in three published reports: Hammer and Blankenship 2001, Table 1; Clark 2004, Table 2; Newman et al. 2004, section 3 (page 11). Hammer and Blankenship 2001 reports results in 1999 US dollars and uses survey and interview data from managers in Canada, Oregon, Washington, the USFWS, and the NWIFC. They give a unit cost of $0.136 per fish for "CWT + adipose fin clip WITH TRAILER" (not an auto-tagging trailer). Adjusted to 2014 dollars this is $0.1938 per fish. Clark 2004 reports results in 2004 US dollars and reports estimated costs from analysis of budgets and expenditures from 13 entities operating hatcheries in southeast Alaska. Nominal costs range from $0.14 to $0.21 per fish, which is equivalent to $0.172 to $0.258 per fish in 2014 dollars. We use the mode of $0.20, which is also the cost reported for the entity that releases the most fish (ADFG), and update it to $0.246 as a representative number from Alaska. The costs reported in Clark 2004 exclude permanent hatchery staff, supervisory staff, support for data analysis and management, and capital equipment costs. Newman et al. (2004) report results in 2003 dollars and use survey data from a contractor in California's Central Valley prior to the adoption of auto-tagging trailers. They report an application cost of $0.14 and a tag purchase price of $0.072 per fish for a total cost of $0.212 per fish. This is equivalent to $0.27 in 2014 dollars. We use the mean value of these three estimates (0.236 = mean(0.194, 0.246, 0.27)) as the unit cost of marking and apply a CWT by hand.

Apply ADC and CWT to juvenile fish released at the hatchery by auto-tagging trailer: $0.154 per fish tagged

This step occurs under the current CWT-based system. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Washington, Idaho, Oregon, and California are tagged via auto-tagging trailer. For marking and tagging via auto-tagging trailer we rely on data from Hammer and Blankenship 2001, calculations from a BPA-funded project report in the Columbia River basin, and interviews with staff at the PSMFC. Real (inflation adjusted) costs from these sources range from 0.144 to 0.165. We use the mean of five estimates of costs from Washington, Oregon, and California as the unit cost to apply an ADC and CWT using an auto-tagging trailer (0.154 = mean(0.144, 0.158, 0.165, 0.149, 0.154)).

Apply ADC only to juvenile fish released at the hatchery by hand: $0.1095 per fish tagged

This step occurs under the current CWT-based system and in some proposed alternative PBT-based systems. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Alaska

and Canada are tagged by hand. To estimate the cost of ADC by hand, we subtract an assumed cost per CWT ($0.09 per tag) from the estimated unit cost of ADC+CWT ($0.236). This difference ($0.146) is the cost to handle a fish when both marking and tagging. We further assume that ADC marking only can occur at a faster rate such that the unit cost of handling fish for ADC only is 75% of that for ADC+CWT. This fraction is based on conversations with DFO staff and on observed differences in fish throughput in a study in Great Lakes fisheries (Webster et al. 2014). We therefore estimate the unit cost of ADC only by hand to be $0.1095 per fish (0.75*$0.146).

Apply ADC only to juvenile fish released at the hatchery by auto-tagging trailer: $0.048 per fish tagged

This step occurs under the current CWT-based system and in some proposed alternative PBT-based systems. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Washington, Idaho, Oregon, and California are tagged via auto-tagging trailer. To estimate the cost of ADC, we subtract an assumed cost per CWT ($0.09 per tag) from the estimated unit cost of ADC+CWT ($0.154). This difference ($0.064) is the cost to handle a fish when both marking and tagging. We further assume that ADC marking only can occur at a faster rate such that the unit cost of handling fish for ADC only is 75% of that for ADC+CWT. This fraction is based on conversations with DFO staff and on observed differences in fish throughput in a study in Great Lakes fisheries (Webster et al. 2014). We therefore estimate the unit cost of ADC only by auto-tagging to be $0.048 per fish (0.75*$0.064).

Apply CWT only to juvenile fish released at the hatchery by hand: $0.236 per fish tagged

This step occurs under the current CWT-based system, often for use in DIT programs. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Alaska and Canada are tagged by hand. We assume the same unit cost for CWT only as for ADC+CWT: $0.236 per fish tagged.

Apply CWT only to juvenile fish released at the hatchery by auto-tagging trailer: $0.154 per fish tagged

This step occurs under the current CWT-based system, often for use in DIT programs. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Washington, Idaho, Oregon, and California are tagged via auto-tagging trailer. We assume the same unit cost for CWT only as for ADC+CWT: $0.154 per fish tagged.

Apply ADC and AWT to juvenile fish released at the hatchery by hand: $0.186 per fish tagged

This step occurs in some proposed alternative PBT-based systems. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Alaska and Canada are tagged by hand. We assume that the cost to apply ADC and AWT are the same as those to apply ADC and CWT, except for the purchase cost per tag. We assume an AWT purchase price of $0.04 and a CWT

purchase price of $0.09 per tag. Therefore the AWT+ADC unit cost of hand tagging is 0.186 per tag (reflective of the 0.05 difference in tag purchase price).

Apply ADC and AWT to juvenile fish released at the hatchery by auto-tagging trailer: $0.104 per fish tagged

This step occurs in some proposed alternative PBT-based systems. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Washington, Idaho, Oregon, and California are tagged via auto-tagging trailer. We assume that the cost to apply ADC and AWT are the same as those to apply ADC and CWT, except for the purchase cost per tag. We assume an AWT purchase price of $0.04 and a CWT purchase price of $0.09 per tag. Therefore the AWT+ADC unit cost of auto-tagging is $0.104 per tag (reflective of the 0.05 difference in tag purchase price).

Apply AWT only to juvenile fish released at the hatchery by hand: $0.186 per fish tagged

This step occurs in some proposed alternative PBT-based systems. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Alaska and Canada are tagged by hand. We assume the same unit cost for AWT only as for ADC+AWT: $0.186 per fish tagged.

Apply AWT only to juvenile fish released at the hatchery by auto-tagging trailer: $0.104 per fish tagged

This step occurs in some proposed alternative PBT-based systems. Two practices are used across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Washington, Idaho, Oregon, and California are tagged via auto-tagging trailer. We assume the same unit cost for AWT only as for ADC+AWT: $0.104 per fish tagged.

Apply ADC and alternative mark to juvenile fish released at the hatchery by hand: $0.146 per fish tagged

This step occurs in some proposed alternative PBT-based systems. Two practices are used for adipose fin clipping across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Alaska and Canada are ADC by hand. It is likely that the alternative mark would be a ventral fin clip. We assume that the unit cost is equal to the cost of applying ADC+CWT, less the purchase price of a CWT ($0.236 – $0.09). This essentially assumes that applying the alternative mark has similar time/labor requirements as applying a CWT. Our estimated unit cost for ADC+alternative mark: $0.146.

Apply ADC and alternative mark to juvenile fish released at the hatchery by auto-tagging trailer: $0.064 per fish tagged

This step occurs in some proposed alternative PBT-based systems. Two practices are used for adipose fin clipping across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Washington, Idaho, Oregon, and California are ADC via auto-tagging trailer. It is likely that the alternative mark would be a ventral fin clip. It is unclear exactly what the incremental cost of an additional

ventral fin clip on a portion of the released fish would be or whether this mark would be applied by hand or by reconfiguring the auto-tagging set up. We assume that the unit cost is equal to the cost of applying ADC+CWT, less the purchase price of a CWT ($0.154 – $0.09). Given that we assume that ADC only costs are 75% of the fish handling cost for ADC+CWT with an auto-tagging trailer (see details of the ADC only unit cost below), our unit cost essentially assumes that applying the alternative mark has 33% greater time/labor requirements than applying ADC only. One way to think of this is that applying the ventral clip reduces the throughput of juvenile fish such that it requires 33% more effort. Our estimated unit cost for ADC+alternative mark: $0.064.

Apply alternative mark only to juvenile fish released at the hatchery by auto-tagging trailer: $0.064 per fish tagged

This step occurs in some proposed alternative PBT-based systems. Two practices are used for marking and tagging fish across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Washington, Idaho, Oregon, and California are marked via auto-tagging trailer. It is likely that the alternative mark would be a ventral fin clip. We assume that the unit cost is equal to the cost of applying ADC+alternative mark: $0.064.

Apply alternative mark only to juvenile fish released at the hatchery by hand: $0.146 per fish tagged

This step occurs in some proposed alternative PBT-based systems. Two practices are used for marking and tagging fish across the Pacific coast: automated tagging trailers and hand tagging. We assume that all fish released in Alaska and Canada are marked by hand. It is likely that the alternative mark would be a ventral fin clip. We assume that the unit cost is equal to the cost of applying ADC+alternative mark: $0.146.

Natural-origin stocks: apply ADC only to juvenile fish by hand: $0.146 per fish tagged

We use the same cost as ADC+CWT, less the tag purchase cost, and the same cost as ADC+alternative mark by hand for hatchery fish: $ 0.146. This assumption is reasonable because each natural-origin smolt must be handled and have two clips (an ADC and a tissue sample). This figure does not include travel to field sites. We assume that these travel costs will be the same in CWT- or PBT-based systems.

Natural-origin stocks: apply ADC and AWT to juvenile fish by hand: $0.186 per fish tagged

We use the same cost as ADC+AWT by hand for hatchery fish. This figure does not include travel to field sites. We assume that these travel costs will be the same in CWT- or PBT-based systems.

Natural-origin stocks: apply AWT only to juvenile fish by hand: $0.186 per fish tagged

We use the same cost as ADC+AWT by hand for hatchery fish. This figure does not include travel to field sites. We assume that these travel costs will be the same in CWT- or PBT-based systems. We assume that the cost to handle and tag fish is the same whether they are ADC or not.

Natural-origin stocks: apply ADC and alternative mark to juvenile fish by hand: $0.146 per fish tagged

We use the same cost as ADC+ alternative mark by hand for hatchery fish. This figure does not include travel to field sites. We assume that these travel costs will be the same in CWT- or PBT-based systems.

Natural-origin stocks: apply alternative mark only to juvenile fish by hand: $0.146 per fish tagged

We use the same cost as ADC+ alternative mark by hand for hatchery fish. This figure does not include travel to field sites. We assume that these travel costs will be the same in CWT- or PBT-based systems. We assume that the cost to handle and tag fish is the same whether they are ADC or not.

II.5.b Estimated Unit Costs of Sampling, Recovery, and Tag Decoding Numbers

This cost analysis assumes that the cost to screen the same proportion of the catch for marks and to remove heads/take genetic samples is equivalent. Therefore the cost to survey harvest and escapement is not considered. Note that we estimate genotyping costs for two cases: $7/fish for GBS and $22.50/fish for ExN. The two genotyping costs reflect different laboratory methods and probably reflect the much of the range of possible values for genotyping costs that are currently possible. Estimated costs are for laboratory labor and materials cost only and exclude capital and overhead costs. This is consistent with treatment of the costs of components of the CWT-based system in this cost evaluation.

Excise and record CWT at head lab: $5.00 per head

Under CWT-based systems tags are usually decoded by excising the tag from the recovered heads in a head lab. These costs include the labor required to excise, read, and record the tag, as well as minor materials expenses. They do not include overhead costs. These costs come from three sources: a process-based estimate from the FWS in California's Central Valley, budgeted amounts from BPA-funded project reports in Oregon, and an estimate developed for the Northwest Power and Conservation Council. The first source is an estimate by the USFWS head lab in Red Bluff, California for excising and reading CWTs at their facility. These costs are estimates based on the amount of labor required to extract and read a CWT. The figures are based on a tag extraction crew and a tag reading crew each consisting of 2 GS-5 level employees and 1 GS-7. The excision rate is assumed to be 100 tags per day. The tag reading rate is assumed to be 200 tags per day. The estimated cost is $3.74 per tag in 2012 US dollars. Adjusted to 2014 dollars the estimate is $3.82 per tag. The second source is from BPA-funded projects annual reports. BPA funds CWT operations in the Columbia River Basin via a number of projects, including BPA Project Number: 1982-013-01, which is paid to the PSMFC and ODFW. A portion of this project partially funds the ODFW head lab at Clackamas. Annual reports include the number of heads processed and the funding amounts budgeted for this task. We use costs attributed only to this portion of the contract (e.g. in 2013 this was Work "Element L:157. ODFW: Collect/Generate/Validate Field and Lab Data"). Unit costs from 2005-2013 ranged from $3.34 to $5.78 per snout processed in nominal dollars. We do not consider budgeted costs from 2007 and 2008,

which seem anomalous and occur in years where much of the salmon fishery was closed. This translates to an unweighted annual average of $4.65 in 2014 dollars. The third source is an estimate developed by Bill Jaeger at Oregon State University as part of project for the Independent Economic Analysis Board of the Northwest Power and Conservation Council. This estimate is $5.14 ($5 in 2011 dollars) and is based on analysis of budget data from ODFW. Based on these three, we assume an estimated unit cost of $5 per head.

