# State of California <br> The Resources Agency DEPARTMENT OF FISH AND WILDLIFE 

ANNUAL REPORT<br>on the<br>MAD RIVER WINTER-RUN STEELHEAD BREEDING PROGRAM<br>and<br>HATCHERY AND GENETIC MANAGEMENT PLAN<br>for<br>2019-2024 BROOD YEARS

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March 2024

Suggested Citation: CDFW (California Department of Fish and Wildlife). 2024. Annual report on the Mad River Hatchery winter-run steelhead breeding program and the hatchery and genetic management plan, 2019-2024 seasons. Prepared for CDFW and the National Oceanic and Atmospheric Administration. Arcata, CA.

## EXECUTIVE SUMMARY

This report is presented as required by the Hatchery and Genetic Management Plan (HGMP) for CDFW's Mad River Hatchery (MRH) winter-run steelhead program. It summarizes general hatchery operations, hatchery steelhead production, and CDFW's efforts to monitor naturalorigin (NOR) and hatchery-origin (HOR) steelhead populations within the Mad River basin. This report covers activities relating to MRH and the HGMP for hatchery brood years 2019-2024 and follows preceding annual reports on HGMP compliance delivered for brood years 2017 and 2018. The MRH HGMP was approved by NOAA Fisheries on February, 17 ${ }^{\text {th }}, 2017$ and can be found by searching "Mad River Hatchery" at:
https://nrm.dfg.ca.gov/documents/docviewer.aspx

## TABLE OF CONTENTS

Executive Summary ..... iii
TABLE OF CONTENTS ..... iv
List of Tables ..... vi
List of Figures ..... ix
List of Appendices ..... xi
Frequently Used Acronyms and Abbreviations ..... xii
1 Introduction ..... 1
2 Hatchery Program Operations ..... 4
2.1 Spawning ..... 4
2.1.1 Fish Ladder ..... 4
2.2 Off-site NOR Broodstock Collection ..... 9
2.2.1 Adult Holding Survival Rate ..... 13
2.3 Mate Pairing ..... 13
2.3.1 Scale pattern stock validation ..... 17
2.4 Incubation ..... 19
2.5 Rearing ..... 22
2.6 Disease ..... 23
2.7 Marking ..... 23
2.8 Smolt Release ..... 23
3 Facilities Management ..... 25
3.1 Smolt Release Strategy ..... 25
3.2 Predation Incidents ..... 25
3.3 Water Quality Monitoring ..... 25
3.4 Public Relations ..... 26
3.5 Facilities Maintenance and Upgrades ..... 26
4 Biological Monitoring ..... 27
4.1 Genomic validation of pNOB ..... 27
4.1.1 Parentage Analysis Results ..... 27
4.2 Population Monitoring Methods ..... 29
4.2.1 Sonar-based run size estimation ..... 30
4.2.2 All calculations were executed in program $R$ ( $R$ Core Team 2023).Capture-mark- recapture run size estimation of steelhead stock abundance ..... 34
4.2.3 Tangle Netting on the Mad River ..... 36
4.3 Population Monitoring Results ..... 38
4.3.1 SONAR-based Run Size Estimates ..... 38
4.3.2 Steelhead Capture-Mark-Recapture estimates. ..... 54
5 Discussion. ..... 55
5.1 Recommendations ..... 61
5.1.1 Strategies to Increase pNOB ..... 61
5.1.2 Strategies to Reduce pHOS ..... 63
5.1.3 Balance socio-economic benefits and HGMP performance criteria ..... 64
6 Literature Cited ..... 65
7 Appendices. ..... 68
Appendix A. ..... 68
Appendix B. ..... 70
Appendix C. ..... 71
Appendix D. ..... 73
Appendix E. ..... 74
Appendix F. ..... 75

## LIST OF TABLES

Table 1. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility in the 2019 brood year. .. 6

Table 2. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2020 brood year. . 6

Table 3. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2021 brood year. . 7

Table 4. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2022 brood year. . 7

Table 5. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2023 brood year. . 8

Table 6. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2024 brood year. . 8

Table 7. Summary of angling effort and steelhead catches reported by Mad River Steelhead Stewards during the 2023-24 fishing season.

Table 8. Number, holding time and survival of broodstock delivered to MRH from 2019-2024. From 2019 to 2023, all broodstock delivered to MRH were caught via hook and line by volunteer anglers. In 2024, counts include steelhead caught by anglers and tangle nets.

Table 9. The number and percentage of hatchery origin (HOR) and natural origin (NOR) winterrun steelhead (based upon visual identification of adipose fin clips and scale analysis) used for breeding at Mad River Hatchery in 2019. The proportion of HOR breeders ( pHOB ) and proportion of NOR breeders ( $p N O B$ ) are presented by week and season.

Table 10. The number and percentage of HOR and NOR winter-run steelhead (based on visual identification of adipose fin clips and scale analysis) used for breeding at Mad River Hatchery in 2020. The proportion of HOR breeders ( $p H O B$ ) and proportion of NOR breeders ( $p N O B$ ) are presented by week and season.

Table 11. The number and percentage of HOR and NOR winter-run steelhead (based upon visual identification of fin clips and scale analysis) used for breeding at Mad River Hatchery in 2021. The proportion of HOR breeders ( pHOB ) and proportion of NOR breeders ( pNOB ) are presented by week and season. 15

Table 12. The number and percentage of HOR and NOR winter-run steelhead (based upon visual identification of fin clips and scale analysis) used for breeding at Mad River Hatchery in 2022. The proportion of HOR breeders ( pHOB ) and proportion of NOR breeders ( pNOB ) are presented by week and season.
Table 13. The number and percentage of HOR and NOR winter-run steelhead (based upon visualidentification of fin clips and scale analysis) used for breeding at Mad River Hatchery in 2023.The proportion of HOR breeders ( pHOB ) and proportion of NOR breeders ( pNOB ) are presentedby week and season.16
Table 14. The number and percentage of HOR and NOR winter-run steelhead (based upon visual identification of fin clips and scale analysis) used for breeding at Mad River Hatchery in 2024. The proportion of HOR breeders ( pHOB ) and proportion of NOR breeders ( pNOB ) are presented by week and season. ..... 16
Table 15. Comparison of origin assignments (HOR vs. NOR) by adipose fin clip status and scale analysis for NOR steelhead collected for broodstock from 2019-2024. Totals include all NOR steelhead brought into the hatchery for broodstock from angling or tangle netting (regardless of whether they were spawned). Scale analysis correction rates represent the proportion of adipose-fin-intact (i.e., NOR-assumed) steelhead confirmed as HOR using scale analysis. ..... 18
Table 16. Winter-run steelhead egg survival (i.e., fertility rate) summaries by spawning week at Mad River Hatchery for brood year 2019. ..... 19
Table 17. Winter-run steelhead egg survival summaries by spawning week at Mad River Hatchery for brood year 2020 ..... 20
Table 18. Winter-run steelhead egg survival summaries by spawning week at Mad River Hatchery for brood year 2021. ..... 20
Table 19. Winter-run steelhead egg survival summaries by spawning week at Mad River Hatchery for brood year 2022. ..... 21
Table 20. Winter-run steelhead egg survival summaries by spawning week at Mad River Hatchery for brood year 2023. ..... 21
Table 21. Winter run steelhead egg, fry and smolt production totals at Mad River Hatchery for brood years 2019-2023. The Mad River HGMP (CDFW 2016) stipulates annual smolt releases to be no more than 150,000 adipose fin-clipped smolts. ..... 22
Table 22. Stage-based survival rates for steelhead production at Mad River Hatchery for brood years 2019-2023. ..... 22
Table 23. HOR steelhead smolt release summaries by brood year, release date and river flows .....  24
Table 24. Summary of winter-run steelhead Mad River Hatchery broodstock genotyped from 2018-2022 returns including the number of samples missing genotypes at more than 12 loci, and the number of genetically unique male and female individuals used in the parentage analysis ..... 28
Table 25. Estimates of the proportion of NOR steelhead used as broodstock at Mad River Hatchery 2013-2022 from pedigree reconstruction and from adipose fin clip status. ..... 28
Table 26. Comparison of drifted and fixed tangle netting techniques based on 2023-24 sampling.37
Table 27. SONAR derived estimates of total net upstream passage of salmonids in temporal strata A, B, C, D of survey year 2019-20 at river mile 7 on the Mad River, Humboldt County, CA. ..... 40
Table 28. SONAR derived estimates of total net upstream passage of salmonids in temporal strata A, B, C, D of survey year 2020-21 at river mile 7 on the Mad River, Humboldt County, CA.43
Table 29. SONAR derived estimates of total net upstream passage of salmonids in temporalstrata A, B, C, D of survey year 2021-22 at river mile 7 on the Mad River, Humboldt County, CA.46Table 30. SONAR derived estimates of total net upstream passage of salmonids in temporalstrata A, B, C, D of survey year 2022-23 at river mile 7 on the Mad River, Humboldt County, CA.
Table 31. SONAR derived estimates with upper (UCI) and lower (LCI) confidence intervals of total net upstream passage of salmonids from 5 Sept 2023 to 15 March 2024 at river mile 7 on the Mad River, Humboldt County, CA. ..... 52
Table 32. Catch per unit effort using tangle nets per hour, day and month for each species throughout the 2023-24 monitoring season. ..... 52

