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# An Evaluation of Otolith Thermal Marking at the Feather River Hatchery, California

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## ABSTRACT

California's Feather River Hatchery (FRH) propagates two runs of Chinook salmon (*Oncorhynchus tshawytscha*): spring run and fall run. Loss of spawning habitat and historical hatchery practices have led to introgression of these runs. Recent efforts to reform hatchery operations at the FRH are focused on reducing introgression and increasing the proportion of natural-origin spawners in the broodstock. Implementing these reforms, however, requires a means of distinguishing FRH fish from natural-origin fish, and FRH spring-run fish from FRH fall-run fish. Coded-wire tagging and parentage-based genetic tagging can be used for this purpose, but are labor-intensive and expensive. Otolith thermal marking (OTM) is a 100% marking technique widely used in the Pacific Northwest, Alaska, and Russia that can be effective and relatively inexpensive. We initiated an OTM program at the FRH in 2005 to determine its viability as a 100% marking tool for a hatchery with an annual production goal of 10 million smolts. Our analysis of otoliths collected from returning adults at the FRH demonstrated that OTM could be successfully applied to identify the origin (FRH or natural) and, for FRH fish, the run type (spring run or fall run). Otoliths collected between 2009 and 2011 show run-type mixing between 12% to 20% in both spring-run and fall-run FRH broodstock. Additionally, results suggest natural-spawner contribution to hatchery broodstock is very low (<1% to 10%). OTM may provide another way to reduce the rate of introgression between FRH spring-run and fall-run Chinook salmon, and increasing the proportion of natural origin spawners in hatchery broodstock, both of which should improve the long-term viability of FRH spring-run and fall-run Chinook salmon.

## KEY WORDS

Chinook salmon, fall-run, spring-run, otolith thermal marking, broodstock, hatchery management, introgression, natural origin, bias.

## INTRODUCTION

The Feather River drainage is located in the Central Valley of California and supports both spring-run and fall-run Chinook salmon (*Oncorhynchus tshawytscha*) populations. Fall-run salmon migrate from the ocean in fall and historically spawned in the lower foothill reaches. Alternatively, spring-run salmon migrate

from the ocean in the spring and early summer and historically spawned in the uppermost reaches in cold mountain tributaries (Yoshiyama et al. 2001). Thus, life history strategies of spring run and fall run have historically created both temporal and spatial reproductive isolation. However, construction of the Oroville Dam in 1967 cut off all historical spring-run spawning habitat (Reynolds et al. 1993) and a portion of fall-run habitat. As a result, all natural-origin river spawning of spring run and fall run is now concentrated in the first 12 river kilometers (rkm) below the Oroville Dam. Spring-run still migrate in spring and early summer but now hold over in cold deep pools of the lower Feather River during the hot summer months, a by-product of Oroville Dam operations. Because the spawning times of spring-run and fall-run salmon overlap, both spatial and temporal reproductive isolation have been lost.

The Feather River Hatchery (FRH) began operation shortly after construction of the dam as mitigation for the loss of upstream spawning habitat of salmon and steelhead. Early hatchery management practices attempted to separate spring-run and fall-run salmon during spawning; however, considerable mixing of spring-run and fall-run populations has occurred (CDFG 1998). Habitat modifications such as those resulting from the construction of dams and their associated mitigation hatcheries are particularly problematic because these factors appear to be important contributors to increases in hybridization rates (Rhymer and Simberloff 1996). The most recent genetic data indicates spring-run and fall-run Feather River salmon are genetically homogenous based on neutral microsatellite data, although a genetic distinction does exist at the circadian rhythm gene locus (a gene influencing run timing) (O'Malley et al. 2013).

Even though FRH spring-run are included in California's Central Valley spring-run Chinook salmon Evolutionary Significant Unit (ESU), which is listed as threatened under both the state and federal Endangered Species Acts (ESAs), FRH operations are considered to be a significant threat to the recovery of spring-run salmon within the ESU (NMFS 2014). This is largely attributable to the potential for FRH spring-run, which are introgressed with fall-run and predominately of hatchery origin, straying onto the spawning grounds of natural populations elsewhere within the ESU, particularly the Deer, Mill, and Butte Creek populations (NMFS 2014). The California Hatchery Scientific Review Report (California HSRG 2012) includes major concerns regarding the Chinook salmon programs at FRH:

1. Offsite release of juvenile production and its tendency to promote straying of returning spawners to areas outside the Feather River Basin (and to the Yuba River),
2. Introgression of the two run-types, and
3. Lack of inclusion of natural-origin fish at sufficient levels in the broodstock to lessen the genetic risks associated with domestication selection to FRH spring-run and fall-run and the Feather River natural populations they are integrated with, respectively.

Having the means of identifying an individual's origin and run type is necessary for addressing the latter two of these concerns: introgression and domestication selection.