Genotyping: GBS cost estimate = $7 per fish

The low cost estimate of cost per genotyped fish assumes use of GBS techniques. We use estimated costs from a specific method of GBS, GT-seq as outlined in Campbell et al. (2015). Table II.I.9 reproduces Table 1 from that article and contains detailed materials cost information for GT-seq. This is a relatively new method that provides lower-cost genotyping and likely represents a lower bound for genotyping costs in the near future and is perhaps is most nearly reflective future PBT-based system costs could be.

Table II.I.9 Materials costs for GT-seq method

Step for GT-seq	Cost per 96-well plate	Cost per sample
DNA extraction	$48.18	$0.51
PCR 1: pre-amp and sequencing adapters	$61.19	$0.64
Dilution of amplicons	$19.38	$0.20
PCR 2: dual barcoding	$96.42	$1.00
Sample normalization & pooling samples	$87.51	$0.91
Bead size selection	$18.75	$0.20
qPCR & normalize each pooled 96-well plate	$7.73	$0.08
Illumina HiSeq (SR100 reads)	$42.26	$0.44
Total Materials Cost	$381.41	$3.98

Labor costs are calculated in Table II.I.10 as follows. Campbell et al. (2015) report that genotyping one library of 2,068 samples requires one technician working for 4-6 weeks, or 8-12 percent of a technician's time. We assume a cost per technician of $70,000. The starting salary for a GS-7 level technician is approximately 35,000 and we double that to account for benefits and other expenses. Based on this figure and the estimated output shown in Table II.I.10, we assume a labor cost of $3 per genotype. Total materials plus labor costs estimated for the GT-seq method are $7.

Table II.I.10 Estimated labor costs for GT-seq method

Samples per library	Technicians per library	$/technician	Labor cost per library	$/sample
2,068	0.08 4 weeks/50 weeks	$70,000	$5,600	$2.71
2,068	0.12 6 weeks/50 weeks	$70,000	$8,400	$4.06

Genotyping: ExN cost estimate = $22.50 per fish

The high cost estimate of the unit costs for genotypes is based on use of exonuclease reactions (e.g., Fluidigm SNP-type assays). Campbell et al. (2015) estimate the materials cost to be $16.50 for 192 SNPs. We assume that the labor costs associated with this method are roughly double the costs for the GT-seq procedure above. We therefore use a labor cost of $6 per genotype. Total costs per genotype for the exonuclease genotyping option are $22.50.

II.I.6 Capital Costs Discussion

The cost analysis presented above does not consider capital costs. In this section we briefly present examples of the types of capital equipment required for CWT-based and PBT-based systems. These capital costs are difficult to attribute to different systems for comparison and tagging trailers may be used under both systems. Tagging trailers also represent a sunk cost (i.e., a cost that is already incurred) for some jurisdictions, regardless of which system is used, but may represent an additional cost for other jurisdictions depending on the marking and tagging requirements of the system chosen. There are multiple possible capital configurations even within the 11 proposed alternative systems for which we have estimated costs. For example, agencies may also choose to build their own trailers or develop alternative marking and tagging equipment, depending on the marking and tagging requirements of the proposed alternative system rather than incur the purchase costs quoted here. Further, when the marking and tagging equipment reaches the end of its useful life, existing equipment may be reconditioned or reconfigured. Agencies that do not currently utilize auto-tagging trailers for marking or tagging, may choose to obtain this equipment if marking or tagging requirements change. Therefore, the discussion below is not intended to be a direct comparison of capital costs, but rather reference information on the types of capital equipment used.

II.I.6.1 Tagging Trailers

NMT lists the purchase price for an auto-tagging trailer (AutoFish SCT6) as $1,345,000. These trailers may have a useful life of 20 or more years (though currently none have been in existence for that long). Assuming an interest rate of 3 percent (consistent with Office of Management and Budget Circular No. 94) and a useful life of 20 years implies an annualized capital cost of $90,000 per trailer per year.

NMT also lists the purchase price for a manual tagging trailer as $390,000. Assuming an interest rate of 3 percent and a useful life of 20 years implies an annualized capital cost of $26,000 per trailer per year.

II.I.6.2 Genetic sequencing equipment

Illumina, Inc. is the primary vendor for the type of high-throughput DNA sequencing equipment needed to generate genotypes for PBT using a GBS approach. The volume of genotypes required for a coastwide PBT system could be performed using one of Illumina's intermediate capacity sequencers, the NextSeq 500 (http://www.illumina.com/systems/sequencing.html). The list price for the NextSeq 500 is $250,000. Assuming a useful life of 10 years, and an interest rate of 3 percent this implies an annualized capital cost of $30,000 per sequencer per year.

Under a coastwide PBT-based tagging system it is unclear how many genotyping facilities would be required, though currently several Pacific coast agencies currently have full-service fishery genetic laboratories. Agencies may want to maintain jurisdictional control over the tagging process so multiple instruments (perhaps in the range of 5-10) would be necessary for a PBT-based system to be implemented coastwide.

II.J. "Break-even" cost-per-fish of genotyping that would generate approximately equal costs for support of CWT-based and PBT-based systems.

II.J.1 Introduction

We estimate the unit cost per genotype that would generate equal total costs between the current CWT-based system and the proposed alternative PBT-based systems, i.e, the "break-even" cost of each PBT-based scenario. To put this another way, we propose an answer to the question: "How low would the cost per genotype need to be for the two systems to have equal costs?" Lower genotyping costs would imply an operating cost advantage for a PBT-based system. We estimate break-even costs under two sets of assumptions: 1) total marking/tagging costs are variable (i.e., total costs increases linearly with the number of fish) and 2) total mark/tagging (which include the labor costs of fish handling, ad clipping, and inserting a tag but do not include the cost of the wire tag itself) costs are fixed (i.e., total costs do not increase as the number of fish increases).

To estimate the break-even genotyping cost when total marking and tagging costs are variable, we divide the difference between the estimated costs of the current CWT-based system (System 0) and the estimated non-genotype costs of each proposed alternative PBT-based system by the number of genotypes required. Non-genotype costs are the costs of ADC marking, AWT tagging, and alternative mark marking hatchery releases. This assumption is consistent with the cost analysis presented in section II.I.

To estimate the break-even genotyping cost when total marking and tagging costs are fixed, we assume that CWT- and PBT-based systems differ only in the purchase cost of wire tags, the cost of excising and decoding tags, and in genotyping costs (labor costs to mark and tag fish at hatcheries or in natural-origin smolts is fixed). To estimate the break-even genotype costs, we divide the total estimated cost of purchasing CWTs and processing heads in System 0 less the purchase cost (if any) of blank wire tags in the PBT-based system by the number of genotypes required for each proposed alternative PBT-based system. The purpose of this second set of break-even costs is to account for the uncertainty in the cost per fish of ADC marking. The cost analysis assumes that the unit cost per fish of ADC marking is uniform. In this case, the total cost of ADC marking therefore increases linearly as the number of fish marked increases. This assumption may not be strictly correct, however, especially in the case of auto-tagging trailers. When auto-tagging trailers are used to mark and tag fish at the hatchery, all releases (or at least all release groups to be tagged) are run through the trailer. A subset of these is clipped and/or tagged. The incremental cost of increasing the proportion of fish ADC marked may not be as high as the unit cost calculated under current conditions because many of the costs of running the tagging trailer are fixed. That is, many of the incurred tagging costs do not change (or increase at a decreasing rate) as the number of fish ADC marked increases. The second set of break-even costs, calculated relative to the cost of CWTs only, therefore assumes that the total cost of marking fish will be the same as the cost of handling and marking fish under the current CWT-based system. Another way of stating this assumption is that the incremental cost of marking and inserting tags under a PBT-based system is equal to zero. The cost of purchasing blank wire tags is not.

It is likely that neither assumption is strictly correct. Calculating break-even genotyping costs under both gives an upper and lower bound on these costs.

Recall that the cost of sampling fisheries and escapement for adult fish is assumed equal for CWT- and PBT-based systems and is not estimated in this analysis.

II.J.2 Break-even genotyping costs assuming all marking/tagging costs are variable

Table II.J.1 Break-even cost per genotype assuming variable costs for ADC mark.

System	Total Cost GBS (Millions US$)	Non-genotype costs (Millions US$)	Cost Difference (Millions US$)	Required Genotypes (Millions)	Break-even cost per Genotype
0 CWT	18.9				
1	25.2	14.8	4.0	1.481	$ 2.72
1a	38.0	22.6	-3.7	2.207	NA
1b	23.9	14.8	4.0	1.295	$ 3.12
1c	22.7	12.3	6.5	1.481	$ 4.41
2	38.1	21.1	-2.3	2.429	NA
2a	43.2	21.1	-2.3	3.158	NA
2b	37.5	21.8	-2.9	2.238	NA
2c	24.4	10.5	8.3	1.979	$ 4.21
2d	33.3	22.9	-4.1	1.481	NA
5	19.0	12.7	6.2	0.902	$ 6.84
5a	16.5	13.6	5.2	0.404	$ 12.97

In Table II.J.1, the Total Cost columns are the estimated total system costs from the analysis in section II.I (GBS is the $7 per genotype assumption). Non-genotype costs are marking and tagging costs for each system (see the "Mark and Tag" and "Handle Wild Fish" columns of Tables II.I.3 and II.I.4). The column in Table II.J.3 labeled "Difference" is the difference in between CWT-based system costs (system 0) and non-genotype costs in the proposed alternative PBT-based systems. The break-even cost per genotype is estimated by dividing the "Difference" by the required number of genotypes.

Some key results regarding break-even costs when all marking/tagging costs are assumed to vary with the number of fish marked:

- The break-even costs for PBT-only systems range from $2.72 – $4.41 per genotype
- Five systems (1a, 2, 2a, 2b, and 2d) have non-genotyping costs that are greater than the estimated cost of the current CWT-based system. This means that genotyping costs cannot be low enough to offer a cost advantage for those systems. These high costs are due to the 100% ADC mark rate. Under the assumption that all ADC costs are variable, this greatly increases the total marking cost for these scenarios. Increasing the ADC mark rate also increases the number of fish genotyped during later sampling.
- The hybrid systems that do not require genotyping of natural-origin stocks have the highest break-even costs, indicating that they are the closest to being cost-equivalent to the current CWT-based system. System 5a has a break-even cost higher than the GBS-based genotyped cost ($7) assumed in the cost analysis in section II.I.

II.J.3 Break-even genotyping costs assuming total marking/tagging costs are fixed

Table II.J.2 Break-even cost per genotype assuming fixed total costs (0 incremental) for ADC mark.

System	Millions of Tags (CWT,AWT)	Total Tag Purchase Cost (Mill. US$)	Head Lab Costs (Mill. US$)	Difference (Mill. US$)	Required Genotypes (Millions)	Break-even Cost per Genotype
0 CWT	62.572	5.6	0.9			
1	62.572	2.5		4.0	1.481	$ 2.72
1a	14.359	0.6		6.0	2.207	$ 2.70
1b	62.572	2.5		4.0	1.295	$ 3.12
1c	0			6.5	1.481	$ 4.41
2	0			6.5	2.429	$ 2.69
2a	0			6.5	3.158	$ 2.07
2b	0			6.5	2.238	$ 2.92
2c	0			6.5	1.979	$ 3.30
2d	0			6.5	1.481	$ 4.41
5	14.359	1.3		5.2	0.902	$ 5.81
5a	14.359	1.3		5.2	0.404	$ 12.97

In Table II.J.2, "Number of Tags" is the total number of tags required in a given system. In the current CWT-based system (system 0) this is the total number of CWTs (Chinook and coho, hatchery and wild, including DIT releases). In systems 1, 1a, and 1b these are AWTs (blank wire tags). In System 5 this is the CWTs needed to tag natural-origin stocks and DIT fish. Systems 1c, 2, 2a, 2b, 2c, and 2d require no wire tags.

Total tag costs are the number of tags multiplied by the assumed purchase price. The assumed purchase price for CWTs $0.09 per tag. The assumed purchase price for AWTs $0.04 per tag. It is important to note that several agencies report purchasing CWTs at a discount, sometimes in the range of $ 0.07 – 0.08 per tag. "Head Lab Costs" are the cost of recovering tags at $5 per head, as detailed in section II.I.