## LIST OF FIGURES

Figure 1. A contour plot displaying the effects of the proportion of NOR broodstock in the hatchery population (pNOB) and the proportion of HOR spawning in the wild population (pHOS) on Proportionate Natural Influence (PNI).3

Figure 2. Comparison of Natural Origin (NOR) and Hatchery Origin (HOR) juvenile growth patterns of adult return steelhead scales as read from scale microfiches.18

Figure 3. Estimated clip rates of Mad River Hatchery smolt releases by brood year (BY) based on Quality Control measures. Clip rates are estimated by assessing a subsample of $\geq 1 \%$ (approx. 1,500 ) of the ponded juveniles for quantity and quality of fin clips.24

Figure 4. Diagram of the breeding system for anadromous steelhead (Oncorhynchus mykiss) in the Mad River basin. The hatchery origin (HOR) sub-population reared and released at the hatchery is made up of a proportion of hatchery-origin broodstock ( pHOB ) mixed with naturalorigin broodstock ( pNOB ) brought into the hatchery from the wild. The natural origin (NOR) subpopulation that emerges and rears in the wild is made up of a proportion of natural-origin spawners (pNOS) mixed with hatchery-origin spawners that "stray" from the hatchery. Proportionate Natural Influence (PNI), originally described as the 'approximate PNI index' by HSRG (2009) as an approximation of the proportional genetic influence of the natural environment on a population.

Figure 5. Top panel depicts daily expanded net movement of salmonids greater than 54 cm total length for the 2019-2020 season derived from SONAR images (left primary Y axis) and Mad River discharge (right secondary $Y$ axis). Vertical lines separate the time series into temporal stratum A, B, C, and D. Middle panel depicts daily snorkel counts of salmonid species (CO=Coho Salmon, KS=Chinook Salmon, $\mathrm{SH}=$ steelhead) greater than 54 cm . Bottom panel displays daily hatchery and natural steelhead catches reported on the CDFW Steelhead Report Card from completed trips
Figure 6. Daily expanded net movement of salmonids greater than 54 cm for the 2020-2021 season derived from SONAR images (left primary $Y$ axis) and Mad River discharge (right secondary Y axis) (Top Panel). Vertical lines separate the time series into temporal stratum A-D for used for estimates of total net movement. Daily snorkel counts of salmonid species greater than 53 cm (Middle panel). Bottom panel displays daily hatchery and wild steelhead catches reported on the CDFW Steelhead Report Card from completed trips.

Figure 7. Top panel depicts daily expanded net movement of salmonids greater than 54 cm for the 2021-2022 season derived from SONAR images (left primary $Y$ axis) and Mad River discharge (right secondary Y axis). Vertical lines separate the time series into temporal stratum A-D for used for estimates of total net movement. Middle panel depicts daily snorkel counts of salmonid species greater than 54 cm . Bottom panel displays daily hatchery and wild steelhead catches $\geq 42 \mathrm{~cm}$ reported on the CDFW Steelhead Report Card from completed trips.

Figure 8. Top panel depicts daily expanded net movement of salmonids greater than 54 cm for the 2022-2023 season derived from SONAR images (left hand $Y$ axis) and Mad River discharge (right hand $Y$ axis). Vertical lines separate the time series into temporal stratum A-D for used for estimates of total net movement. Middle panel depicts daily snorkel counts of salmonid species greater than 54 cm . Steelhead Report Card data is not yet available for this year.

Figure 9. Time series of data used to estimate the net number of upstream migrating salmonid species and hatchery and natural steelhead $\geq 42 \mathrm{~cm}$ total length in the Mad River 2023-2024 season. (A) Net daily passage of unknown salmonid fish species $\geq 42 \mathrm{~cm}$ total length imaged by SONAR (green bars left y axis) and average daily Mad River discharge (blue line, right y axis), (B) daily counts of salmonid species $\geq 42 \mathrm{~cm}$ fork length observed during snorkel surveys and, (C) tangle net captures of Coho and Chinook Salmon and steelhead in the Mad River below Blue Lake bridge used for species apportionment, (D) the number of hatchery and natural steelhead observed during both snorkeling and tangle net surveys used to apportion steelhead into hatchery or natural run components, and (E) resulting daily SONAR net upstream moving salmonids apportioned to both species and steelhead hatchery and natural run components. 51

Figure 10. Stacked bars presenting catch per unit effort using tangle nets per hour and month for adult salmonids throughout the 2023-24 Mad River monitoring season53

Figure 11. Estimated abundance of HOR, NOR and harvested HOR steelhead generated with Capture-Mark-Recapture (CMR) and SONAR derived estimates of NOR and HOR steelhead in the Mad River, 2023-2024

Figure 12. Total counts of first-pass HOR steelhead at the MRH fish ladder, reported HOR steelhead landings from steelhead report cards, and NOR steelhead brought to MRH for broodstock (with origin verified by scale inspection). Report card data from 2017, 2018, 2023 and 2024 were not available at the time of this reporting. *NOR origin assignments for steelhead brought to MRH in 2017-18 were not substantiated by scale pattern verification.

## LIST OF APPENDICES

Appendix A. Annual MRH HGMP reporting requirements. ..... 68
Appendix B. Species apportionment snorkel survey counts of total species observed by date and ..... 70reach on the Mad River during the 2023-24 season.
Appendix C. Species captured by tangle net date and reach on the Mad River during the 2023-24
season. ..... 71
Appendix D. Total hatchery-origin (adipose fin absent) and natural-origin (adipose fin present)steelhead $\geq 42 \mathrm{~cm}$ total length observed during snorkel surveys and tangle netting by Julian weekfor the 2023-24 monitoring season.73
Appendix E. Number of fish $\geq 42 \mathrm{~cm}$ total length by species and Julian week observed duringsnorkel surveys and tangle netting throughout the 2023-24 monitoring season.74
Appendix F. Weekly comparison of HOR broodstock, NOR broodstock, and the proportion of NORsteelhead used as broodstock at Mad River Hatchery 2013-2022 by visual inspection of adiposefin and scale sample pattern ( $\mathrm{HOR}_{\mathrm{v}}, \mathrm{NOR}_{\mathrm{v}}, \mathrm{pNOB} \mathrm{V}_{\mathrm{v}}$ ), and from genetic pedigree reconstruction( $\mathrm{HOR}_{\mathrm{g}}$, NORg $_{\mathrm{g}}, \mathrm{pNOB}_{\mathrm{g}}$ )75