In the spring of 2004, the California Department of Water Resources (CDWR) started a spring-run tagging program to increase reproductive isolation. The early-arriving spring-run salmon phenotype is allowed to ascend the FRH fish ladder in May and June, when they are tagged externally with Hallprint© dart tags and immediately released back into the lower Feather River to over-summer naturally. In fall, when the FRH fish ladder re-opens, only previously dart-tagged individuals are selected as broodstock for the FRH spring-run program. The spring-run tagging program occurs annually and provides a mechanism to visually distinguish spring-run during spawning operations. However, introgression with fall-run fish still occurs because the spring-run tagging program is imperfect; not all spring-run fish returning to the hatchery in the fall are dart-tagged and not all dart-tagged fish are spring-run, based on coded-wire tags (CWTs).

Marking with adipose fin-clips and tagging with CWTs is the most common method used to identify origin and run type among Central Valley hatcheries (Nandor et al. 2010). Currently, 100% of FRH spring-run and 25% of the fall-run fish are marked

with an adipose-fin clip and tagged with CWT. About six million fall-run fish are released without a mark or tag. CWT tagging of all hatchery fish is recommended by the California HSRG (2012), but it has yet to be implemented and would add a substantial cost to the current FRH operation budget. During the fall spawning operations, CWTs are used to cull early egg groups found to have high percentages of spring–fall mating crosses. While this greatly reduces introgression, hybrid crosses still occur. Operational constraints require daily egg collections to be mixed in incubators trays, resulting in an inability to remove individual run-type mating crosses without sacrificing large groups of eggs.

Though genetic stock identification techniques based on allele frequencies would not be sufficient to accurately identify the run type and origin of individuals at the FRH (Banks et al. 2000; Williamson and May 2005; Garza and Pearse 2008), recent work with single nucleotide polymorphisms (SNPs) has demonstrated the possibility of using a genetics-based method to determine the origin and run type of FRH-produced fish (Clemento et al. 2011). Parentage-based tagging using SNPs is a method that requires not only genotyping of the parent broodstock, but also the genotyping and parentage analysis of returning offspring (Anderson and Garza 2006). Although an effective 100% tagging method, parentage-based tagging is labor-intensive and relatively expensive.

Otolith thermal marking (OTM) is another method of 100% marking juvenile salmonids in hatcheries that is widely used among the Pacific Rim nations (Volk et al. 2005). The use of controlled, short-term, water temperature variations in the hatchery rearing environment to induce otolith marks is appealing because it is benign, it is simultaneously delivered to all incubating fry, it is permanent, and it is possible to induce a variety of mark codes. In comparison with CWT and parentage-based tagging (PBT), OTM is a relatively straightforward way of 100% marking, is relatively inexpensive to apply, and while not as informative as CWT or PBT and subject to reader error, may be sufficient for the FRH Chinook salmon programs.

In 2005, we implemented an OTM program at the FRH to mark all Chinook salmon released from the hatchery. At the time of implementation in 2005, the FRH had no 100% marking programs. The program was designed to provide a means of identifying origin and run type for each individual. Our main objective was to investigate whether OTM was a viable option for marking all 10 million fish released annually from the FRH. Specifically, we benchmarked our ability to identify individuals within the broodstock by origin and run-type from the thermally marked otoliths of CWT fish, of known origin and run-type. We also analyzed the OTM data, together with CWT data, to determine the proportion of natural-origin spawners and the degree of run-type mixing within the broodstock.

## MATERIALS AND METHODS

### Thermal Marking

We thermally marked otoliths of Chinook salmon alevins by exposing them to a scheduled series of chilled-water treatments while in their incubation trays at the FRH (Figure 1) (Volk et al. 1994). The treatment series were initiated approximately 1 week after hatching. Each treatment was applied by lowering the ambient water temperature supplied to the trays approximately 2 °C for 7 hrs using a large-capacity chiller (Figure 2). The incubating stacks are plumbed with two separate lines of supply water—one from ambient river water and one from the chiller. Instantaneously, the water supplied to the stacks is switched from ambient water to chilled water by closing the ambient water valve and opening the chilled water valve over the appropriate stacks. The chiller does not have the capacity to chill all the stacks at one time nor are the fish all at the same developmental stage at the same time. Therefore, fish were grouped together based on similar development stage and total number of stacks (or fish) for the treatment.