The column in Table II.J.2 labeled "Difference" is the difference in tag purchase cost and head lab cost in the current CWT-based system and each proposed alternative PBT-based system. This represents the estimated foregone cost of tags and tag recovery achieved by moving to a PBT-based system. The break-even cost per genotype is estimated by dividing the "Difference" by the required number of genotypes. Note that system 2b sacrifices any information provided by DIT. Other systems may offer larger or smaller sample sizes (and thus precision) than the CWT-based system, but system 2b is the only one to completely sacrifice an entire category of information.

Some key results regarding break even costs when all marking/tagging costs are assumed to be fixed regardless of the number of fish marked:

- The break-even costs for PBT-only systems range from $2.07 – $4.41 per genotype
- All of the proposed alternative systems have an estimated break even cost greater than zero, which illustrates the effect of assuming fixed marking/tagging costs. Systems with high ADC mark rates (1a, 2, 2a, 2b, and 2c) are more cost-effective under this assumption.

- The hybrid systems that do not require genotyping of natural-origin stocks have the highest break-even costs, indicating that they are the closest to being cost-equivalent to the current CWT-based system. System 5a has a break-even cost higher than the GBS-based genotyped cost ($7) assumed in the cost analysis in section II.I.

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Appendix 1. The Snake River Experience Transitioning to PBT

Parentage Based Tagging was initiated in the Snake River basin following requests by IDFG managers to investigate alternative tagging methods to increase tagging rates and recoveries to estimate stock contributions of Chinook salmon and steelhead hatchery stocks returning over Lower Granite Dam back to Idaho and in-State fisheries. In addition, during this same period, several committees and science review groups specifically recommended that large-scale evaluations of the technology be performed (PFMC 2008; PSC 2008; ISRP/ISAB 2009). In 2010, IDFG and CRITFC received funding from the BPA to initiate and evaluate PBT technology in the Snake River basin. The major objectives of this project are to:

- Test the feasibility of PBT sampling and inventorying all hatchery broodstock spawned in the Snake River basin.

- Test whether a set of 96 SNP loci (including a sex marker) for each species can provide robust genotyping and sufficient power for accurate parentage assignment.

- Test whether the SNP loci used for PBT can be integrated within a set of SNP loci used for GSI.

- Test whether parental baselines can be constructed each year and that high genotyping and tagging rates can be obtained for each species.

- Describe the application and versatility of this technology through the summary of multiple back end projects that use completed PBT baselines to assign parentage to samples of unknown origin.

Details relevant to meeting these objectives are described in journal articles and annual reports to BPA. We briefly summarize some of the major achievements meeting these objectives below:

- Steele et al. (2013a) empirically demonstrated that fewer than 100 SNPs are needed to accurately conduct steelhead PBT in the Snake River basin. They also demonstrated that the 95 SNP set provides accurate parental assignment with low false negative (3.9%) and false positive rates (0.0%), and that stock assignments made with this SNP panel matched those made using CWTs (52/52).

- The utility of a 192 SNPs (2 panels) to characterize genetic variability throughout the Snake and Columbia rivers and to perform GSI analyses at Lower Granite Dam, Bonneville Dam, and in lower Columbia River mixed fisheries (both Chinook salmon and steelhead) has been demonstrated (Ackerman et al. 2012; Hess et al. 2012; Steele et al. 2012). This 192 SNP set includes the 96 SNP loci used for PBT and allows dual interrogation of both wild and hatchery fish. Importantly, the screening of all unmarked adults against the Snake River PBT baseline, allows for improved estimation of wild abundance. For example, non-lethal sampling at Bonneville Dam has demonstrated that approximately 16% of the returning steelhead adults phenotypically identified as “wild”

(unmarked) were unclipped hatchery fish using PBT. Similar non-lethal sampling has been done at Lower Granite Dam, were approximately 12% of the "wild" steelhead returning to the Snake River basin in Idaho were identified as unclipped hatchery fish.

- This project has demonstrated high concordance between sex-specific markers used for steelhead and Chinook salmon and known phenotypic sex of broodstock. In the most recent comparison, the sex-specific assay for steelhead matched phenotypic sex in 99.6% of the samples (4,727 comparisons) and the sex-specific assay for Chinook Salmon matched phenotypic sex in 97.3% of the samples (8,888 comparisons) (Steele et al. 2014).

- This project has demonstrated that multiple labs can adopt similar protocols and procedures for inventorying and genotyping samples, conducting QA/QC procedures, storing standardized meta- and genetic data in secure in-house databases, and combine PBT data into single, secure databases that make PBT baselines available to agencies throughout the Columbia River Basin.
    a. To produce Snake River PBT and GSI baselines for Chinook salmon and steelhead, CRITFC and IDFG labs divide the annual broodstock genotyping work requirements equally (each lab genotypes ~1/2 of the total broodstock).
    b. Each lab follows similar laboratory methods/protocols for genotyping samples and performing QA/QC procedures. These protocols are published on Monitoring Methods.org (https://www.monitoringmethods.org/).
    c. Standardized genotypes are stored on Progeny database servers housed at IDFG and CRITFC. Progeny software (http://www.progenygenetics.com/) is currently in use by a large number of labs throughout the Pacific Northwest: CRITFC, IDFG, WDFW, University of Washington and USFWS. The commonality of database software promotes seamless sharing of genetic data among labs.
    d. Snake River PBT baselines are stored on a publicly available database repository (www.FishGen.net).

- In addition to SNP genotyping concordance between IDFG and CRITFC, this project has also demonstrated >99% SNP genotyping concordance with both PBT and GSI SNP panels among five labs (WDFW, NMFS Northwest Fisheries Science Center, CRITFC, IDFG, and Abernathy Fish Technology Center). This confirms that SNP genotype data is accurately reproducible among labs.

- Since 2008, 7 agencies: IDFG, ODFW, WDFW, USFWS, Idaho Power Company, Shoshone-Bannock Tribe and the Nez Perce Tribe have coordinated the genetic sampling of hatchery Chinook salmon and steelhead broodstock spawned in the Snake River basin. Over this time, approximately 63,000 hatchery Chinook salmon broodstock have been sampled (~9000/year) and 38,000 hatchery steelhead broodstock have been sampled (~5,500/year). Genotyping and tagging rates for Chinook salmon have averaged 98.4% and 96.5%, respectively. Genotyping and tagging rates for steelhead have averaged 97.6% and 95.0%, respectively.

- This sampling and genotyping PBT tags approximately:
    a. 9.5 million hatchery steelhead smolts, comprising approximately 65% of all annual hatchery steelhead releases in the Columbia River basin.
    b. 13.8 million hatchery spring/summer Chinook salmon smolts, comprising approximately 36% of all annual hatchery spring/summer releases in the Columbia River basin.
    c. 5.8 million hatchery Fall Chinook salmon smolts, comprising approximately 9% of all annual hatchery fall releases in the Columbia River basin.
- Backend sampling projects using the Snake River PBT baselines generated from this project have:
    a. Demonstrated that PBT technology can be used to estimate the stock and age composition of Chinook salmon within freshwater mixed stock fisheries in Idaho (Cassinelli et al. 2013). Because of the increased tagging rates provided by PBT in this study, precision was greater with PBT-derived estimates than with CWT-estimates.
    b. Demonstrated that PBT baselines can be used to determine the origin of stray hatchery steelhead in the Deschutes River. Because samples can be taken non-lethally, the relative reproductive success of stray hatchery adults can be compared to wild steelhead (Smith and Hawkins 2012; 2013). Of note, the Abernathy Lab (USFWS) uses Snake River PBT SNP baselines generated by IDFG and CRITFC, but generates its own SNP genotype data from hatchery adults sampled in the Deschutes. This demonstrates that multiple labs can "tag" broodstock for PBT baselines, and that multiple labs can "recover" PBT tagged fish.
    c. Demonstrated that estimates of the stock proportion of hatchery Chinook salmon crossing Lower Granite Dam can be performed non-lethally using PBT (Cassinelli et al. 2013). This is important because underrepresentation of stock- and age-specific untagged returns by PIT-tagged fish has been an ongoing issue, and PIT detections have been the only non-lethal tag previously available. It is believed that PIT tags generally underestimate the number of actual returns due to tag shedding and differential mortality (IDFG unpublished data). In 2012 returns, in-season PIT tag estimates accounted for 72.6% of the PBT-based stock/age-specific estimates at Lower Granite Dam.
    d. Hess et al. (2012) demonstrated that PBT can be used in combination with GSI in a tiered approach for stock identification. Sampling of Chinook salmon and steelhead at Bonneville Dam for stock identification uses PBT to identify Snake River hatchery-origin fish and then uses GSI to estimate stock-of-origin for all other hatchery fish that were not assigned with PBT (i.e. non-Snake River hatchery-origin) and all wild fish. In this way PBT and GSI are very complimentary, and using them in combination takes full advantage of the strengths of each method, while resolving many of their limitations. Wild stocks can only be effectively PBT tagged when adults can be sampled at weirs, but can often be discriminated using GSI methodologies if sufficient differentiation exists. Many hatchery stocks are difficult to discriminate using GSI methods because they were either founded using the same stock or have experienced recent stock transfers. PBT can completely address this limitation, allowing the discrimination

of hatchery stocks that exhibit no genetic differentiation (Steele et al. 2011; 2012; 2013b).

e. Demonstrated that PBT can be used to estimate the proportion of hatchery-origin Chinook salmon adults in natural spawning areas, and that in the South Fork Salmon River application, PBT-based estimates were more precise than CWT-based estimates because there were 340% more PBT than CWT recoveries (Hinrichsen et al. in review). This work was accomplished using carcass samples. In the first year of the study, 87% of the carcasses were successfully genotyped. In the second year of the study, 74% of the carcasses were successfully genotyped.
f. Demonstrated that a coordinated/collaborative PBT sampling effort involving 5 agencies (IDFG. WDFW, ODFW, CRITFC, Yakama Nation and PSMFC) could provide robust stock composition estimates of Snake River steelhead harvested in mixed stock tribal and non-tribal fisheries in the Columbia and Snake rivers (Byrne et al. 2014a; 2014b). Importantly, this work also highlights the utility of a combined SNP panel set that can perform both PBT and GSI. In this study, steelhead caught in the tribal Zone 6 fall fishery included clipped and unclipped hatchery adults and wild adults. Sampling and genotyping of clipped hatchery adults allowed assignment back to the PBT baseline and estimation of the harvest of individual Snake River hatchery stocks. All unclipped fish were genotyped with the complete 192 SNP set, which allowed estimation of the proportion of unclipped Snake River hatchery origin adults, which were included in total hatchery harvest estimates. Unclipped/unmarked adults that did not assign to the PBT baseline were presumed wild and were assigned to a genetic reporting unit using the GSI baseline to allow the estimation of the harvest of wild steelhead by genetic stock (reporting unit).
g. Demonstrated that PBT of Snake River hatchery stocks allows the generation of detailed stock-specific information on broodstock demographics and heritability of specific traits that have not been previously available with other tagging methodologies (Steele et al. 2013c). This is possible, because most broodstock spawned in the Snake River basin are PBT tagged, and in turn, they are genetically sampled to create the next generation of PBT tagged offspring. Because all of these broodstock can be assigned parents, PBT provides an efficient, powerful and comprehensive means for evaluating and monitoring biological and life-history traits of the hatchery broodstock. This has also been clearly demonstrated for PBT programs outside the Snake River basin (Abadía-Cardoso et al. 2013).

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Appendix 2 – Variation in parentage-based tagging and coded-wire tagging rates, and its consequences for the variance of sample-expanded tag recovery estimates

1 Summary

Realized rates of tagging are investigated for scenarios using PBT and CWTs. Using CWTs the fraction of fish tagged can usually be accurately estimated at the time of release, though there will be some variation in the realized proportion of tagged versus untagged fish at the time of adult sampling. In a PBT scenario, the *expected* fraction of fish tagged at the time of tagging can be estimated from the number of successfully-genotyped families that contributed to the release group. However, unless 100% of the families producing the release group are successfully genotyped, the realized PBT tagging rate can differ from the expected rate because different families produce different numbers of offspring that survive at different rates from mating to the time of release. Additional variation is possible due to differential survival of family groups from the time of release to the time of adult recovery sampling. These issues, as well as their consequences for the variance of sample-expanded tag recovery estimates, are explored through simulation. The results indicate that it is best to maintain a high ($> 96\%$) fraction of successfully-genotyped families, especially when the number of parents producing the release group is small (< 30) and the product of the sampling rate in the recovery stratum and the marking rate in the release group is high ($\geq 50\%$).