## FREQUENTLY USED ACRONYMS AND ABBREVIATIONS

| BRT | Biological Review Team |
| :--- | :--- |
| BY | Brood year |
| CDFW | California Department of Fish and Wildlife |
| cfs | cubic feet per second |
| CPUE | Catch Per Unit Effort |
| DPS | Distinct Population Segment |
| ESA | Federal Endangered Species Act |
| ESU | Evolutionarily Significant Unit |
| FMEP | Fishery Management and Evaluation Plan |
| HGMP | Hatchery and Genetic Management Plan |
| HOR | Hatchery Origin Return |
| MOA | Memorandum of Agreement |
| MRH | Mad River Hatchery |
| MRSS | Mad River Steelhead Stewards |
| NOAA | National Oceanic \& Atmospheric Administration |
| NMFS | National Marine Fisheries Service (aka "NOAA Fisheries") |
| NOR | Natural Origin Return |
| pHOS | Proportionate hatchery origin spawners |
| PNI | Proportionate natural influence |
| pNOB | Proportionate natural origin broodstock |
| QAQC | Quality Assurance/Quality Control |
| RM | River mile |
| RWQCB | (North Coast) Regional Water Quality Control Board |

## 1 INTRODUCTION

The Mad River Hatchery (MRH) was built in 1971 and has been used to enhance angling opportunities for declining winter-run Northern California (NC) steelhead (Oncorhynchus mykiss), which were listed as Threatened under the federal Endangered Species Act (ESA) in the year 2000. Beginning in 2009, MRH began implementing new spawning matrices to incorporate returning natural-origin (NOR) Mad River NC steelhead broodstock into the program as well as shift production goals to limit impacts to the wild salmonid populations of the Mad River. The Hatchery Genetics Management Plan (HGMP) for the MRH was finalized in 2016, formalizing this spawning regime and creating a new foundation for continued operation of the hatchery as an integrated hatchery program with conservation potential. Although NOR NC steelhead are a federally Threatened species, hatchery propagated stock are not considered listed stock and are all marked (adipose fin removed) and available for in-river recreational harvest by hook and line angling.

The goal of the MRH and the California Department of Fish and Wildlife (CDFW) is to continue to provide enhanced angling opportunities in the Mad River, including the potential to harvest hatchery steelhead, while maintaining and promoting the NOR population through continued integration of NOR genetics into hatchery stock and minimizing to the degree practicable, the influence of hatchery origin (HOR) steelhead on the NOR stock. Maintaining MRH as an integrated program is necessary because gene flow between HOR and NOR stocks cannot be eliminated in the natural river setting. Additionally, genetic divergence has been shown between HOR and NOR Mad River stocks due to nearly four decades of hatchery production using primarily HOR stock (Reneski 2011). Continued integration of NOR genetics can mitigate the deleterious effects of genetic drift.

As described in the HGMP, a variety of performance indicators define a successfully functioning hatchery program including a high Proportionate Natural Influence (PNI) (Ford 2002, HSRG 2009), high NOR adult abundance, convergent NOR and HOR adult run- and spawn-timing, limited competition among HOR and NOR juveniles, large size and smolt condition of released HOR smolts, high survival at numerous life-stages, low number and severity of disease outbreaks, low HOR escapement, and low HOR straying rate (assessed as pHOS). These performance indicators were developed by National Marine Fisheries Service (NMFS) and California Department of Fish and Wildlife (CDFW) along with a set of associated benefits and risks to the populations.

PNI, which involves several of these performance indicators, is defined in this report as:

$$
P N I=\frac{p N O B}{p N O B+p H O S}
$$

Where $p N O B$ is the ratio of Natural Origin Broodstock (NOB) broodstock to the total number of both NOB and Hatchery Origin Broodstock (HOB) used to generate a cohort of hatchery stock,
and $p H O S$ is the ratio of HOR adults, less HOR harvest $(\mathrm{H})$, to the total number of natural area returning adults. A PNI value of 0.5 or greater ensures that genetic diversity is maintained, decreases inbreeding, and decreases domestication in the HOR stock (HSRG 2009). Higher levels of PNI can be accomplished by achieving low levels of pHOS and/or high levels of pNOB (Figure 1). Under the current HGMP the maximum number of steelhead used for broodstock is 125 female fish and 125 male fish, of which $50 \%$ or greater would be NOR to ensure a pNOB of at least 0.5 . The resulting progeny of this broodstock would ideally generate a target smolt population of 150,000 yearlings.

Following best management hatchery practices, this hatchery stock should have high survival from egg to smolt and should emigrate from the hatchery in good condition to survive and return as adults with a high smolt to adult return rate (SAR). High survival and good body condition through all of these life stages are considered indicators of a properly functioning MRH with good spawning and culture practices. Additionally, the hatchery stock will be reared to a size, condition, and age that promotes smolting and a swift emigration to the Ocean environment to reduce competitive impacts on naturally occurring juvenile salmonids.

A successful hatchery operation should yield fish which not only exhibit the genetic diversity of the NOR population, but also mirror the run-timing and reproductive success of the wild population. MRH aims to spawn broodstock in a manner that reflects the natural run and spawn timing of the NOR population to avoid divergence of NOR and HOR stock. Continued integration of NOR broodstock collected throughout the observed spawning season should facilitate expression of the variation in run timing that has been successful in the Mad River.

The behavior and fate of HOR returning adults is also considered as an indicator of hatchery function; a low straying rate and low $p H O S$, or low escapement rate, reduce the potential risk to NOR populations in the Mad River and other coastal streams. Reducing the number of HOR steelhead that spawn in the wild through use in broodstock collection and angler harvest reduces HOR gene flow to the Mad River NOR population, and high fidelity of returning adults to the Mad River and MRH reduces gene flow to other NC steelhead populations. Additionally, high site fidelity ensures some broodstock will be available, reducing the need to excessively use NOR broodstock.

Lastly, a high NOR adult abundance is a natural indication that the MRH is not having a deleterious effect on the NOR population and that the natural environment is sufficient to maintain high productivity. Additionally, as the NOR population increases, pHOS is naturally driven down which could result in higher PNI values assuming constant $p N O B$ and HOR escapement (Figure 1). As the MRH is being operated with conservation potential, supporting and improving the NOR population is a parallel goal along with enhancement of the fishery and the two objectives are not mutually exclusive. Further, if MRH is effectively functioning as an integrated hatchery when the NOR stock becomes severely depressed, the HOR stock can provide a conservation benefit through supplementation, providing improved genetic diversity in the overall population.

This report summarizes operations and facilities management of MRH, and the biological monitoring conducted by CDFW to track and assess the performance indicators outlined in the HGMP.


Figure 1. A contour plot displaying the effects of the proportion of NOR broodstock in the hatchery population ( pNOB ) and the proportion of HOR spawning in the wild population ( pHOS ) on Proportionate Natural Influence (PNI).

## 2 HATCHERY PROGRAM OPERATIONS

As specified in the Mad River HGMP, Mad River Hatchery operations support maintaining an integrated hatchery program that targets a NOR steelhead integration rate of $\geq 50 \%$, and maintaining PNI values $\geq 0.5$. Adult steelhead arriving at the hatchery are predominately HOR fish and are spawned with NOR fish collected from the river by volunteer anglers (spawn years 2020-2023) from the Mad River Steelhead Stewards (MRSS) and brought to the hatchery by CDFW staff. Beginning in 2024, the use of a tangle net by CDFW was initiated to collect additional NOR steelhead to further improve the ability to achieve the target pNOB value of $>50 \%$.The fish ladder at the MRH facility was generally open for fish to enter from lateDecember through late-March of each spawning season. After spawning and throughout the following year, progeny are incubated, reared in ponds, marked with adipose fin clips and released into the Mad River the following March.