The otolith banding patterns we selected followed the “interleaved two of five” bar code symbology, consisting of six bands and five spaces (Palmer 1989; Volk et al. 1994). A series of six unique codes were used to indicate the run-type and brood year for all

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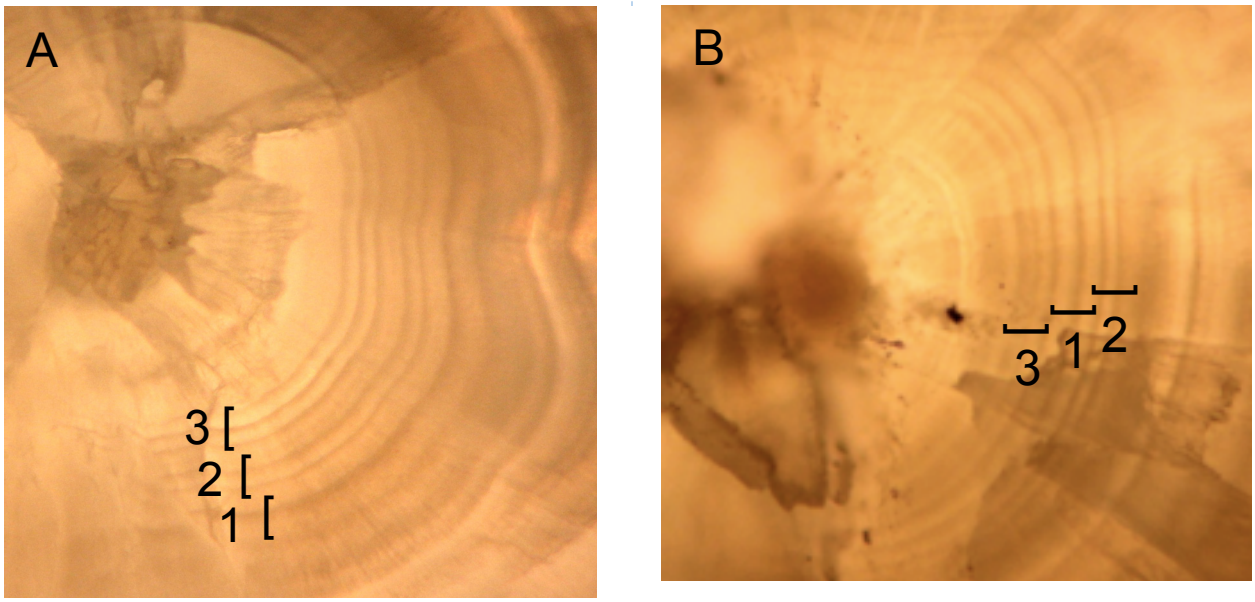


**Figure 1** Otolith thermal mark examples for Feather River Hatchery Chinook salmon: (A) fall-run, broodyear 2006 mark (3-2-1) and (B) spring-run, broodyear 2007 mark (3-1-2).

**Table 1** Marking patterns for spring-run and fall-run broods in 2005–2009. Parentheses ")" denote the individual mark, which are separated by either a narrow or wide space. Numeric coding convention represents a single mark (1), double mark (2), and triple mark (3), with the hyphen (-) representing the larger space between marks.

| | 2005 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|
| Fall run | 1-2-3 ) )) ))) | 3-2-1 ))) )) ) | 2-1-3 )) ) ))) | 1-2-3 ) )) ))) | 3-2-1 ))) )) ) |
| Spring run | 1-3-2 ) ))) )) | 2-3-1 )) ))) ) | 3-1-2 ))) ) )) | 1-3-2 ) ))) )) | 2-3-1 )) ))) ) |

Chinook produced at the FRH (Table 1). Every third brood year the series of codes was repeated. Our objective was to develop thermal marks that would identify run type and brood year from every FRH fish we recovered, essentially providing the same information as a CWT tag.

OTM fish were then moved from incubator trays to outdoor hatchery raceways as determined by FRH standard operating procedures. CWT tagging operations occurred once fish meet minimum size requirements. Spring-run CWT tagging was performed by manual tagging crews at a target rate of 100%, fall-run CWT tagging occurred in automated tagging trailers at a target rate of 25%. Because of tag shed rates and mechanical and human error, the true CWT mark/tag rate is often near but under the target rate (Buttars 2009, 2010, 2011).

To investigate mark quality, a small proportion of OTM juvenile fish were sampled before their release. Of the 77 otoliths examined, all were confirmed to have the intended mark; however, mark groups from some years were not checked because of an equipment failure that resulted in the loss of samples. Because of the nature of OTM water treatments (all incubator trays are treated, no tag shedding) we assume a 100% mark rate.

The FRH was the only hatchery in the Central Valley conducting an OTM program; thus, when adult OTM fish were detected at the FRH they could be identified as either FRH-origin or non-FRH-origin fish.