2 Introduction

We develop a simple system of simulations to describe, explore, and understand variation in the realized tagging rates achieved when using PBT or CWTs for hatchery-raised salmon. Here we describe simulation routines and the assumptions made in them for both CWTs and PBT. We subsequently investigate the effective number of breeders in hatchery populations, and the distribution of release group sizes, marking rates and sampling rates to understand the potential consequences of this tagging rate variation.

We pursue two (related) simulations. In the first, we focus exclusively on the variance in the tagging rate itself. In the second we examine the effect of this variation on sample-expanded tag recovery estimates to assess the practical importance of PBT tagging rate variation.

3 Simulation 1. Tagging rate variation

3.1 Tagging rates of CWTs

Coded wire tags are typically deployed when fish are large enough to be tagged and to have their adipose fins clipped, frequently just days before release. In large hatchery programs, the tagging of release groups may be undertaken with specialized tagging trailers that automate the tagging and marking process and allow for a precise count of the number of fish tagged and marked, as well as the total number of fish in the release group. Within a release group, the tags are assumed to be randomly assigned to individual fish, so there is no association between the family the fish came from and whether or not it carries a tag. For these simulations, we will assume that the CWT tagging rate, p_{cwt}, is known without error at the time of release and that the rate of tag shedding has been accounted for in it.

Most CWT recaptures occur in adult fish. Each tagged fish at the time of recovery can be expanded by $1/p_{\mathrm{cwt}}$ into an estimate of the total number of release group fish (tagged and untagged) that the tag recovery represents. Usually, p_{cwt} will provide a good estimate of the fraction of adult fish from the release group that are tagged at the time of recovery, a proportion we will call the *realized* tagged fraction, $\tilde{p}_{\mathrm{cwt}}$. It should be clear that the realized tagged fraction is the relevant quantity when expanding tag recoveries of adult fish, but is typically not known. We will assume that tagged and untagged fish have the same rate of survival so that adult fish can be regarded as a random sample from the fish in the release group (hence, we are not modeling the effects of mark selective fisheries, etc.). Although the sampling is technically without replacement, it is reasonable to model the sampling as with replacement because relatively few fish survive to adulthood for any given cohort. For our simulations, if we consider the N_A adults in a particular sample stratum that have survived from a release group tagged at rate p_{cwt} we will model $\tilde{p}_{\mathrm{cwt}}$ as having a scaled binomial distribution: $\tilde{p}_{\mathrm{cwt}} \equiv X/N_A$, where X is the number of N_A that are in fact tagged,

$$X \sim \mathrm{Binomial}(N_A, p_{\mathrm{cwt}}).$$

3.2 Tagging rates with PBT

Determining expected and realized PBT tagging rates proceeds in a different fashion. In PBT, the offspring of a set of male and female spawners are raised separately in the hatchery as a single release group. Let us assume that there are S male-female pairs of spawners whose eggs are contributed to the release group. PBT will work most efficiently with 100% tagging—in other words, when all the parents of a release group are genotyped. However, there may be some rate at which genotypes are not obtained for certain individuals (e.g., incomplete tissue sample collection, unscoreable genotypes, etc.). Currently, genotype data from both parents is required for PBT; however, with newer genotyping systems, that can economically provide more genotypes per individual, it will be possible to accurately perform assignments of offspring to single parents. Regardless of the exact mechanism, we can still focus on the tagging rate of families (the offspring of two distinct parents) in the hatchery: under current practices a family is tagged if both parents are genotyped; with single-parent assignments a family would be considered tagged if at least one parent was genotyped. For our simulations, we will let G families out of S be tagged. Under this assumption, the fraction of families that are PBT-tagged is,

$$f_{\mathrm{pbt}} = \frac{G}{S}.$$

This quantity is the expected value of p_{pbt}, the PBT tagging rate, defined to be the fraction of juveniles in the release group that will be tagged at the time of release.

Depending on how much information about spawning is recorded at the hatchery, there are various ways one can go about predicting G or G/S. If cross information is recorded at the hatchery, G can be recorded directly from the mating records and the record of successfully obtained genotypes. If cross information is not recorded, then G/S can be predicted from the total proportion of males and females contributing to the release group that were successfully genotyped (see section II.B of the report). In our simulations we will assume that G is known because we are most interested in the effect of family size; however, if one were interested, it would be possible to add another hierarchy to the simulations in which, although G is fixed for the simulation, f_{pbt} would be estimated from the proportion of males and females successfully genotyped.

The PBT tagging rate both at the juvenile stage, and later, the realized PBT tagging rate at the adult stage, will vary from f_{pbt} because 1) different females produce different numbers of eggs that survive at different rates to the time of release and 2) different families will have different survival rates after release. We

simulate p_{pbt} (recall that this is the fraction of juveniles that are PBT-tagged at the time of release) by first assuming that each family produces a negative-binomial distributed number of offspring with mean μ and overdispersion parameter r, (hence giving a variance of $\mu + \frac{\mu^2}{r}$), and then assigning the parents of those families randomly to successfully- or unsuccessfully-genotyped categories. We assume a 1:1 mating scheme in our simulations. Operationally, we:

1. Set values of the parameters for the simulation: S, G, μ, and r.
2. The number of offspring, J_i, produced by the i^{th} family is independently and identically simulated from a negative binomial distribution. That is:

$$P(J_i = k|\mu, r) = \frac{\Gamma(r+k)}{\Gamma(r)k!}\left(\frac{r}{r+\mu}\right)^r\left(\frac{\mu}{r+\mu}\right)^k$$

   for $k = 1, 2, \ldots$ and $i = 1, \ldots, S$.
3. G of the S families are randomly selected to be successfully PBT-tagged. The indexes of these families are recorded as the set $\mathcal{B}$.
4. The fraction of PBT-tagged juveniles in the release group is recorded as

$$p_{\text{pbt}} = \frac{\sum_{i\in\mathcal{B}} J_i}{\sum_{i=1}^{S} J_i}.$$

At the end of the above steps we have a simulated value of p_{pbt}. Next, we simulate the realized PBT-tagged fraction of adults, $\tilde{p}_{\text{pbt}}$. This is somewhat more complicated than for CWTs, because both the occurrence of tagging (*i.e.*, successful genotyping of parents) and post-release rates of survival are family-specific. Our simulation scheme has a mechanism to introduce this additional variability in the family-specific survival at this stage. We assume that the family-specific survival rate from release to adulthood is independent of the number of individuals each family produced in the release group (a reasonable assumption, as the selective forces in the wild are very different from those in the hatchery). To simulate $\tilde{p}_{\text{pbt}}$, using quantities that were simulated above, we:

1. Set the value of an additional parameter needed for this part of the simulation: N_A, the number of adults (tagged and untagged) from the release group present at the time of recovery sampling in a particular stratum, and α, a parameter that controls variation in survival rate among families.
2. Let

$$Y = (Y_1, \ldots, Y_S) \sim \text{Dirichlet}(\alpha, \ldots, \alpha)$$

   be a symmetrically-distributed Dirichlet random vector with S components (one for each family). Note that $\sum_{i=1}^{S} Y_i = 1$. The value Y_i represents the probability that a member of the i^{th} family group will survive to adulthood. As $\alpha \to \infty$, the vector Y approaches a vector with all components equal to $1/S$ (all individuals survive at the same rate, regardless of family). As $\alpha \to 0$, Y will have all its mass on one i, suggesting that only a single family has individuals that survive.
3. Simulate W_i, the number of individuals from family i that survive and are present in the sample stratum, from a multinomial distribution with N_A trials and cell probabilities $q_i = \frac{Y_i J_i}{\sum_{i=1}^{S} Y_i J_i}$:

$$W = (W_1, \ldots, W_S) \sim \text{Mult}_S(N_A, (q_1, \ldots, q_S)).$$

4. The realized PBT-tagging rate amongst adult fish is recorded as:

$$\tilde{p}_{\text{pbt}} = \frac{\sum_{i \in \mathcal{B}} W_i}{\sum_{i=1}^{S} W_i}.$$

3.3 Interpretation of parameters r and α

The parameters r and α are the two that directly affect variance in family size. r controls the variance in the number of juveniles from each family at the time of tagging. It is meant to reflect variation between females in fecundity and family-specific variation in smolt to adult survival. Given a mean number of juveniles, μ, a good rule of thumb is that roughly $\frac{2}{3}$ of the variation will be contained within the interval $\mu \pm \sqrt{\mu + \mu^2/r}$. For example, if the average number of smolts produced per family is 3000, then setting r to 9 means that roughly $\frac{2}{3}$ of the families will have between 2000 and 4000 juveniles in the release group.

The interpretation of α will perhaps be even less familiar to most, and it also might not be transparent how one should contextualize the joint effect of r and α on the distribution of family sizes at the time of adult sampling. To aid in interpretation of how much variance in family size is conferred by the combination of r and α in any scenario, we estimate the effective size of the population for each scenario. This is done by simulating the number of gene copies per family, $C_1, \ldots, C_S$, for a constant sized population from a multinomial distribution:

$$(C_1, \ldots, C_S) \sim \text{Mult}_S(2S, (q_1, \ldots, q_S))$$

and then calculating the probability that two gene copies sampled from amongst those offspring gene copies are identical by descent (IBD) as:

$$P(\text{IBD}) = \frac{1}{4} \sum_{i=1}^{S} \frac{C_i}{2S} \frac{C_i - 1}{2S - 1},$$

derived as follows: $\frac{C_i}{2S}$ is the probability that the first gene copy is sampled from family i; $\frac{C_i - 1}{2S - 1}$ is the probability that the second gene copy is sampled from family i; if those two gene copies are to be IBD, then they must both be from the same parent (probability 1/2) and they must be copies of the same gene within that parent (probability 1/2), which gives the term of 1/4. $P(\text{IBD})$ is recorded for each replicate at a particular value of S, r, and α, the average of those values, $\widehat{P(\text{IBD})}$, is taken and the effective number of breeders is estimated by $N_b = [2\widehat{P(\text{IBD})}]^{-1}$. The effect of the parameters controlling variance in family size can thus be interpreted as the ratio of the effective number of spawners to the actual number of spawners, $\frac{N_b}{2S}$.

3.4 Simulation 1 settings

For simulation 1 we present simulations at 6 different values of $S = 10, 25, 50, 100, 250, 1000$. For each value of S, we assume two different values for the number G out of S families that are genotyped: $G = \lfloor 0.8S \rfloor$ and $G = \lfloor 0.96S \rfloor$ and we explore two different scenarios of variance in family size. The first is a scenario in which each family produces a Poisson-distributed number of juveniles at the release stage (equivalent to $\mu = 3000$, and $r \to \infty$) and there is no additional variance due to differential family survival, post-release (*i.e.*, $\alpha \to \infty$)—this is equivalent to reproduction in a "Wright-Fisher" population (Hartl and Clark 2007, p. 102). The second scenario, has $\mu = 3000$, $r = 30$, and $\alpha = 1$, and produces an

$N_b/(2S)$ ratio close to 0.5. In all simulations, N_A, the number of adults present in the recovery stratum, was set to be $10S$, or 5 times above replacement for the parents of the release group. This figure is likely many more fish than would be encountered in a small stratum; however it was chosen so as to have enough simulated adults to permit reliable estimation of relative differences in realized CWT and PBT adult tagging rates. One thousand replicate simulations were run for each of these 24 different combinations of simulation parameters.