### 2.1 Spawning

Hatchery-origin (HOR) steelhead volitionally enter MRH by ascending a fish ladder which leads to a holding pen. Each Tuesday after January 1 until late March, fish are crowded from the holding pen into the hatchery spawning shed for counting and spawning purposes. Depending on the number of fish ascending the ladder, fish may be crowded into the spawning shed in multiple passes to prevent overloading the fish bin in the spawning shed. Fish are anesthetized in water with $\mathrm{CO}_{2}$ (buffered with sodium bicarbonate) before they are assessed and selected for either spawning, holding for another week, or release into the Mad River. Fish processing includes examining individuals for species, clips, marks, tags, sex, size, and reproductive maturity. If a fish is selected for spawning, a genetic tissue sample is taken. Steelhead pairs are spawned at a 1:1 ratio in most instances with a goal of spawning one NOR steelhead with one HOR steelhead for each pair. Fish not selected for spawning are either returned to the holding pen and allowed to ripen for another week or released back into the river after recovery from handling. Many fish released in the river return to ascend the ladder again. To ensure accurate counts of individuals passing the ladder for the first time and prevent double counting, fish are marked with a hole punch on the caudal fin after each ascent. Fish receive caudal fin punches each time they are counted throughout the season, with some fish receiving several hole punches. Occasionally, NOR fish (or fish with intact adipose fins) volitionally enter MRH and are processed similarly. Scale and genetic tissue samples are taken from these fish to verify their origin as NOR or HOR and they are used in the spawning matrix accordingly.

### 2.1.1 Fish Ladder

From 2019-2024, new (i.e., first pass up fish ladder) adult steelhead were caught in nearly every week during trap operations from early January to late March. The median capture dates of adult steelhead at the hatchery for each season were 5-Feb-2019 (Table 1), 11 Feb 2020 (Table 2), 2 Feb 2021 (Table 3), 15 Feb 2022 (Table 4), 21 Feb 2023 (Table 5), and 20 Feb 2024 (Table 6). Fish that ascend the fish ladder are identified by size, sex and examined for adipose clips and
other marks. Table 1-Table 6 display fish ladder operation summaries for brood years 20192024, respectively.

Table 1. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility in the 2019 brood year.

| Week | Date | AD-Clip |  | No AD-Clip |  | Juvenile (<18 in) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Female | Male | Female | Male | Female | Male |
| 1 | 1/2/19 | 13 | 24 | 5 | 4 | 0 | 0 |
| 2 | 1/8/19 | 15 | 32 | 4 | 11 | 0 | 0 |
| 3 | 1/15/19 | 89 | 137 | 2 | 6 | 0 | 1 |
| 4 | 1/22/19 | 153 | 187 | 2 | 1 | 0 | 0 |
| 5 | 1/29/19 | 189 | 134 | 4 | 3 | 0 | 2 |
| 6 | 2/5/19 | 279 | 180 | 11 | 6 | 0 | 5 |
| 7 | 2/12/19 | 81 | 50 | 15 | 6 | 1 | 2 |
| 8 | 2/19/19 | 201 | 140 | 7 | 5 | 11 | 0 |
| 9 | 2/26/19 | 176 | 76 | 3 | 2 | 3 | 43 |
| 10 | 3/5/19 | 109 | 49 | 2 | 0 | 4 | 53 |
| 11 | 3/12/19 | 73 | 17 | 0 | 1 | 1 | 95 |
| 12 | 3/19/19 | 28 | 6 | 0 | 0 | 1 | 44 |
| TOTAL |  | 1,406 | 1,032 | 55 | 45 | 21 | 245 |

Table 2. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2020 brood year.

| Week | Date | AD-Clip |  | No AD-Clip |  | Juvenile (<18 in) |  |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  |  | Female | Male | Female | Male | Female | Male |
| 1 | $1 / 7 / 20$ | 1 | 8 | 4 | 3 | 0 | 0 |
| 2 | $1 / 14 / 20$ | 72 | 183 | 1 | 3 | 0 | 0 |
| 3 | $1 / 21 / 20$ | 39 | 94 | 1 | 1 | 0 | 1 |
| 4 | $1 / 28 / 20$ | 221 | 299 | 0 | 0 | 0 | 0 |
| 5 | $2 / 4 / 20$ | 194 | 149 | 1 | 2 | 0 | 0 |
| 6 | $2 / 11 / 20$ | 67 | 36 | 12 | 8 | 0 | 2 |
| 7 | $2 / 18 / 20$ | 148 | 95 | 18 | 13 | 0 | 2 |
| 8 | $2 / 25 / 20$ | 102 | 52 | 17 | 10 | 0 | 0 |
| 9 | $3 / 3 / 20$ | 166 | 121 | 18 | 4 | 0 | 0 |
| 10 | $3 / 10 / 20$ | 228 | 107 | 20 | 6 | 1 | 3 |
| 11 | $3 / 17 / 20$ | 160 | 77 | 2 | 3 | 1 | 5 |
| TOTAL |  | $\mathbf{1 , 3 9 8}$ | $\mathbf{1 , 2 2 1}$ | $\mathbf{9 4}$ | $\mathbf{5 3}$ | $\mathbf{2}$ | $\mathbf{1 3}$ |

Table 3. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2021 brood year.

| Week | Date | AD-Clip |  | No AD-Clip |  | Juvenile (<18 in) |  |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  |  | Female | Male | Female | Male | Female | Male |
| 1 | $1 / 5 / 21$ | 38 | 51 | 1 | 1 | 0 | 0 |
| 2 | $1 / 12 / 21$ | 9 | 10 | 2 | 1 | 0 | 1 |
| 3 | $1 / 19 / 21$ | 38 | 38 | 6 | 3 | 0 | 4 |
| 4 | $1 / 26 / 21$ | 2 | 1 | 9 | 2 | 0 | 0 |
| 5 | $2 / 2 / 21$ | 150 | 169 | 10 | 3 | 2 | 52 |
| 6 | $2 / 9 / 21$ | 39 | 30 | 8 | 1 | 1 | 18 |
| 7 | $2 / 16 / 21$ | 71 | 51 | 4 | 4 | 1 | 108 |
| 8 | $2 / 23 / 21$ | 31 | 11 | 6 | 0 | 3 | 107 |
| 9 | $3 / 2 / 21$ | 4 | 3 | 6 | 2 | 1 | 22 |
| 10 | $3 / 9 / 21$ | 24 | 8 | 11 | 2 | 12 | 106 |
| 11 | $3 / 16 / 21$ | 22 | 4 | 4 | 0 | 6 | 78 |
| 12 | $3 / 23 / 21$ | 5 | 3 | 3 | 0 | 1 | 59 |
| TOTAL | $\mathbf{4 3 3}$ | $\mathbf{3 7 9}$ | $\mathbf{7 0}$ | $\mathbf{1 9}$ | $\mathbf{2 7}$ | $\mathbf{5 5 5}$ |  |

Table 4. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2022 brood year.

| Week | Date | AD-Clip |  | No AD-Clip |  | Juvenile (<18 in) |  |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  |  | Female | Male | Female | Male | Female | Male |
| 1 | $1 / 4 / 22$ | 54 | 90 | 0 | 1 | 1 | 0 |
| 2 | $1 / 11 / 22$ | 90 | 177 | 1 | 0 | 1 | 1 |
| 3 | $1 / 18 / 22$ | 59 | 64 | 5 | 6 | 0 | 1 |
| 4 | $1 / 25 / 22$ | 48 | 42 | 6 | 5 | 0 | 0 |
| 5 | $2 / 1 / 22$ | 24 | 9 | 1 | 1 | 0 | 0 |
| 6 | $2 / 8 / 22$ | 34 | 17 | 5 | 4 | 0 | 0 |
| 7 | $2 / 15 / 22$ | 173 | 155 | 3 | 4 | 0 | 3 |
| 8 | $2 / 22 / 22$ | 68 | 63 | 5 | 3 | 0 | 0 |
| 9 | $3 / 1 / 22$ | 183 | 174 | 5 | 2 | 1 | 0 |
| 10 | $3 / 8 / 22$ | 144 | 126 | 0 | 3 | 0 | 1 |
| 11 | $3 / 15 / 22$ | 147 | 47 | 2 | 1 | 0 | 1 |
| TOTAL | $\mathbf{1 , 0 2 4}$ | $\mathbf{9 6 4}$ | $\mathbf{3 3}$ | $\mathbf{3 0}$ | $\mathbf{3}$ | $\mathbf{7}$ |  |

Table 5. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2023 brood year.