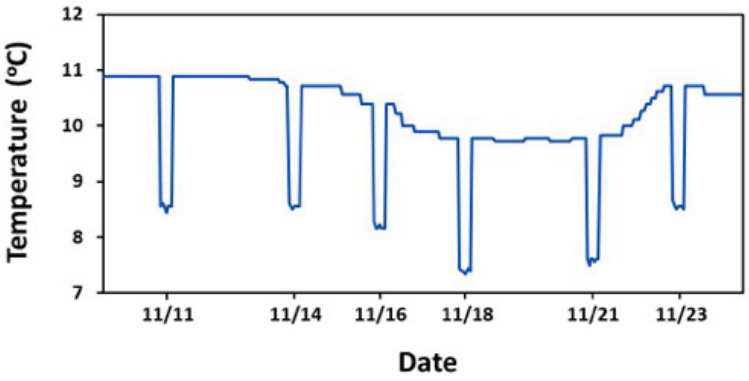


**Figure 2** An example of temperature fluctuations during marking operations at the Feather River Hatchery in Oroville, CA. This particular schedule of fluctuations generated the mark code 1-3-2.

Presumably a high proportion of the non-FRH-origin fish entering the FRH are Feather River natural-origin fish, with a small proportion originating from outside the Feather River Basin, either from a hatchery or natural area.

## Otolith Collection

Otoliths of known origin and run type were required for use in validating the OTM method and were collected from CWT fish. Otoliths from CWT fish were collected on Feather River natural area spawner surveys. Some brood year, run-type combinations were below our target sample size of 20. Brood years 2005 and 2009 contained the smallest sample size because of their availability in only one sampling season, i.e., brood year 2005 as 4-year-olds in 2009 and brood year 2009 as 2-year-olds in 2011.

Otoliths used to estimate composition of hatchery returns were collected from post spawned adults at the FRH between 15 September and 1 November in 2009 thru 2011. Otolith collections focused on the non-clipped (non-CWT) portion of both spring-run and fall-run hatchery returns because these fish represented salmon of unknown origin and run-type. For clipped fish returning to the FRH, CWTs were used to determine origin and run-type. During spring-run spawning operations, our goal was to sample 100% of non-clipped salmon. External Hallprint© tag numbers, sex, and length were recorded. During fall-run spawning operations, non-clipped fish were sub-sampled, because of the large number of fall-run Chinook spawned at the FRH. A target rate of approximately 20% of non-clipped fish was sampled throughout the fall-run spawning season.

## Otolith Preparation

Otolith mounting and polishing was based on the protocols of Stevenson and Campana (1992) with the addition of an electric grinder/polisher. Otoliths were attached to glass microscope slides using thermal plastic (Crystal Bond) and ground on both sides using a Ted Pella, Inc. XP 8 Grinder/Polisher with 1200 grit polishing paper until the primordium was just visible. The otoliths were then manually polished using 9 micron and 3 micron polishing paper until thermal marks were distinct under 200× magnification. An image of each otolith was taken using a Canon EOS Rebel T3i digital camera and downloaded to a computer. The contrast of the image was enhanced before reading using Canon's Digital Photo Professional. A 22-inch, high definition, HP computer monitor was used to display the images for reading.

## Otolith Reading

Otolith reading was conducted by a single experienced reader because the reliability of otolith and scale readings by a single experienced reader has been found to be more accurate and more precise than the combined efforts of several readers of varying experience (Flain and Glova 1988). For each year, otolith images from fish of known (CWT), and unknown, origin and run type were merged and sorted by collection date, which yielded a mixed sequence of images with respect to known/unknown, origin, run-type, and brood year. Image names were changed to a numeric value and data sheets were created that contained image number, sex, and fork length. Sex and fork length data were provided to the reader in order to help narrow down the range of possible OTM codes, given that the codes recycle every 3 years. Otolith size and annular marks were not visible from images thus there was no further consideration of size by the reader. With sex and fork length data the reader evaluated each image

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and reported the mark if present. Individuals with no discernible thermal mark were classified as "Feather River natural-origin" fish, acknowledging that the lack of a thermal mark could also indicate stray hatchery or natural-origin fish from outside the Feather River Basin, or FRH-origin fish that were not effectively thermally marked.

## Reader Accuracy

A validation matrix was used to evaluate reader accuracy using blind reads of known origin, run type, and brood year ($n = 215$). Construction of the matrix compared the reader-assigned origin, run type, and brood year with CWT-known run type and brood year (Table 2). The matrix represents reader accuracies for given run-type/brood-year combinations, as well as, associated inaccuracies.

We used Kimura and Chikuni's (1987) method to estimate proportions of FRH returns. Their method uses the validation matrix to adjust the proportions of read thermal marks from the unknown samples using an algorithm that corrects for reader bias. Using this method, reader bias is quantified and adjusted for. One difference between the Kimura and Chikuni (1987) validation matrix and ours is their assumption that the matrix is known vs. estimated, as in our case.

No reader validation data was available for the "No OTM, known" category in the matrix because of an inability to locate known natural-origin adult otoliths. However, we did include them in the validation matrix because the algorithm requires accuracy values for all groups. Thus, the model assumes the reader is 100% accurate at identifying non-thermal-marked otoliths.