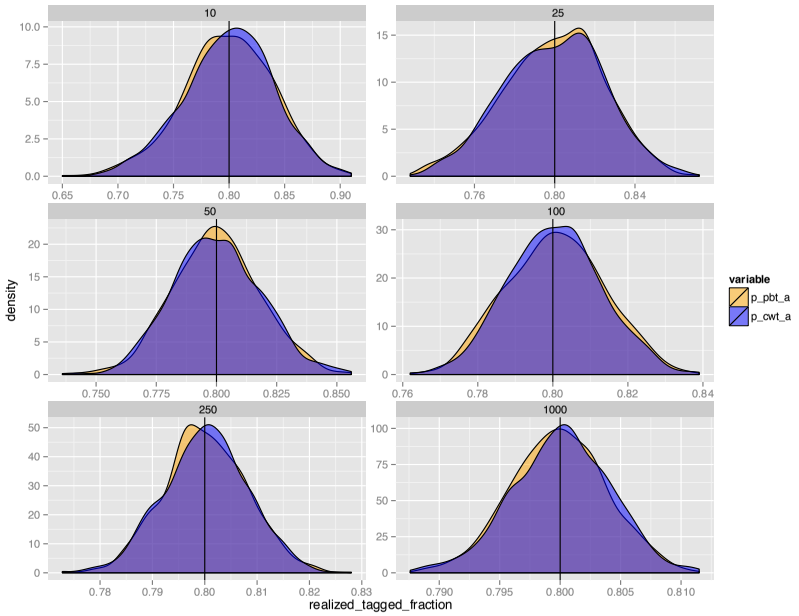
3.5 Simulation 1 results

The resulting distribution of realized adult tagging rates ($\tilde{p}_{\mathrm{pbt}}$ and $\tilde{p}_{\mathrm{cwt}}$) are shown in Figures 1 and 2. These simulations show that, if salmon populations reproduced in a Wright-Fisher fashion (*i.e.*, Poisson distribution of family sizes), then the variation in realized adult tagging rates for PBT would be commensurate with those for CWTs. However, salmon populations typically exhibit variance in reproductive success beyond that expected in a Wright-Fisher population, with $N_b/(2S)$ ratios in Oregon coho hatcheries reported to be between 0.76 and 0.84 (Moyer et al. 2007). In such cases with overdispersed variance in reproductive success, for release groups arising from few families (< 50 families, for example), there is an appreciable increase in the variance of the realized tagged fraction of adults using PBT when not all of the parents were successfully genotyped. However, the consequence of this for different applications needs to be assessed. Accordingly a second set of simulations was conducted in which the goal was to assess the effect on sample-expanded tag recovery estimates.

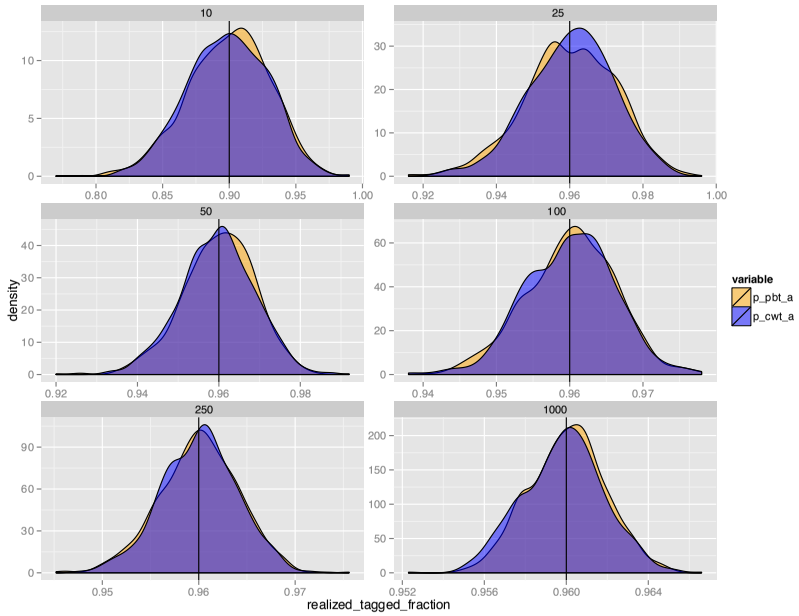
4 Simulation 2. Implications for sample-expanded tag recovery estimates

Simulation 1 assesses the variance in the realized adult tagging rate that corresponds to a situation in which the goal is to PBT-tag 100% of a release group, but due to a variety of possible reasons, some fraction of families are not tagged (parents were not successfully genotyped). The comparison between CWTs and PBT in those simulations make it clear that for a given tagging rate of a release group, PBT will always have a higher variance in the realized adult tagging rate than CWTs. However, some quantities of interest are estimated based on mark-, tag-, and sample-expanded recoveries including, e.g., the release group contribution to a stratum, and the release group early-life survival rate (release-to-age first vulnerable to fisheries). The purpose of the expansion is to scale up the observed number of recoveries in a stratum to an estimate of the total number of release group fish present in that stratum (whether or not marked+tagged, and whether or not sampled). The expansion is calculated by dividing the number of release group-specific recoveries by the product of the corresponding mark rate, tag rate, and sample rate (or, equivalently, multiplying the number of recoveries by the product of the corresponding inverse rates). Therefore, to understand the consequences of variation in the realized adult tagging rate on the uncertainty of release group-specific estimates of the total number of fish present in a recovery stratum, it is necessary to understand the sampling context in which tagged fish are recovered. Simulation 2 attempts to provide that context.

Some other quantities of interest, e.g., release group-specific estimated exploitation and maturation rates, are also based on sample-expanded tag recoveries, but the estimators are independent of the mark and tag rates (since the release group-specific mark and tag rates appear in every term in the denominator, as well as in the numerator). However, variation in the number of tag recoveries itself for a given sampling rate would

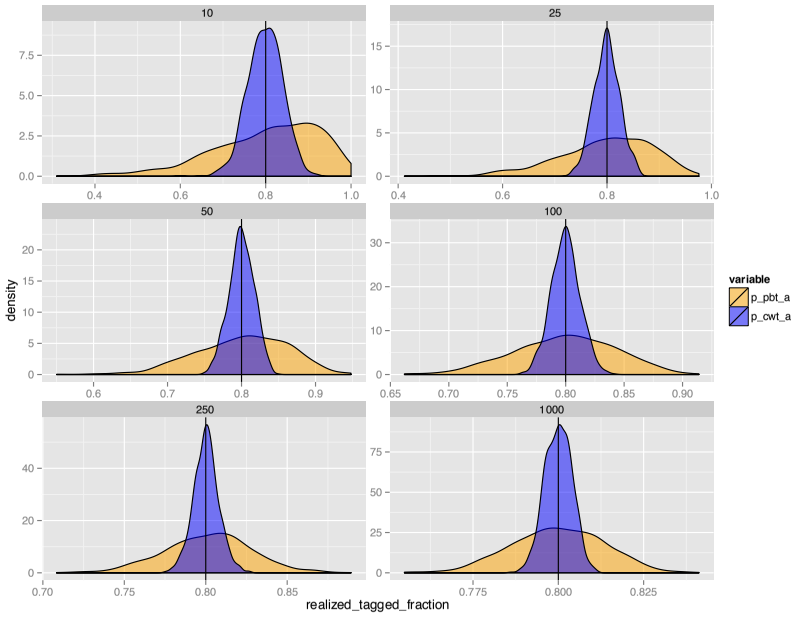


(a) $G = \lfloor 0.80S \rfloor$

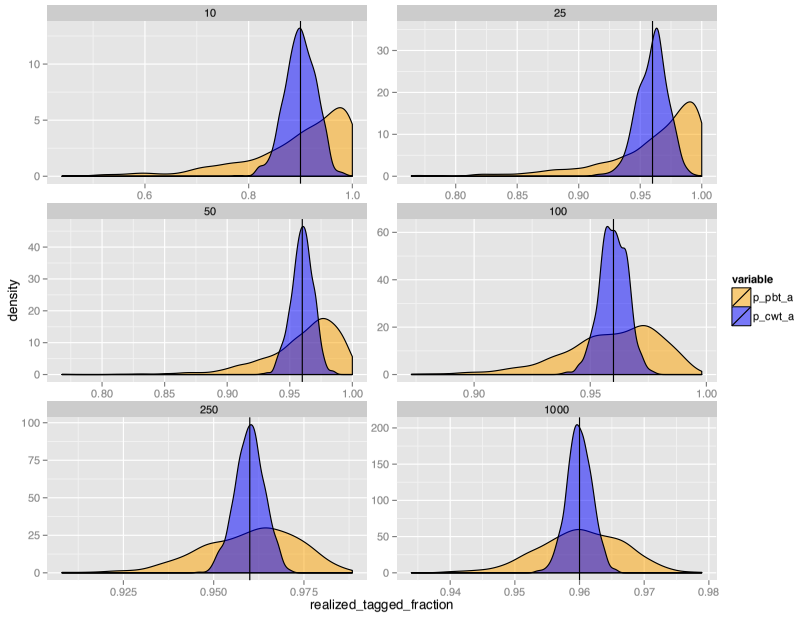


(b) $G = \lfloor 0.96S \rfloor$

Figure 1: Density estimates of the distribution of realized tagging fractions under PBT (orange) and CWT (blue) scenarios in Simulation 1 with $\frac{N_b}{2S} = 1$ (i.e., Wright-Fisher variance in reproductive success). Number of successfully tagged families equal to $G = \lfloor 0.80S \rfloor$ in (*a*) and $G = \lfloor 0.96S \rfloor$ in (*b*). Numbers atop each panel give S, the number of families contributing to the release group. The black vertical line gives the true fraction of families successfully genotyped or tagged at release by CWTs. Note that scales differ between panels.



(a) $G = \lfloor 0.80S \rfloor$



(b) $G = \lfloor 0.96S \rfloor$

Figure 2: Density estimates of the distribution of realized tagging fractions under PBT (orange) and CWT (blue) scenarios in Simulation 1 with $\frac{N_b}{2S} = 0.5$. Number of successfully tagged families equal to $G = \lfloor 0.80S \rfloor$ in (*a*) and $G = \lfloor 0.96S \rfloor$ in (*b*). Numbers atop each panel give S, the number of families contributing to the release group. The black vertical line gives the true fraction of families successfully genotyped or tagged at release by CWTs. Note that scales differ between panels.

depend on the variance of the realized adult tagging rate.

4.1 Simulating sample-expanded tag recovery estimates

In Simulation 2, under the PBT scenario, each release group is, as before, descended from S families, of which G are successfully tagged. However, a fraction c of the release group is marked in such a way as to be subject to sampling. In the simulated recovery stratum, there are N_C fish from the release group in total, but only a fraction v of the total is sampled for marks. Accordingly, the expected fraction of fish from the release group expected to yield tag recoveries is $\frac{cvG}{S}$. If n_{pbt} tags were recovered, the expanded estimate of N_C, the total number of fish from the release group in the recovery stratum, would be $\hat{N}_{\text{pbt}} = \frac{n_{\text{pbt}}S}{cvG}$. The PBT scenario is compared against a CWT scenario in which a fraction c of the release group is marked, and 100% of those marked fish carry CWTs (i.e., assume for simplicity that the rate of tag shedding is 0). N_C fish from the release group appear in the recovery stratum, and a fraction v of the total is sampled for marks, yielding n_{cwt} recoveries and an expanded estimate of $\hat{N}_{\text{cwt}} = \frac{n_{\text{cwt}}}{cv}$ fish from the release group in the recovery stratum.

It is worth mentioning that while we refer to fish as being "marked" these simulations apply equally well to both visually and electronically sampled fisheries. In the visual case, a fraction c of the fish are ad-clipped and all of those carry CWTs (in the CWT scenario), or a fraction G/S are expected to be tagged with PBT (in the PBT scenario). In the electronic case, the PBT scenario is equivalent to a random fraction c of the fish in the release group receiving AWTs that will trigger electronic detectors and lead to genotyping of the fish, while the CWT scenario is equivalent to a fraction c of the release group carrying CWTs.

The effect of c and v on the estimates always acts through their product, and, in Simulation 2 we can simply model their product $m = cv$, the fraction of the release group in the recovery stratum that is expected to be marked and sampled. For PBT simulation replicates we first simulate $\tilde{p}_{\text{pbt}}$ as in Simulation 1, and then we simulate the number of PBT-tagged fish recoveries as $n_{\text{pbt}} \sim \text{Binomial}(N_C, m\tilde{p}_{\text{pbt}})$, from which we compute and record $\hat{N}_{\text{pbt}} = \frac{n_{\text{pbt}}S}{mG}$. For the CWT simulation replicates, we simulate the number of sampled and tagged fish in the total recovery stratum as $n_{\text{cwt}} \sim \text{Binomial}(N_C, m)$, and from that compute $\hat{N}_{\text{cwt}} = \frac{n_{\text{cwt}}}{m}$.