| Week | Date | AD-Clip |  | No AD-Clip |  | Juvenile (<18 in) |  |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  |  | Female | Male | Female | Male | Female | Male |
| 1 | $1 / 3 / 23$ | 10 | 8 | 1 | 0 | 0 | 0 |
| 2 | $1 / 10 / 23$ | 24 | 16 | 0 | 0 | 0 | 1 |
| 3 | $1 / 17 / 23$ | 3 | 3 | 0 | 1 | 0 | 2 |
| 4 | $1 / 24 / 23$ | 1 | 0 | 0 | 0 | 0 | 0 |
| 5 | $1 / 31 / 23$ | 13 | 3 | 0 | 0 | 0 | 0 |
| 6 | $2 / 7 / 23$ | 15 | 8 | 0 | 0 | 0 | 3 |
| 7 | $2 / 14 / 23$ | 16 | 8 | 0 | 0 | 0 | 3 |
| 8 | $2 / 21 / 23$ | 26 | 14 | 3 | 1 | 1 | 3 |
| 9 | $2 / 28 / 23$ | 15 | 4 | 0 | 0 | 0 | 4 |
| 10 | $3 / 7 / 23$ | 7 | 5 | 0 | 0 | 0 | 4 |
| 11 | $3 / 14 / 23$ | 35 | 29 | 0 | 0 | 0 | 15 |
| 12 | $3 / 21 / 23$ | 7 | 8 | 0 | 0 | 1 | 15 |
| TOTAL | $\mathbf{1 7 2}$ | $\mathbf{1 0 6}$ | $\mathbf{4}$ | $\mathbf{2}$ | $\mathbf{2}$ | $\mathbf{5 0}$ |  |

Table 6. Weekly first-time winter-run steelhead captures (i.e., first pass up fish ladder only) by sex, adipose fin clip status, and age at the Mad River Hatchery facility during 2024 brood year.

| Week | Date | AD-Clip |  | No AD-Clip |  | Juvenile (<18 in) |  |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  |  | Female | Male | Female | Male | Female | Male |
| 1 | $1 / 2 / 24$ | 6 | 15 | 0 | 1 | 0 | 1 |
| 2 | $1 / 9 / 24$ | 2 | 8 | 0 | 0 | 0 | 1 |
| 3 | $1 / 16 / 24$ | 15 | 29 | 0 | 1 | 0 | 0 |
| 4 | $1 / 23 / 24$ | 17 | 22 | 1 | 1 | 0 | 0 |
| 5 | $1 / 30 / 24$ | 39 | 32 | 1 | 2 | 1 | 1 |
| 6 | $2 / 6 / 24$ | 42 | 36 | 1 | 0 | 0 | 0 |
| 7 | $2 / 13 / 24$ | 56 | 31 | 3 | 3 | 0 | 0 |
| 8 | $2 / 20 / 24$ | 63 | 42 | 0 | 1 | 0 | 1 |
| 9 | $2 / 27 / 24$ | 38 | 25 | 1 | 2 | 0 | 0 |
| 10 | $3 / 5 / 24$ | 56 | 12 | 2 | 1 | 0 | 0 |
| 11 | $3 / 12 / 24$ | 66 | 21 | 0 | 0 | 0 | 0 |
| 12 | $3 / 19 / 24$ | 38 | 8 | 0 | 0 | 0 | 0 |

### 2.2 Off-site NOR Broodstock Collection

Founded in 2015, the Mad River Steelhead Stewards (MRSS) volunteer angler program has been instrumental for assisting CDFW in collecting NOR broodstock in the Mad River Basin for delivery to MRH. Under the guidance and supervision of CDFW and MRH, MRSS volunteers had particularly major impacts on NOR steelhead integration at MRH during the 2019-2022 brood years. Stewards capture potential NOR fish using hook-and-line techniques and place the fish in a flow-through tube in the river. On-call CDFW personnel or CDFW volunteers respond to angler calls by driving a CDFW truck equipped with an oxygenated water tank to the river access point nearest to the fish. The captured fish is then transported from the river to the truck in a water bag tagged within an individually numbered spaghetti tag, and delivered to MRH where it is held in the hatchery pen until the next spawning day.

In both 2023 and 2024, consistently high water lead to poor angling conditions throughout the spawning season, minimizing the effectiveness of the volunteer angler program to bring NOR fish to the hatchery. In 2024, one adult steelhead was caught for every 9.63 hours fished by MRSS volunteers (Table 7). CDFW supplemented the existing efforts in 2024 to acquire NOR broodstock using tangle nets throughout the winter. The tangle nets were 96 feet long by 12 feet deep with 2 -inch square mesh and were drifted downstream with the current or fixed in eddies or slow water environments. Similar to the volunteer angler program, fish captured with tangle nets were transported to the hatchery for spawning. Additional information on the use of tangle nets is presented later including their use in species apportionment sampling (Section 4.2.1.2), their advantages and disadvantages (Section 4.2.3) and their effectiveness on the Mad River in the 2023-24 season (Section 4.3.1.5).

At the hatchery, CDFW personnel critically examine the fish to determine its sex and origin. If further analysis using scales determines the fish to be HOR, it is released back into the river near the hatchery facility unless HOR steelhead are needed for spawning. If the fish is confirmed to be NOR, CDFW personnel place the fish into the hatchery pen and hold it until the next spawning event. NOR fish that are not ripe for spawning are often held for multiple weeks to allow them to fully ripen for spawning. However, spawning results from previous years suggest that most steelhead that are not ready to spawn within their first week or two at the hatchery are never successfully spawned-particularly females. Based on the limited spawning success achieved at MRH with these holdover steelhead, fish are rarely held for longer than two weeks. NOR fish that do not ripen are released back into the river near the hatchery facility after a few to several weeks (Table 8).

Table 7. Summary of angling effort and steelhead catches reported by Mad River Steelhead Stewards during the 2023-24 fishing season.

| Reach | Location | \# Angling <br> Days | Total <br> Hours | Adipose-clipped <br> adults | Adipose-intact <br> adults | Half- <br> pounders |
| :--- | :--- | ---: | ---: | ---: | ---: | ---: |
| 3 | Hwy 101 Bridge to <br> Hwy 299 Bridge | 4 | 9.76 | 0 | 0 | 2 |
| Hwy 299 Bridge to <br> Railroad Bridge | 0 | 0.00 | 0 | 0 | 0 |  |
| Railroad Bridge to <br> Quarry Creek | 24 | 75.10 | 11 | 1 | 3 |  |
| Hatchery Area <br> (Quarry Cr to <br> Boundary Cr) | 70 | 297.27 | 25 | 3 | 0 |  |
| n/a | Upstream of <br> Boundary Creek <br> Location Not <br> Reported | 1 | 0.33 | 0 | 0 | 0 |
| TOTALS |  | 101 | 385.46 | 0 | 0 | 0 |

Table 8. Number, holding time and survival of broodstock delivered to MRH from 2019-2024. From 2019 to 2023, all broodstock delivered to MRH were caught via hook and line by volunteer anglers. In 2024, counts include steelhead caught by anglers and tangle nets.



### 2.2.1 Adult Holding Survival Rate

All NOR fish are released back into the Mad River in hopes they continue their life cycle and spawn again, regardless of whether they successfully ripen at the hatchery and are used as broodstock or fail to ripen and are not spawned. However, fish collected for broodstock occasionally do not survive the process. Mortality causes range from dying (1) in hatchery truck transport, (2) in the holding pen at the hatchery facility due to stress or predation (e.g., otter), or (3) after spawning during recovery. The Mad River HGMP established a goal to maintain adult holding and spawning survival rate of $\geq 90 \%$, which was reached each year (Table 8).

### 2.3 Mate Pairing

Since the 2009 brood year, MRH has used a spawning matrix that aims to spawn enough adult steelhead to reach production goal of 150,000 yearling release smolts. The current HGMP sets a maximum take of 250 adult steelhead ( 125 mating pairs) for use as broodstock and distributes spawning pairs along a normal distribution over time beginning in January, peaking in February with the last pairs spawned in March. This pattern of hatchery spawning is intended to be in proportion to the NOR run-timing in the lower river. Mate pairing aims to integrate $50 \%$ or greater NOR fish into the hatchery broodstock. Adult steelhead numbers and origins (HOR/NOR) based on visual identification of adipose fin clips are presented for each year's broodstock (Table 9-Table 14). Beginning in brood year 2019, fish ascending the ladder identified as NOR based on the presence of an adipose fin had their scales inspected for typical HOR/NOR growth patterns. This additional check was implemented in real time in an effort to improve NOR identification over using adipose fin clip status assignment alone (Kinziger et al. 2022) (see sec. 2.3.1).