## Estimation of Proportions

Our estimation of origin and run-type proportions used a maximum likelihood estimator originally developed to estimate age distributions of fish populations (Kimura and Chikuni 1987). This method uses the validation matrix described above to adjust reader-assigned proportions for the unclipped sampled fish. The matrix uses conditional probabilities to adjust reader-assigned proportions by distributing raw reads based on accuracies and inaccuracies of the validation matrix.

Validation matrices were applied separately to the 2009, 2010, and 2011 data sets and included only the origin/run-type combinations possible for the given

**Table 2** Validation matrix based on the accuracy of otolith reads from sampled CWT fish of known run-type and brood-year samples ($n = 215$). "Read As" data was unavailable for umarked individuals ("KNOWN, No OTM") and thus was assumed (here shown to be read with 100% accuracy).

| | | F05 | S05 | F06 | S06 | F07 | S07 | F08 | S08 | F09 | S09 | No OTM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| READ AS | F05 | 79% | 20% | 0% | 20% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | S05 | 7% | 60% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | F06 | 7% | 20% | 100% | 15% | 4% | 0% | 0% | 0% | 0% | 0% | 0% |
| | S06 | 0% | 0% | 0% | 55% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | F07 | 0% | 0% | 0% | 5% | 93% | 0% | 6% | 7% | 0% | 0% | 0% |
| | S07 | 0% | 0% | 0% | 0% | 0% | 100% | 3% | 0% | 0% | 0% | 0% |
| | F08 | 0% | 0% | 0% | 0% | 0% | 0% | 83% | 41% | 0% | 0% | 0% |
| | S08 | 0% | 0% | 0% | 0% | 0% | 0% | 6% | 48% | 0% | 0% | 0% |
| | F09 | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 78% | 22% | 0% |
| | S09 | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 17% | 67% | 0% |
| | No OTM | 7% | 0% | 0% | 5% | 4% | 0% | 3% | 4% | 4% | 11% | 100% |
| | # Samples | 14 | 10 | 13 | 20 | 27 | 37 | 35 | 27 | 23 | 9 | 0 |

year. For example, the 2009 analysis was limited to the 2005–2009 marks/reads; for 2010 limited to the 2005–2008 marks/reads; and for 2011 limited to the 2006–2009 marks/reads.

Adjusted proportions of origin and run-type from the Kimura and Chikuni (1987) method were expanded to include all non-clipped, unsampled returns by year and run-type. These data represent the OTM estimate of origin and run-type proportions for unclipped FRH returns. CWT recovery data was determined from the Regional Mark Information System database (RMIS; *http://www.rmpc.org*). To calculate the proportion of origin and run-type of all spring-run and fall-run Chinook returning to the FRH we used the OTM estimate of unclipped fish and CWT data for clipped fish. The estimated proportions of origin and run type from both OTM and CWT data were combined by weighting the relative abundance of the clipped and unclipped total hatchery escapement for each year.

Although known reads of unmarked (natural-origin) otoliths were not performed, the distribution of possible reads for unmarked otoliths must be included in the validation matrix in order to apply the method of Kimura and Chikuni (1987). Therefore, we specified this distribution *a priori* and then evaluated the sensitivity of the resulting estimated proportions to a range of alternative assumptions for the read accuracy on fish not thermally marked: 100%, 75%, and 50% correct. The complement of the percentage—the percentage that was incorrectly designated as marked fish—was distributed evenly across the possible OTM categories.

## RESULTS

### Reader Accuracy

A total of 1,051 (527 from the spring-run spawning period, 524 from the fall-run spawning period) otoliths from non-clipped hatchery returns in 2009–2011 was prepared and analyzed for the presence of OTMs. An additional 215 otoliths from CWT fish of known origin, run-type and brood year were prepared and used as validation samples.

Based on the validation matrix (Table 2), our success in identifying FRH thermally marked fish varied from 89% to 100% depending on the mark. Our success in correctly identifying run type of hatchery individuals varied from 48% to 100% for spring-run and 78% to 100% for fall-run (Table 2). Reading accuracy of individual broods based on validation samples varied. For example, spring-run 2009 (S09) known samples were inconsistent and difficult to read. On the other hand, spring-run 2007 (S07) samples were all "bold" and "distinguished." In the case of S09, of the nine known samples, the reader correctly identified the mark 67% of the time; 22% of the time the reader identified S09 as fall-run 2009 (F09), and 11% of the time as no thermal mark. In contrast, of the 37 S07 known samples, 100% were read correctly.

### Estimation of Proportions

Based on the expansion of the estimated proportions from the Kimura and Chikuni (1987) method, natural-spawner contributions to hatchery broodstock are low (Table 3). The estimated percentage of non-FRH produced fish entering the hatchery ranged from 0.2% (4 of 1,969) in spring-run 2011 to 10.2% (2,032 of 19,972) in fall-run 2010 (Table 3).