4.2 Simulation 2 settings

500 replicate simulations were performed for every combination of the following set of values:

- $S \in \{10, 20, 30, 50, 100, 200, 400, 1000\}$
- $N_C = N_A = 50$
- $G \in \{\lfloor aS \rfloor\}$ where $a \in \{0.80, 0.82, \ldots, 0.98, 1.00\}$, and with p_{cwt} set to the same value as $\lfloor aS \rfloor$ in each case.
- $\mu = 3000$, $r = 30$, and $\alpha \in \{0.3, 0.5, 1.0, 2, 10\}$, as well as an additional scenario corresponding to Wright-Fisher reproduction.
- $m \in \{0.125, 0.25, 0.5, 0.75, 1.0\}$

Each of the different scenarios of family size variance produced a different ratio of $N_b/(2S)$ covering a broad range: $(0.25, 0.34, 0.50, 0.66, 0.88, 1.00)$, we summarize the results in terms of these $N_b/(2S)$ ratios rather than in terms of α, μ, and r.

Figures 3–7 display the standard deviation of the estimators $\hat{N}_{\rm pbt}$ and $\hat{N}_{\rm cwt}$. Several clear trends emerge. First, when $m \leq 0.25$, the effect of the extra variance in the PBT tagging rate has a relatively small effect on the variance of $\hat{N}_{\rm pbt}$, even when S is as small as 10. When $m \geq 0.5$ the effect of the PBT tag rate variance is more noticeable, especially at values of S less than 30. If $m = 1.0$, then the PBT tag rate variance is relatively important at all values of S. This is not too surprising, since there is no variance in $\hat{N}_{\rm cwt}$ in that case. However, it is also clear that the extra variance incurred from PBT is not very large for family tagging fractions greater than 0.96 or 0.98. It must also be kept in mind that CWTs will seldom achieve a 100% tag rate, since there is usually some measurable degree of tag shedding. As expected, the influence the of PBT tag rate variance increases when $N_b/(2S)$ decreases.

Overall, the results indicate that the additional variance in expanded estimates, $\hat{N}_{\rm pbt}$ due to the variance in the PBT realized adult tagging rate is somewhat unremarkable when the fraction of tagged families is greater than 98%, regardless of S. If G/S is less than 0.98, however, then the additional variance only becomes truly noteworthy when $S < 50$, $N_b/(2S) < 0.7$ and $m > 0.5$.

Thus, the relative importance of the PBT tagging rate variance to the overall uncertainty of sample-expanded estimates depends on the range of values of those parameters that are likely to be encountered in practice. These values are explored in the following section.

5 Likely range of parameter values

5.1 Effective size of hatchery populations

Only a few estimates of the ratio of the effective number of breeders to the census number of breeders ($N_b/(2S)$) in salmon hatcheries are available. MOYER *et al.* (2007) report estimates of between 0.76 and 0.84 for coho salmon in two different Oregon hatcheries. For Chinook salmon, Mike Ackerman (IDFG Eagle Fish Genetics Lab) provided us with estimates of the number of fish from different families returning to 5 different hatcheries in Idaho. From these data it is possible to estimate a range of $N_b/(2S)$ values from 0.3 to 0.5.

5.2 Number of families per release group

The results in Table 1 gives the size of all CWT-tagged release groups from 2000 forward for Chinook and coho salmon. For this analysis, we have assumed that if each release group were to be tagged via PBT that it arose from matings at the hatchery in which the number of male and female parents is equal and that

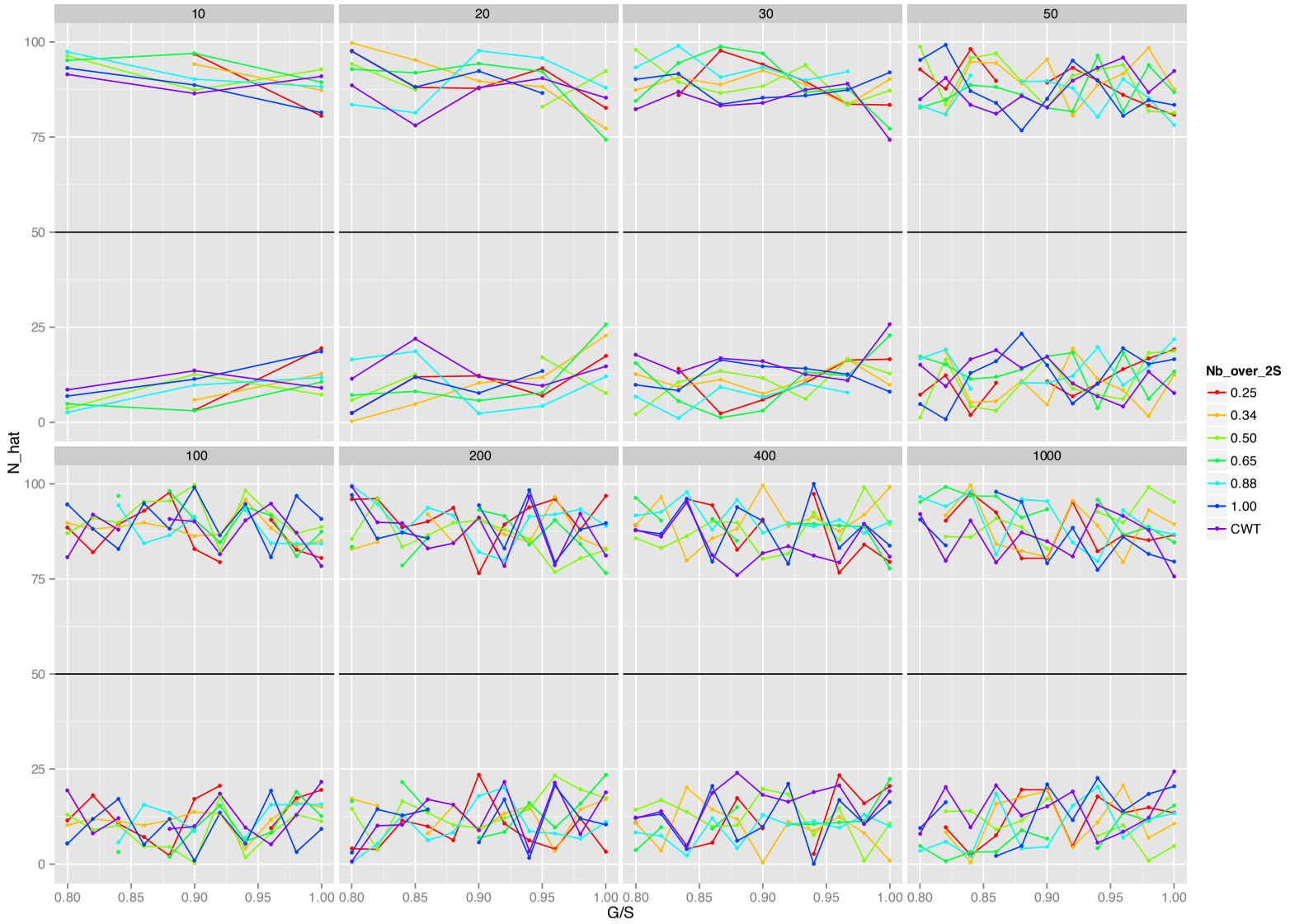


Figure 3: The uncertainty around expanded estimates using PBT or CWTs when $m = 0.125$. x axis shows the expected tagged fraction $\lfloor G/S \rfloor$ in each simulation. Upon the y-axis is the number of fish from the release group in the recovery stratum (shown as the black horizontal line) and the mean estimate of that number from each different set of conditions plus or minus 2 standard deviations. Thus, the vertical spread between lines of the same color gives a representation of the uncertainty in the estimate of the expanded number of fish. Colors denote different $N_b/(2S)$ ratios for PBT, or denote the CWT estimate. Different panels correspond to different numbers of S, as denoted on the top of each panel.

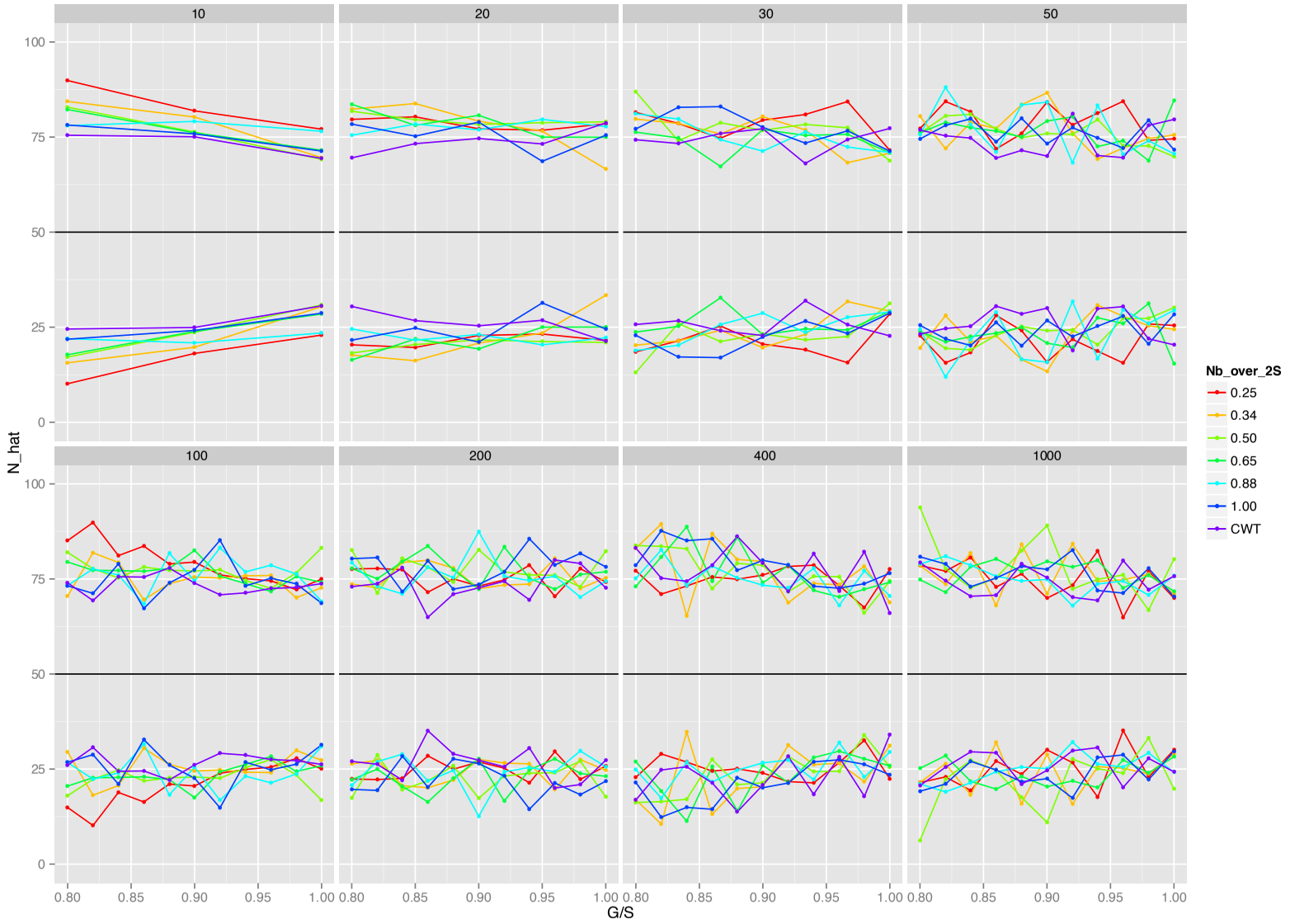


Figure 4: The uncertainty around expanded estimates using PBT or CWTs when $m = 0.25$. x axis shows the expected tagged fraction $\lfloor G/S \rfloor$ in each simulation. Upon the y-axis is the total number of fish from the release group in the recovery stratum (shown as the black horizontal line) and the mean estimate of that number from each different set of conditions plus or minus 2 standard deviations. Thus, the vertical spread between lines of the same color gives a representation of the uncertainty in the estimate of the expanded number of fish. Colors denote different $N_b/(2S)$ ratios for PBT, or denote the CWT estimate. Different panels correspond to different numbers of S, as denoted on the top of each panel.