Since many fish from the ladder identified as NOR by adipose fin presence were subsequently deemed HOR based on scale analysis and excluded from spawning (brood years 2020-2022), NOR steelhead collected by MRSS using hook and line were critical for NOR steelhead genetic preservation. Overall, fish caught by MRSS used in mate pairing at MRH amounted to 20 steelhead ( $24 \%$ of total broodstock) in 2019 (Table 9), 59 steelhead ( $45 \%$ broodstock) in 2020 (Table 10), 23 steelhead ( $44 \%$ broodstock) in 2021 (Table 11), 53 steelhead ( $49 \%$ ) in 2022 (Table 12), 1 steelhead ( $1 \%$ broodstock) in 2023 (Table 13), and 4 steelhead ( $2 \%$ broodstock) in 2024 (Table 14). Additionally, tangle netting supplied 8 steelhead (4\%) for breeding in 2024 (Table 14).

Table 9. The number and percentage of hatchery origin (HOR) and natural origin (NOR) winterrun steelhead (based upon visual identification of adipose fin clips and scale analysis) used for breeding at Mad River Hatchery in 2019. The proportion of HOR breeders ( pHOB ) and proportion of NOR breeders ( $p N O B$ ) are presented by week and season.

| Week | Date | HOR Spawned |  | NOR <br> (Ladder Caught) |  | NOR Spawned <br> (Angler Caught) |  | pHOB | pNOB |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Female | Male | Female | Male | Female | Male |  |  |
| 1 | $1 / 2 / 19$ | 3 | 0 | 0 | 1 | 0 | 2 | $50 \%$ | $50 \%$ |
| 2 | $1 / 8 / 19$ | 5 | 0 | 0 | 2 | 0 | 3 | $50 \%$ | $50 \%$ |
| 3 | $1 / 15 / 19$ | 8 | 1 | 1 | 4 | 0 | 2 | $56 \%$ | $44 \%$ |
| 4 | $1 / 22 / 19$ | 1 | 2 | 2 | 1 | 0 | 0 | $50 \%$ | $50 \%$ |
| 5 | $1 / 29 / 19$ | 3 | 0 | 0 | 1 | 0 | 2 | $50 \%$ | $50 \%$ |
| 6 | $2 / 5 / 19$ | 5 | 2 | 1 | 1 | 0 | 4 | $54 \%$ | $46 \%$ |
| 7 | $2 / 12 / 19$ | 6 | 0 | 0 | 2 | 0 | 3 | $55 \%$ | $45 \%$ |
| 8 | $2 / 19 / 19$ | 4 | 0 | 0 | 2 | 0 | 1 | $57 \%$ | $43 \%$ |
| 9 | $2 / 26 / 19$ | 2 | 1 | 1 | 0 | 0 | $1^{\text {b }}$ | $60 \%$ | $40 \%$ |
| 10 | $3 / 5 / 19$ | 0 | 0 | 0 | 0 | 0 | 0 | -- | - |
| 11 | $3 / 12 / 19$ | 1 | 0 | 0 | 0 | 0 | 1 | $50 \%$ | $50 \%$ |
| 12 | $3 / 19 / 19$ | 2 | 0 | 0 | 0 | 0 | $1^{\text {b }}$ | $67 \%$ | $33 \%$ |
| TOTAL |  | $\mathbf{4 0}$ | $\mathbf{6}$ | $\mathbf{5}$ | $\mathbf{1 4}$ | $\mathbf{0}$ | $\mathbf{2 0}$ | $\mathbf{5 4 \%}$ | $\mathbf{4 6 \%}$ |

${ }^{\text {a }}$ Spawned fish in BY 2019 were assumed to be NOR based on presence/absence of their adipose fin
${ }^{\mathrm{b}}$ Indicates gamete lots were split where single individuals were spawned with multiple partners

Table 10. The number and percentage of HOR and NOR winter-run steelhead (based on visual identification of adipose fin clips and scale analysis) used for breeding at Mad River Hatchery in 2020. The proportion of HOR breeders ( $p H O B$ ) and proportion of NOR breeders ( $p N O B$ ) are presented by week and season.

| Week | Date | HOR Spawned |  | NOR Spawned (Ladder Caught) |  | NOR Spawned (Angler Caught) |  | pHOB | pNOB |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Female | Male | Female | Male | Female | Male |  |  |
| 1 | 1/7/20 |  | 0 | 0 | 0 | 0 | 1 | 50\% | 50\% |
| 2 | 1/14/20 | 2 | 0 | 0 | 1 | 0 | 1 | 50\% | 50\% |
| 3 | 1/21/20 | 1 | 1 | 0 | 0 | 1 | 1 | 50\% | 50\% |
| 4 | 1/28/20 | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| 5 | 2/4/20 | 3 | 0 | 0 | 0 | 0 | 2 | 60\% | 40\% |
| 6 | 2/11/20 | 7 | 2 | 0 | 0 | 2 | 7 | 50\% | 50\% |
| 7 | 2/18/20 | 14 | 1 | 0 | 0 | 1 | 13 | 52\% | 48\% |
| 8 | 2/25/20 | 11 | 2 | 0 | 0 | 2 | 10 | 52\% | 48\% |
| 9 | 3/3/20 | 4 | 1 | 0 | 0 | 2 | 4 | 45\% | 55\% |
| 10 | 3/10/20 | 4 | 6 | 0 | 0 | 7 | 5 | 45\% | 55\% |
| 11 | 3/17/20 | 3 | 2 | 2 | 3 | 0 | 0 | 50\% | 50\% |
| 12 | 3/24/20 | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| TOTAL |  | 50 | 15 | 2 | 4 | 15 | 44 | 50\% | 50\% |

Table 11. The number and percentage of HOR and NOR winter-run steelhead (based upon visual identification of fin clips and scale analysis) used for breeding at Mad River Hatchery in 2021. The proportion of HOR breeders ( $p H O B$ ) and proportion of NOR breeders ( $p \mathrm{NOB}$ ) are presented by week and season.

| Week | Date | HOR Spawned |  | NOR Spawned <br> (Ladder Caught) |  | NOR Spawned <br> (Angler Caught) |  | pHOB | pNOB |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Female | Male | Female | Male | Female | Male |  |  |
|  |  | $1 / 5 / 21$ | 0 | 0 | 0 | 0 | 0 | 0 | -- |
| 2 | $1 / 12 / 21$ | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| 3 | $1 / 19 / 21$ | 3 | 0 | 0 | 0 | 0 | 3 | $50 \%$ | $50 \%$ |
| 4 | $1 / 26 / 21$ | 1 | 0 | 0 | 0 | 0 | 1 | $50 \%$ | $50 \%$ |
| 5 | $2 / 2 / 21$ | 3 | 0 | 0 | 1 | 0 | 2 | $50 \%$ | $50 \%$ |
| 6 | $2 / 9 / 21$ | 1 | 2 | 0 | 1 | 2 | 0 | $50 \%$ | $50 \%$ |
| 7 | $2 / 16 / 21$ | 3 | 2 | 0 | 0 | 1 | 3 | $56 \%$ | $44 \%$ |
| 8 | $2 / 23 / 21$ | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| 9 | $3 / 2 / 21$ | 2 | 3 | 0 | 0 | 2 | 2 | $56 \%$ | $44 \%$ |
| 10 | $3 / 9 / 21$ | 4 | 2 | 0 | 0 | 2 | 4 | $50 \%$ | $50 \%$ |
| 11 | $3 / 16 / 21$ | 0 | 1 | 0 | 0 | 1 | 0 | $50 \%$ | $50 \%$ |
| 12 | $3 / 23 / 21$ | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| TOTAL |  | $\mathbf{1 7}$ | $\mathbf{1 0}$ | $\mathbf{0}$ | $\mathbf{2}$ | $\mathbf{8}$ | $\mathbf{1 5}$ | $\mathbf{5 2 \%}$ | $\mathbf{4 8 \%}$ |