Estimates of FRH returns using raw CWT and expanded OTM data revealed consistent mixing of run type in both spring-run and fall-run broodstock (Table 3). Mixing in the spring-run broodstock included 12.1%, 14.5%, and 20.3% fall-run fish, respectively, for each year (Table 4). Fall-run broodstock mixing included 19.4%, 12.0%, and 16.2% spring-run fish, respectively, for each year (Table 4).

The differences between the unadjusted and adjusted estimates of origin and run-type proportion using the Kimura and Chikuni (1987) method was less than 3.2% in all but two cases, in 2011 the spring-run adjustments increased the estimated proportion of spring-run by 6.7% and decreased the estimated proportion of fall-run by 5.7%.

Results of the sensitivity analysis suggest a maximum of 3.9% change in the estimates for natural-spawner contribution for all years based on a 50% accuracy rate vs. a 100% accuracy rate of reading non-marked samples. For example, if the reader correctly identified non-OTM otoliths at a rate of 50% (vs. 100%,

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**Table 3** Run type and origin designations based on coded-wire tag (CWT) and otolith thermal mark (OTM) data from hatchery returns to the Feather River Hatchery between 2009–2011

| | | SPRING | | | | | | FALL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Adipose fin status** | **Category** | **2009** | | **2010** | | **2011** | | **2009** | | **2010** | | **2011** | |
| **Clipped** | CWT Fall run | 86 | 8.7% | 35 | 2.1% | 164 | 8.3% | 2,140 | 21.5% | 4,343 | 21.7% | 6,852 | 21.0% |
| | CWT Spring run | 660 | 66.7% | 1,191 | 71.7% | 1,037 | 52.7% | 1,095 | 11.0% | 1,597 | 8.0% | 2,321 | 7.1% |
| | CWT Natural origin | 0 | 0.0% | 0 | 0.0% | 1 | 0.1% | 5 | 0.1% | 6 | 0.0% | 9 | 0.0% |
| | CWT Stray | 1 | 0.1% | 0 | 0.0% | 3 | 0.2% | 62 | 0.6% | 91 | 0.5% | 264 | 0.8% |
| | CWT Hybrid | 0 | 0.0% | 0 | 0.0% | 127 | 6.4% | 0 | 0.0% | 0 | 0.0% | 546 | 1.7% |
| | Unknown CWT | 46 | 4.7% | 36 | 2.2% | 94 | 4.8% | 99 | 1.0% | 279 | 1.4% | 310 | 1.0% |
| **Un-clipped** | OTM Fall run | 34 | 3.4% | 206 | 12.4% | 236 | 12.0% | 4,757 | 47.7% | 10,831 | 54.2% | 17,925 | 55.0% |
| | OTM Spring run | 76 | 7.7% | 185 | 11.1% | 304 | 15.4% | 839 | 8.4% | 794 | 4.0% | 2,956 | 9.1% |
| | No OTM | 86 | 8.7% | 8 | 0.5% | 4 | 0.2% | 966 | 9.7% | 2,032 | 10.2% | 1,419 | 4.4% |
| | Total | 989 | | 1,661 | | 1,969 | | 9,963 | | 19,972 | | 32,602 | |

**Table 4** Run type and origin estimations based on coded-wire tag (CWT) and otolith thermal mark (OTM) data from hatchery returns to the Feather River Hatchery between 2009–2011. Results were obtained by aggregation of the Table 3 all-category results.

| | SPRING | | | | | | FALL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Category** | **2009** | | **2010** | | **2011** | | **2009** | | **2010** | | **2011** | |
| **Fall run** | 120 | 12.1% | 241 | 14.5% | 400 | 20.3% | 6,897 | 69.2% | 15174 | 76.0% | 24,777 | 76.0% |
| **Spring run** | 736 | 74.4% | 1,376 | 82.9% | 1,341 | 68.1% | 1,934 | 19.4% | 2391 | 12.0% | 5,277 | 16.2% |
| **Other** | 47 | 4.8% | 36 | 2.2% | 225 | 11.4% | 166 | 1.7% | 376 | 1.9% | 1,129 | 3.5% |
| **No OTM** | 86 | 8.7% | 8 | 0.5% | 4 | 0.2% | 966 | 9.7% | 2032 | 10.2% | 1,419 | 4.4% |
| **Total** | 989 | | 1661 | | 1,969 | | 9,963 | | 19,973 | | 32,602 | |

as our model assumes), the spring-run 2009 estimate of natural-spawner contribution would change from 86 to 118 fish out of 989 (or a 3.2% increase in total natural-spawner contribution). Total changes in natural-spawner contribution estimates in 2010 would decrease by 3.4%, and in 2011 would decrease by 1.1%. For fall-run, natural-spawner contribution estimates would increase by 3.0% in 2009, increase by 3.5% in 2010, and decrease by 3.9% in 2011. Similarly, sensitivity analyses of run-type estimates suggests a maximum of 3.0% change in contribution estimates for all years based on a 50% vs. 100% accuracy rate of identifying non-OTM samples.