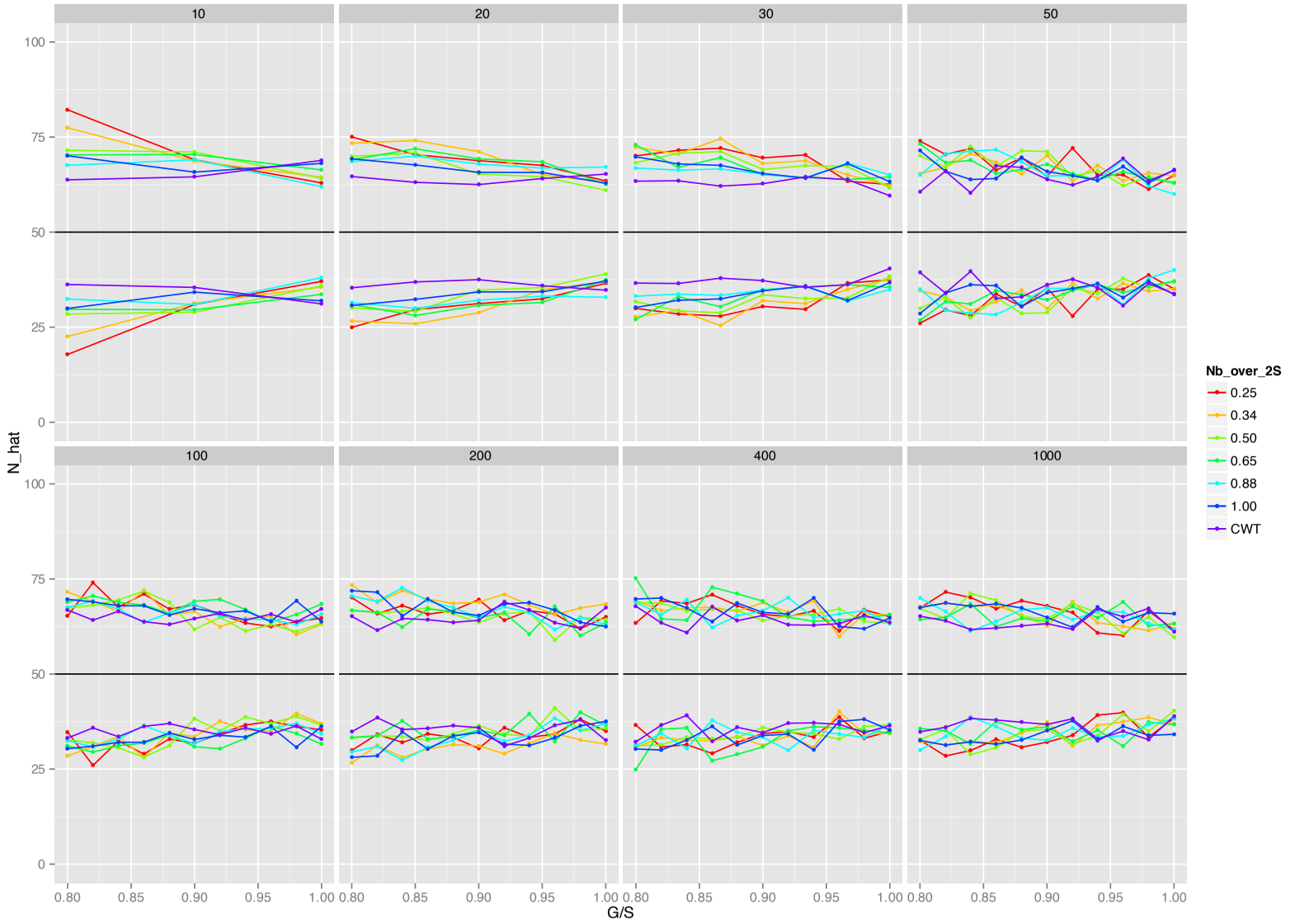


Figure 5: The uncertainty around expanded estimates using PBT or CWTs when $m = 0.5$. x axis shows the expected tagged fraction $\lfloor G/S \rfloor$ in each simulation. Upon the y-axis is the total number of fish from the release group in the recovery stratum (shown as the black horizontal line) and the mean estimate of that number from each different set of conditions plus or minus 2 standard deviations. Thus, the vertical spread between lines of the same color gives a representation of the uncertainty in the estimate of the expanded number of fish. Colors denote different $N_b/(2S)$ ratios for PBT, or denote the CWT estimate. Different panels correspond to different numbers of S, as denoted on the top of each panel.

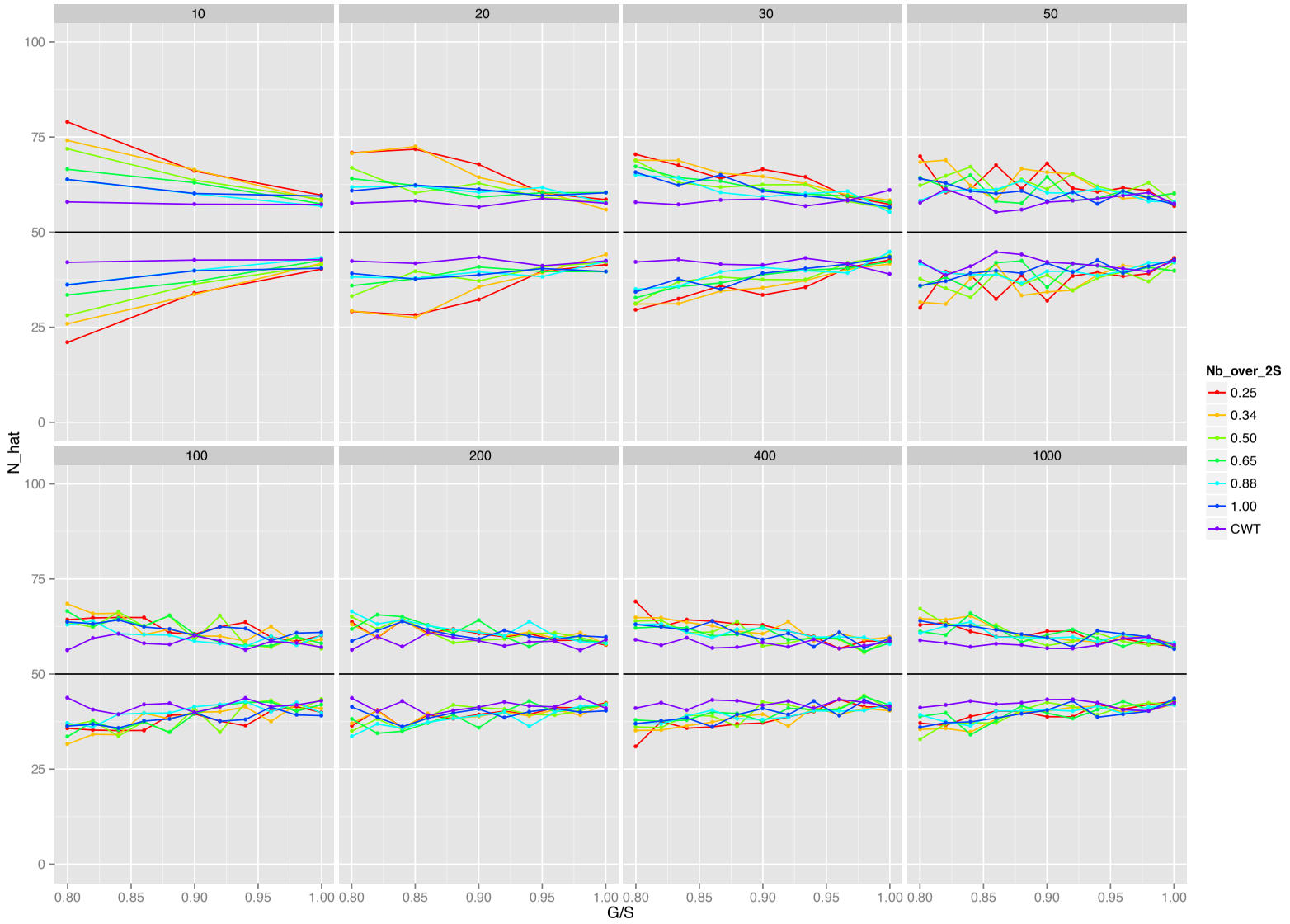


Figure 6: The uncertainty around expanded estimates using PBT or CWTs when $m = 0.75$. x axis shows the expected tagged fraction $\lfloor G/S \rfloor$ in each simulation. Upon the y-axis is the total number of fish from the release group in the recovery stratum (shown as the black horizontal line) and the mean estimate of that number from each different set of conditions plus or minus 2 standard deviations. Thus, the vertical spread between lines of the same color gives a representation of the uncertainty in the estimate of the expanded number of fish. Colors denote different $N_b/(2S)$ ratios for PBT, or denote the CWT estimate. Different panels correspond to different numbers of S, as denoted on the top of each panel.

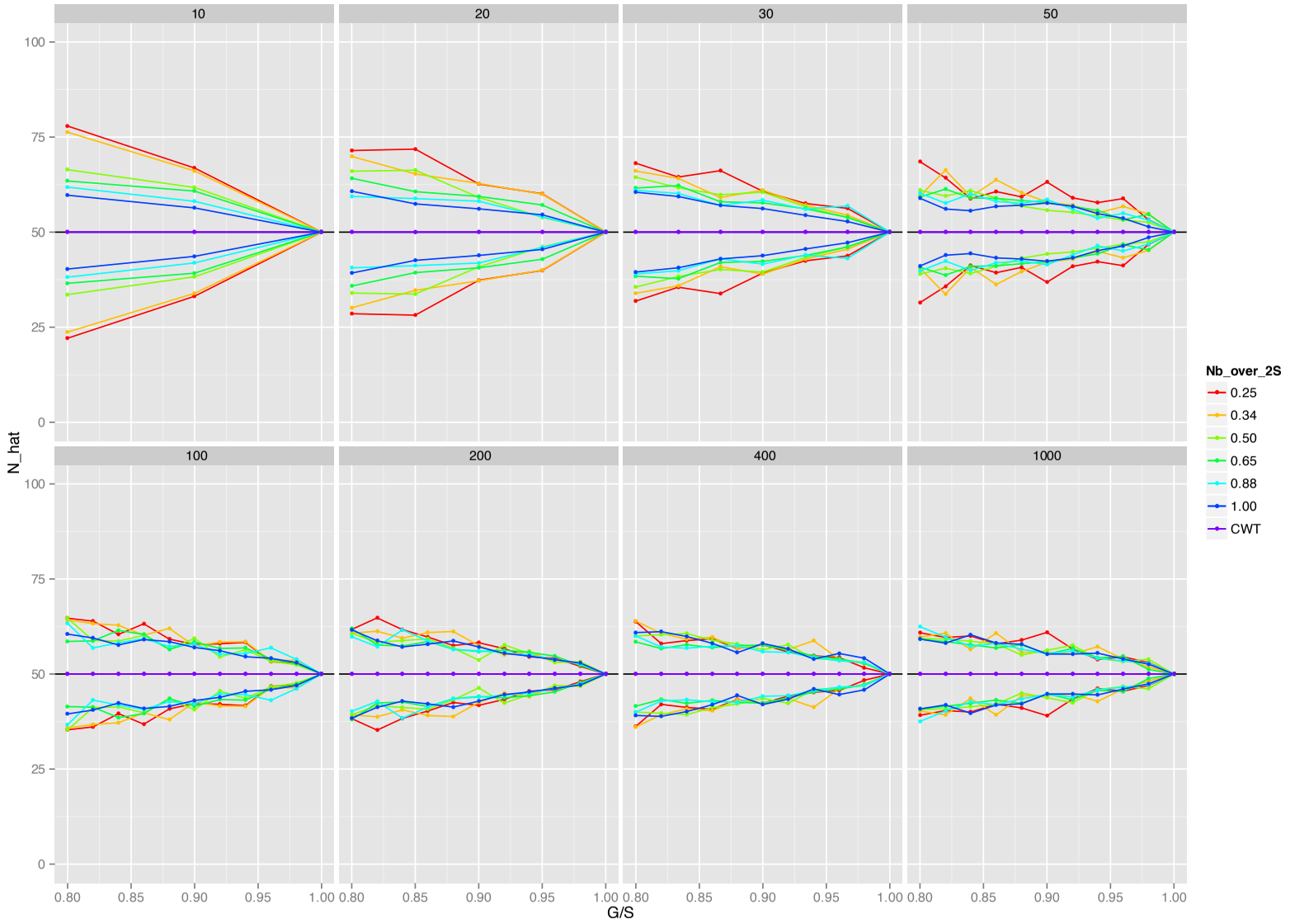


Figure 7: The uncertainty around expanded estimates using PBT or CWTs when $m = 1.00$. x axis shows the expected tagged fraction $\lfloor G/S \rfloor$ in each simulation. Upon the y-axis is the number of fish from the release group in the recovery stratum (shown as the black horizontal line) and the mean estimate of that number from each different set of conditions plus or minus 2 standard deviations. Thus, the vertical spread between lines of the same color gives a representation of the uncertainty in the estimate of the expanded number of fish. Colors denote different $N_b/(2S)$ ratios for PBT, or denote the CWT estimate. Different panels correspond to different numbers of S, as denoted on the top of each panel.