Table 12. The number and percentage of HOR and NOR winter-run steelhead (based upon visual identification of fin clips and scale analysis) used for breeding at Mad River Hatchery in 2022. The proportion of HOR breeders ( pHOB ) and proportion of NOR breeders ( pNOB ) are presented by week and season.

| Week | Date | HOR Spawned |  | NOR Spawned (Ladder Caught) |  | NOR Spawned (Angler Caught) |  | pHOB | pNOB |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Female | Male | Female | Male | Female | Male |  |  |
| 1 | 1/4/22 | 2 | 0 | 0 | 1 | 0 | 1 | 50\% | 50\% |
| 2 | 1/11/22 | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| 3 | 1/18/22 | 4 | 0 | 0 | 0 | 0 | 4 | 50\% | 50\% |
| 4 | 1/25/22 | 4 | 0 | 0 | 0 | 0 | 4 | 50\% | 50\% |
| 5 | 2/1/22 | 4 | 0 | 0 | 0 | 0 | 4 | 50\% | 50\% |
| 6 | 2/8/22 | 12 | 2 | 0 | 0 | 2 | 12 | 50\% | 50\% |
| 7 | 2/15/22 | 6 | 1 | 0 | 0 | 1 | 6 | 50\% | 50\% |
| 8 | 2/22/22 | 3 | 1 | 0 | 0 | 1 | 3 | 50\% | 50\% |
| 9 | 3/1/22 | 3 | 3 | 0 | 0 | 3 | 3 | 50\% | 50\% |
| 10 | 3/8/22 | 5 | 1 | 0 | 1 | 2 | 5 | 43\% | 57\% |
| 11 | 3/15/22 | 1 | 1 | 0 | 0 | 1 | 1 | 50\% | 50\% |
| 12 | 3/22/22 | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| TOTAL |  | 44 | 9 | 0 | 2 | 10 | 43 | 49\% | 51\% |

Table 13. The number and percentage of HOR and NOR winter-run steelhead (based upon visual identification of fin clips and scale analysis) used for breeding at Mad River Hatchery in 2023. The proportion of HOR breeders ( $p H O B$ ) and proportion of NOR breeders ( $p \mathrm{NOB}$ ) are presented by week and season.

| Week | Date | HOR Spawned |  | NOR Spawned (Ladder Caught) |  | NOR Spawned (Angler Caught) |  | pHOB | pNOB |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Female | Male | Female | Male | Female | Male |  |  |
| 1 | 1/3/23 | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| 2 | 1/10/23 | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| 3 | 1/17/23 | 1 | 0 | 0 | 1 | 0 | 0 | 50\% | 50\% |
| 4 | 1/24/23 | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| 5 | 1/31/23 | 0 | 0 | 0 | 0 | 0 | 0 | -- | -- |
| 6 | 2/7/23 | 6 | 0 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| 7 | 2/14/23 | 5 | 5 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| 8 | 2/21/23 | 8 | 8 | 1 | 0 | 0 | 1 | 89\% | 11\% |
| 9 | 2/28/23 | 7 | 7 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| 10 | 3/7/23 | 7 | 7 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| 11 | 3/14/23 | 5 | 5 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| 12 | 3/21/23 | 4 | 4 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| TOTAL |  | 43 | 36 | 1 | 1 | 0 | 1 | 96\% | 4\% |

Table 14. The number and percentage of HOR and NOR winter-run steelhead (based upon visual identification of fin clips and scale analysis) used for breeding at Mad River Hatchery in 2024. The proportion of HOR breeders ( pHOB ) and proportion of NOR breeders ( pNOB ) are presented by week and season.

| Week | Date | HOR Spawned |  | NOR Spawned |  |  |  |  |  | pHOB | pNOB |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Ladder |  | Angler |  | Tangle Net |  |  |  |
|  |  | F | M | F | M | F | M | F | M |  |  |
| 1 | 1/2/24 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 50\% | 50\% |
| 2 | 1/9/24 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| 3 | 1/16/24 | 8 | 7 | 0 | 1 | 0 | 0 | 0 | 0 | 94\% | 6\% |
| 4 | 1/23/24 | 12 | 12 | 1 | 1 | 0 | 0 | 0 | 0 | 92\% | 8\% |
| 5 | 1/30/24 | 13 | 11 | 0 | 1 | 0 | 0 | 0 | 1 | 92\% | 8\% |
| 6 | 2/6/24 | 11 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| 7 | 2/13/24 | 10 | 10 | 0 | 0 | 1 | 1 | 1 | 1 | 83\% | 17\% |
| 8 | 2/20/24 | 13 | 12 | 0 | 0 | 0 | 0 | 0 | 1 | 96\% | 4\% |
| 9 | 2/27/24 | 9 | 9 | 0 | 0 | 1 | 0 | 0 | 1 | 90\% | 10\% |
| 10 | 3/5/24 | 9 | 9 | 0 | 0 | 0 | 0 | 1 | 1 | 90\% | 10\% |
| 11 | 3/12/24 | 6 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 92\% | 8\% |
| 12 | 3/19/24 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100\% | 0\% |
| TOTAL |  | 97 | 91 | 1 | 3 | 2 | 2 | 2 | 6 | 92\% | 8\% |

### 2.3.1 Scale pattern stock validation

Steelhead smolts reared at MRH must receive an adipose fin clip prior to their release so that anglers can easily identify which individuals can be harvested when fish return as adults. This marking is also used by hatchery personnel to assign fish origin at spawning. Kinziger et al. (2022) discovered discrepancies in the proportion of HOR broodstock individuals as estimated by pedigrees (i.e., genetic testing) versus traditional adipose-fin clipping (i.e., visual observation). In their study, they genotyped tissue samples from fish spawned in brood years 2009-2017 and found that visual observations of fin clips suggested $58 \%$ of the broodstock was HOR, while genetic testing found the broodstock to be composed of $87 \%$ HOR. This proportional discrepancy could be explained by preferentially selecting returns to MRH with adipose fins for breeding in order to meet broodstock integration targets. Because many more adults typically return than are used as broodstock, the missed clips as juveniles prior to release were likely being mis-assigned as NOR.

Most Northern California steelhead spend two years in freshwater experiencing periods of faster and slower growth through the seasons, whereas hatchery fish are released at age 1 at similar size having experienced faster, more consistent growth. Beginning in spawning year 2019 CDFW began using real-time scale pattern analysis on all adipose intact fish prior to spawning to help avoid mis-identifying missed clip (adipose intact) HOR that ascend the ladder at MRH as NOR. CDFW staff mounted the scales taken above the lateral line and between the posterior insertion of the dorsal fin and anterior insertion of the anal fin on the left side of all prospective spawners. Mounted scales were then visually interpreted in real time on each spawning day, on site at MRH. The juvenile growth pattern of circuli prior to the ocean entry check was inspected for the number of juvenile annuli and for any growth checks. Growth checks are observed as a band of narrowly spaced circuli or a discontinuously formed circulus relative to a similar number of consistently spaced circuli. In Figure 2, the two individuals represented both possessed intact adipose fins, suggesting both fish were NOR. However, presence of annuli in freshwater growth pattern suggests the individual on the right is instead, HOR. Any adipose-intact fish, regardless of collection technique (e.g. ladder return, angler caught, tangle net capture), that were judged to be potential HOR from scale pattern interpretation were rejected as broodstock.

From 2019-2022, scale analysis changed the initial assignment of origin based on adipose fin status (from NOR to HOR) on $13 \%, 29 \%, 26 \%$ and $2 \%$ of presumed NOR steelhead, respectively (Table 15). In 2023 and 2024, the origins assigned for all individuals captured elsewhere and brought to the hatchery were supported by scales identified as NOR.


Figure 2. Comparison of Natural Origin (NOR) and Hatchery Origin (HOR) juvenile growth patterns of adult return steelhead scales as read from scale microfiches.