## DISCUSSION

Based on our study we believe OTM in a large production hatchery like the FRH is an efficient way of marking 100% hatchery production. From water treatments in the incubator trays of juvenile salmon, to the collection and preparation of otoliths and the identification and analysis of otolith reads, the OTM program at the FRH was effective. While operationally successful, optimizing OTM as a broodstock collection tool would require improvements to reader accuracy and precision.

We found the accuracy and ease of reading thermal marks to be related to the quality of the thermal marks. It was relatively easy to identify the pres-

ence of thermal marks, the false negative rate ranged from 0% to 11% across run-types and brood years, but it was more challenging to distinguish between some of the patterns we used. The difficulty with the bar-code symbology was the reliance on spaces between marks, i.e. 48 hrs vs. 72 hrs. For example, the difference between marks in 2005 was 1-2-3 for fall-run (F05) and 1-3-2 for spring-run (S05) (Table 1), meaning the only difference was a larger space between the third vs. fourth mark. Given the wide variation in otolith microstructure and depending on the polishing plane, these spaces could be unsubstantial in magnitude. A more robust method would limit similarities i.e., 5 regularly spaced marks [ ))))) ] for spring-run and 10 regularly spaced marks [ )))))))))) ] for fall-run for a given year. Alternatively, a 3-3 mark [ ))) ))) ] for spring-run and a 5-5 mark [ )))))) ))))) ] for fall-run would also limit similarities and aid in reader accuracy. Any combination of these mark types would also allow for brood year-specific marks. Since poor marks create bar codes very similar to each other, a simpler marking scheme may improve reader accuracy.

Given the OTM reader assignment error rates, the current FRH OTM program is not suitable as a brood stock selection tool. We suggest that if OTM were to be implemented again at the FRH or in any other hatchery, that marking symbology be reevaluated. If mark selection were simplified to identify only run type (as described above) and reader accuracy and precision improved it is possible that an OTM program, once established, could exist as a broodstock collection tool even without a CWT program. While not practical for all fish, real-time OTM reading could assist with broodstock collection during overlapping spring-run and fall-run spawning times.

The ability to supply cold water (relative to the ambient temperature) in a hatchery setting directly affects the ability to make bold and distinguishing marks, and is another important consideration for any future OTM program. For example, the water chiller at the FRH is less effective at cooling water below the ambient temperature if the supply water reaches the mid- to high 40s (°F).

Our analysis of otolith reads using the Kimura and Chikuni (1987) method corrected some of the vagaries associated with poor mark quality, and we feel it is an effective method of correcting for reader bias. This method is useful for adjusting the estimates of overall composition (origin and run type), but not the read assignments of individual fish. The method also requires the specification of the validation matrix, which in this case was based on the otolith reads of coded-wire-tagged fish, and therefore, relies on a CWT program or other means of obtaining "known" samples.

In the Feather River, we were unable to collect and identify known natural-origin fish otoliths for use in the voucher sample because of the hatchery's fall-run being marked at only 25% (and thus a high proportion of non-clipped FRH fish being in the system). We did not attempt to use natural-origin samples from nearby tributaries such as Mill, Deer, or Butte creeks because FRH stays are recovered in these tributaries, as are stays from other Central Valley hatcheries (Kormos et al. 2012; Palmer–Zwahlen and Kormos 2013). We did attempt to use surrogate natural-origin fish from Alaska, for this purpose, but it was determined that a proportion of these fish were thermally marked and thus we were unable to use them as known natural-origin samples. Our sensitivity analysis, however, indicated that the lack of natural-origin fish samples in our validation matrix does not appear to have greatly affected the results of our study, although, the inability to evaluate non-thermal-mark reader success may be more problematic in a population with a significant natural-origin contribution. In the future, there may be value in contrasting the otolith mark patterns in Feather River natural origin juveniles (at which stage it can be certain that they are, in fact, natural-origin fish) with those of the OTM hatchery-origin juveniles for reader-training purposes. Juvenile samples however, were not used in our validation matrix because of significant differences in the processed appearance of adult and juvenile otoliths, i.e., blind reads would not be possible.