Table 1: Summary of tagged Chinook and coho salmon releases since brood year 2000 across all agencies. The No. families column gives the number of families that would have been required to create both the tagged and untagged components of the release group if the number of male and female parents were equal and each female averaged 3,800 smolts and 1,800 smolts surviving to the release stage for Chinook and coho salmon, respectively. The column No. smolts gives the total number of released smolts since 2000 in releases requiring the number of families in the range as given in No. families. The column No. releases gives the number of actual release groups in each No. families category.

		Chinook		
No. families	No. smolts	No. releases	% of all smolts	% of all releases
0–10	73,629,227	5,138	2.40	33.72
10–25	153,197,630	3,035	4.99	19.92
25–50	248,382,387	2,379	8.08	15.62
50–100	463,816,013	2,177	15.10	14.29
100–250	799,348,868	1,758	26.02	11.54
250–500	443,236,057	454	14.43	2.98
500–1,000	383,866,505	186	12.50	1.22
1,000–10,000	506,683,703	105	16.49	0.69

		Coho		
No. families	No. smolts	No. releases	% of all smolts	% of all releases
0–10	9,876,599	1,347	1.82	32.79
10–25	24,318,776	806	4.47	19.62
25–50	45,857,171	718	8.44	17.48
50–100	48,153,546	381	8.86	9.27
100–250	157,022,545	554	28.88	13.49
250–500	138,161,146	220	25.41	5.36
500–1,000	70,913,957	63	13.04	1.53
1,000–10,000	49,340,239	18	9.08	0.44

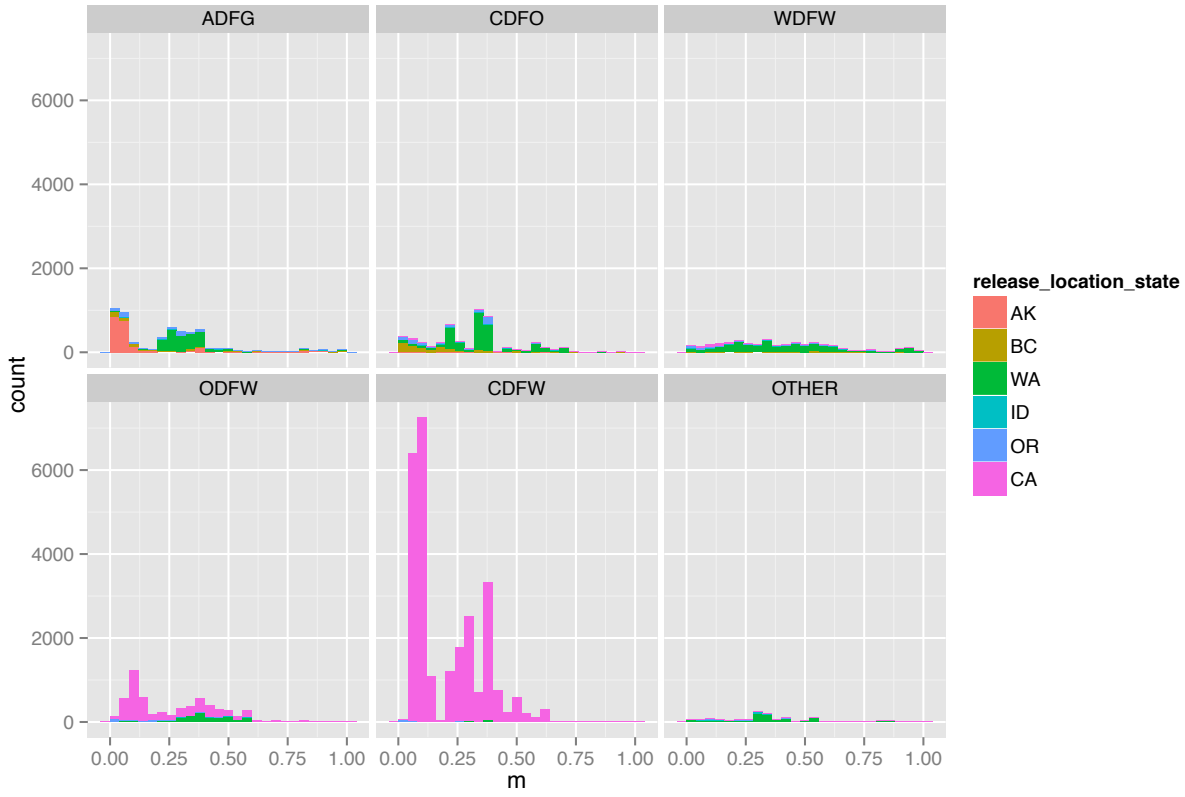


Figure 8: Histograms showing the number of marine fishery recoveries having different values of m. The different panels show recoveries by different agencies. The recoveries are colored according to the state of their release.

each Chinook female produces 3,800 offspring that survive to the release stage, and a coho female produces 1,800 juveniles survive to release.

These tables clearly show that a large fraction ($> 50\%$) of all release groups are very small (requiring fewer than 25 parents to produce the released juveniles).

5.3 Sampling fraction

We calculated the value of m for nearly every non-DIT tag recovered in marine fisheries for Chinook salmon in the year 2012. For each recovery in a marine fishery of type < 50, the value of m was calculated by multiplying the fraction of the release group that received a CWT (c) times the sampling rate (s). The sampling rate for each recovery was derived as the inverse of the value reported for the `estimated_number` field (which provides, "Estimated number of tagged fish in the catch with the same coded wire tag represented by this tag recovery, as estimated by the reporting agency"). This analysis assumes that all fish with CWTs were "marked" so as to be subject to sampling. If a fraction less than 100% of tagged fish are marked, the actual value of m would be smaller than calculated. The calculated m values for each recovery are summarized in the histograms of Figure 8 which show that $m < 0.5$ for most release groups in marine fisheries, though

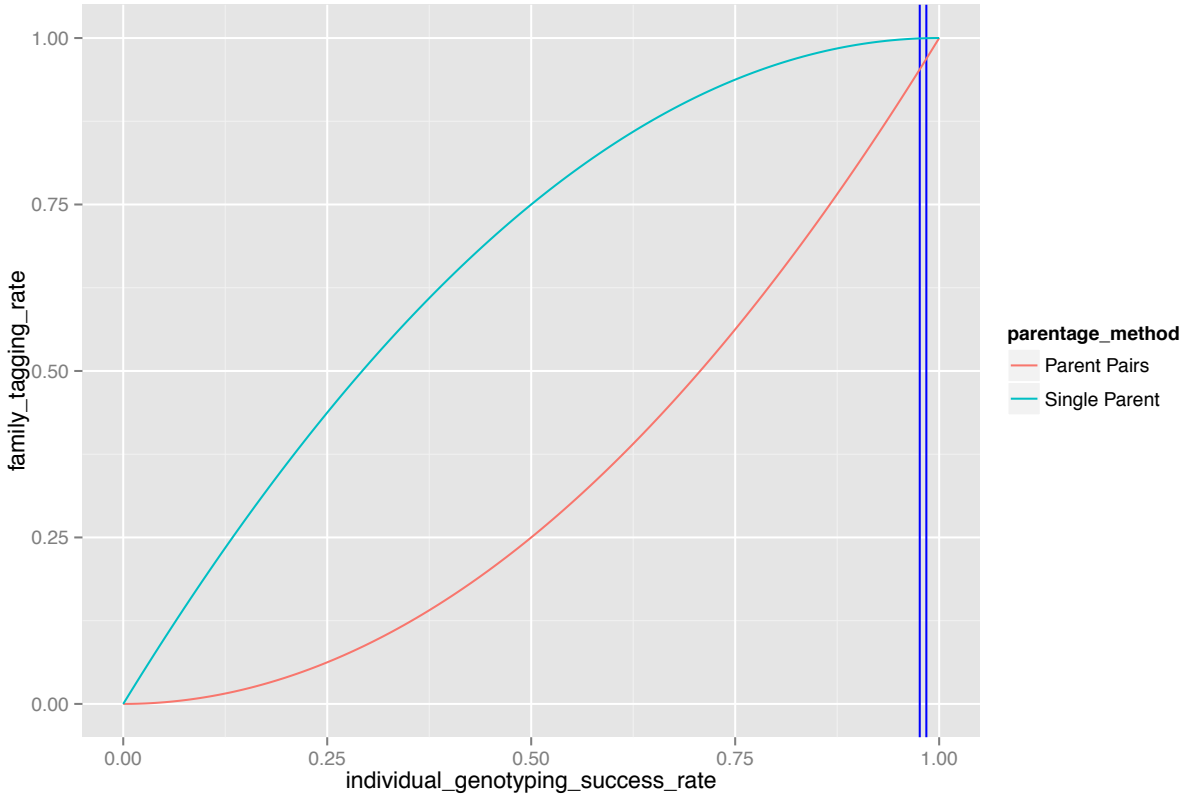


Figure 9: Expected family tagging rates as a function of the rate of successful individual genotyping under two methods of parentage assignment—single parent and parent pair assignment. Vertical blue lines depict the genotyping success rates achieved over the last 5 years in the Chinook salmon and steelhead trout PBT programs in the Snake River basin.

there are some values near 1.0. It should be kept in mind that escapement sampling typically occurs at a higher level than fishery sampling. So, values of m for escapement samples (especially terminal samples at the hatchery) are likely to be higher than in the marine fisheries of Figure 8.

5.4 Fraction of families successfully tagged

It is clear that keeping the successful tagging rate of families as high as possible is important for PBT. One important point this raises is the great advantage that would be achieved if a sufficient number of markers were used such that single-parent assignments could be made accurately. Figure 9 shows the fraction of families tagged as a function of the fraction of the individual genotyping success rate when the parentage assignment is done on the basis of single parents or parent pairs.

If both parents must be successfully genotyped to tag the family, then the number of successfully-tagged families decreases quickly when a few individuals are unsuccessfully genotyped. However, if single-parent assignments were possible, the family tagging rate would decrease much more slowly because only one parent would need to be successfully genotyped to tag the family. For example, even if the individual

genotyping success rate is as low as 0.86, the family tagging rate would still be expected to exceed 0.98.

The most comprehensive data available on the individual genotyping success rate comes from the current Snake River basin PBT programs for Chinook salmon and steelhead trout. In these programs, from 2009 to 2013, 43,770 of 44,468 Chinook salmon (98.43%) were successfully genotyped. During the same period, 26,908 of 27,565 steelhead trout (97.62%) were successfully genotyped. The expected family tagging rates in these programs, using parent-pair assignments is thus 0.969, and 0.953, respectively. Were single parent assignments possible, the expected family tagging rates would be 0.9998 and 0.9994, respectively.

It is worth noting that, because the tagging rate must be less than or equal to one, an expected tagging rate very close to 1 implies that the actual tagging rate must almost always be close to one. This is relevant in consideration of our assumption throughout the simulations that G is known without error. Typically G would only be known without error if all the matings contributing to a release group were recorded, so that each family could be identified as tagged or not, according to whether both parents were successfully genotyped. However, if the expected family tagging rates are very close to 1.0, then it is necessarily the case that the actual tagging rate will, with high probability, also be close to one. Therefore, if a PBT program were able to use single-parent assignments and obtain scorable (successful) genotypes from the broodstock at the same rate that has been achieved over the last five years in the Idaho programs, then (so long as the program was not mating only a single male to each of many tens of females) it would not be necessary to record the release group matings, and still the resulting uncertainty in G would be negligible.

6 Conclusions

Variance in the realized PBT adult tagging rate is most likely to decrease the accuracy of PBT-based estimates of sample-expanded tag recoveries when all four of the following conditions occur:

1. Release groups are composed of offspring of fewer than 30 families,
2. The $N_b/(2S)$ ratio in the hatchery is lower than 0.88,
3. A fraction higher than 50% of all the fish from a release group in a recovery stratum are expected to be marked and sampled,
4. The genotyping success rate is low enough that fewer than 96% of families are successfully tagged.

A review of these conditions and quantities with respect to existing CWT and PBT programs suggest that condition 1 is often encountered, 2 is usually encountered, 3 is infrequently encountered, and most importantly, the existing PBT programs in the Snake River Basin have demonstrated that genotyping success rates can be maintained at high levels.

This analysis indicates that careful sampling and tissue handling protocols to ensure high genotyping success and the use of a sufficient number of markers to allow accurate single parent assignment would reduce PBT realized tag rate variance and its practical consequences to negligible proportions.

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