Our estimate of the mean percentage of fish entering FRH over the 2009–2011 period that were hatchery-origin is 97.9% and 92.9% during the spring-run and fall-run spawning operations, respectively. Other

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studies also indicate high proportions of hatchery-origin fish among Central Valley salmon populations. For example, Barnett–Johnson et al. (2007) found 90% ± 6% hatchery-origin fish in a central California coastal fishery in 2002 based on otolith microchemistry, and 90.7% to 99.3% in the Mokelumne River watershed in 2004 based on otolith microchemistry (Johnson et al. 2012). For 2010, Kormos et al. (2012) estimated the FRH fall-run hatchery origin at 95%, and spring-run hatchery origin at 82%, based entirely on the expansion of CWT recoveries. Similarly for 2011, Palmer–Zwahlen and Kormos (2013) estimated 96% hatchery origin for fall run and 94% hatchery origin for spring run based entirely on the expansion of CWT recoveries. All of their estimates of the percentage of natural-origin fish are similar to ours except for the spring-run 2010 estimate, and, to a lesser extent, the spring-run 2011 estimate. Our estimates for FRH spring-run suggest a higher proportion of hatchery-origin fish: 99.5% versus 82% in 2010, and 99.8% versus 94% in 2011.

One explanation for these discrepancies might be an over-estimate of the fraction of FRH fish that are adipose-fin-clipped and coded-wire-tagged, the inverse of which was used by Kormos et al. (2012) and Palmer–Zwhalen and Kormos (2013) to expand the FRH CWT recoveries to account for the non-clipped and/or non-tagged fish (Mohr and Satterthwaite 2013). While the objective at FRH is to tag 100% of the spring-run production with an adipose-fin clip and a coded-wire tag, the achieved fraction is typically less than that. This fraction is release group-specific and estimated just before the fish are released, and for the brood years returning to FRH in 2010, ranged from 96% to 100% (Kormos et al. 2012, Table 3, %CWT). However, based on this study's estimated composition of spawners entering the hatchery during the spring spawning operations, a composite of multiple release groups and year classes, the fraction of spring-run fish that were adipose-fin-clipped and coded-wire-tagged, CWT Spring / (CWT Spring + Clipped Unk + TM Spring), ranged from 72% to 84% over the 2009–2011 period (Table 3, assuming all unknown clipped fish were spring-run fish). While these estimated fractions are not strictly comparable to one another (the fractions used to expand CWT recoveries are release group-specific, not return-year specific), the differences seem large enough to warrant some concern.

One possible explanation is manual marking operations. Spring-run 2010 hatchery returns consisted of spring-run fish from brood years 2006–2008; marking and tagging operations in those years were done manually. Hand et al. (2010) found only 70% of the manual clips rated "good" when employees had little or no experience, while 95% of automated tagging trailers clips rated "good." Thompson and Blankenship (1997) reported that 23% of Coho salmon completely regenerated their adipose fin when either the back or top two-thirds of the adipose fin was clipped. Adipose fin regeneration would render FRH spring-run fish visually undetectable and result in under-estimating hatchery-origin fish among that population.

Given that the target marking and tagging fraction for FRH spring-run production is 100%, the relatively high proportion of spring-run thermal marks found in non-clipped salmon was unexpected. We submitted 21 of the otoliths from non-clipped, spring-run fish that we identified as hatchery-origin, spring-run fish for microchemistry analysis to confirm our conclusions. The analysis indicated that all 21 otoliths were of hatchery origin. Although the microchemistry analysis was not able to identify the run type of these fish based on the results of this study, we suspect that at least 70% of these samples were are spring-run fish.

The group of unclipped, but thermally marked, spring-run fish presents an issue for FRH broodstock selection. Before the results of this study, it may have been assumed that an unclipped fish in the spring spawning period was either (a) a natural-origin spring-run fish (even though it is known that the effective clip rate for hatchery-origin spring-run fish is less than 100%), or (b) an early returning, unclipped, hatchery-origin fall-run fish. However, it now appears that a significant portion of these fish were hatchery-origin spring-run fish. Otolith thermal marking thus provides the means by which to further resolve the disposition of these unclipped fish, which can be useful for broodstock management purposes.

Our results indicate that mixing of the two run types continues within the FRH spring-run and fall-run spawning periods, with the mixed-run component averaging about 15% of the returns during both periods for the years 2009–2011. This mixing should be an important consideration in the future management of the spring-run and fall-run Chinook salmon programs at the FRH.

Historically, the Feather River supported two presumably genetically distinct populations of spring-run and fall-run Chinook salmon. Despite genetic similarities and years of spatial and temporal overlap in the river and the FRH, there is still a significant portion of Chinook salmon in the Feather River that exhibit spring-run migration timing, arriving at the hatchery in April, May or June every year. Considering that Central Valley spring-run Chinook salmon are listed as threatened under both the state and federal ESAs, and FRH is the only hatchery capable of producing Central Valley spring-run Chinook salmon in the ESU, it is exceedingly important to conserve spring-run Chinook salmon life history type in the Feather River. The use of an OTM program at FRH could identify individual fish by run type and origin, and this may help increase the use of natural-origin fish in FRH broodstock, and help limit the introgression of the two run types there. Improving reproductive isolation between spring-run and fall-run Chinook salmon and increasing the use of natural origin fish in hatchery broodstock are important steps in improving the long-term viability of FRH spring-run Chinook salmon.

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