Table 15. Comparison of origin assignments (HOR vs. NOR) by adipose fin clip status and scale analysis for NOR steelhead collected for broodstock from 2019-2024. Totals include all NOR steelhead brought into the hatchery for broodstock from angling or tangle netting (regardless of whether they were spawned). Scale analysis correction rates represent the proportion of adipose-fin-intact (i.e., NOR-assumed) steelhead confirmed as HOR using scale analysis.

| Brood Year | NOR Assignments <br> (based on fin clip status) | NOR Assignments <br> (based on scale analysis) | Scale Analysis <br> Correction Rate |
| :---: | :---: | :---: | :---: |
| 2019 | 51 | 45 | $13 \%$ |
| 2020 | 151 | 107 | $29 \%$ |
| 2021 | 47 | 35 | $26 \%$ |
| 2022 | 81 | 79 | $2 \%$ |
| 2023 | 4 | 4 | $0 \%$ |
| 2024 | 18 | 17 | $6 \%$ |

### 2.4 Incubation

MRH culture staff keep records of eggs produced by lot throughout each brood year. Survival from green eggs to total egg production for each lot varied each year from as low as $31 \%$ to as high as $94 \%$. The overall survival rates (green eggs-to-total egg production) across each spawning season was consistent, ranging from 73-83\% from 2019 to 2023 (Table 16-Table 20). Survival rates for 2024 are not yet available.

Table 16. Winter-run steelhead egg survival (i.e., fertility rate) summaries by spawning week at Mad River Hatchery for brood year 2019.

| Lot \# | Date | Spawning <br> Pairs | Estimated <br> Green Eggs | Estimated <br> Eyed Eggs | $\%$ <br> Eyed | Culled <br> Eggs | Total Egg <br> Production |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 1 | $1 / 2 / 19$ | 3 | 15,916 | 7,056 | $44 \%$ | 0 | 7,056 |
| 2 | $1 / 9 / 19$ | 5 | 19,964 | 13,284 | $67 \%$ | 0 | 13,284 |
| 3 | $1 / 16 / 19$ | 8 | 46,646 | 37,130 | $80 \%$ | 11,613 | 25,517 |
| 4 | $1 / 23 / 19$ | 3 | 15,408 | 12,669 | $88 \%$ | 0 | 12,669 |
| 5 | $1 / 30 / 19$ | 3 | 15,834 | 11,560 | $78 \%$ | 0 | 11,560 |
| 6 | $2 / 5 / 19$ | 7 | 21,543 | 20,812 | $97 \%$ | 0 | 20,812 |
| 7 | $2 / 12 / 19$ | 6 | 28,124 | 23,040 | $81 \%$ | 0 | 23,040 |
| 8 | $2 / 19 / 19$ | 4 | 17,936 | 12,012 | $67 \%$ | 0 | 12,012 |
| 9 | $2 / 26 / 19$ | 3 | 15,130 | 12,312 | $81 \%$ | 0 | 12,312 |
| 10 | $3 / 5 / 19$ | 0 | 0 | 0 | -- | 0 | 0 |
| 11 | $3 / 12 / 19$ | 1 | 5,340 | 1,668 | $31 \%$ | 0 | 1,668 |
| 12 | $3 / 19 / 19$ | 2 | 8,775 | 2,848 | $32 \%$ | 0 | 2,848 |
| TOTALS |  | $\mathbf{4 2}$ | $\mathbf{2 1 0 , 6 1 6}$ | $\mathbf{1 5 4 , 3 9 1}$ | $\mathbf{7 3 \%}$ | $\mathbf{1 1 , 6 1 3}$ | $\mathbf{1 4 2 , 7 7 8}$ |

Table 17. Winter-run steelhead egg survival summaries by spawning week at Mad River Hatchery for brood year 2020.

| Lot \# | Date | Spawning <br> Pairs | Estimated <br> Green Eggs | Estimated <br> Eyed Eggs | \% <br> Eyed | Culled <br> Eggs | Total Egg <br> Production |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 1 | $1 / 7 / 20$ | 1 | 5,396 | 4,000 | $74 \%$ | 0 | 4,000 |
| 2 | $1 / 14 / 20$ | 3 | 9,016 | 7,772 | $86 \%$ | 0 | 7,232 |
| 3 | $1 / 21 / 20$ | 2 | 14,190 | 10,696 | $75 \%$ | 0 | 10,696 |
| 4 | $1 / 28 / 20$ | 0 | 0 | 0 | -- | 0 | 0 |
| 5 | $2 / 4 / 20$ | 3 | 16,511 | 9,604 | $59 \%$ | 0 | 9,604 |
| 6 | $2 / 11 / 20$ | 9 | 42,126 | 32,850 | $78 \%$ | 0 | 32,850 |
| 7 | $2 / 18 / 20$ | 15 | 84,480 | 54,758 | $65 \%$ | 0 | 54,758 |
| 8 | $2 / 25 / 20$ | 13 | 82,308 | 52,320 | $64 \%$ | 6,000 | 46,320 |
| 9 | $3 / 3 / 20$ | 6 | 34,542 | 30,184 | $87 \%$ | 9,000 | 21,184 |
| 10 | $3 / 10 / 20$ | 11 | 64,640 | 55,272 | $86 \%$ | 18,228 | 37,044 |
| 11 | $3 / 17 / 20$ | 5 | 29,458 | 27,324 | $93 \%$ | 10,350 | 16,974 |
| 12 | $3 / 24 / 20$ | 0 | 0 | 0 | -- | 0 | 0 |
| TOTALS |  | $\mathbf{6 6}$ | $\mathbf{3 8 2 , 6 6 7}$ | $\mathbf{2 8 4 , 7 8 0}$ | $\mathbf{7 4 \%}$ | $\mathbf{4 3 , 5 7 8}$ | $\mathbf{2 4 0 , 6 6 2}$ |

Table 18. Winter-run steelhead egg survival summaries by spawning week at Mad River Hatchery for brood year 2021.

| Lot \# | Date | Spawning <br> Pairs | Estimated <br> Green Eggs | Estimated <br> Eyed Eggs | $\%$ <br> Eyed | Culled <br> Eggs | Total Egg <br> Production |
| :---: | :---: | :---: | ---: | ---: | ---: | ---: | ---: |
| 1 | $1 / 5 / 21$ | 0 | 0 | 0 | -- | 0 | 0 |
| 2 | $1 / 12 / 21$ | 0 | 0 | 0 | -- | 0 | 0 |
| 3 | $1 / 19 / 21$ | 3 | 22,302 | 17,716 | $79 \%$ | 0 | 17,206 |
| 4 | $1 / 26 / 21$ | 1 | 8,568 | 7,400 | $86 \%$ | 0 | 6,680 |
| 5 | $2 / 2 / 21$ | 3 | 25,112 | 18,172 | $79 \%$ | 0 | 18,172 |
| 6 | $2 / 9 / 21$ | 3 | 18,718 | 15,732 | $84 \%$ | 0 | 15,732 |
| 7 | $2 / 16 / 21$ | 5 | 32,308 | 23,936 | $69 \%$ | 0 | 23,936 |
| 8 | $2 / 23 / 21$ | 0 | 0 | 0 | -- | 0 | 0 |
| 9 | $3 / 2 / 21$ | 5 | 29,628 | 21,279 | $72 \%$ | 0 | 21,279 |
| 10 | $3 / 9 / 21$ | 6 | 28,656 | 21,276 | $74 \%$ | 0 | 21,279 |
| 11 | $3 / 16 / 21$ | 1 | 3,850 | 3,560 | $92 \%$ | 0 | 3,560 |
| 12 | $3 / 23 / 21$ | 0 | 0 | 0 | -- | 0 | 0 |
| TOTALS |  | $\mathbf{2 6}$ | $\mathbf{1 6 9 , 1 4 2}$ | $\mathbf{1 2 9 , 0 7 1}$ | $\mathbf{7 6 \%}$ | $\mathbf{0}$ | $\mathbf{1 2 7 , 8 4 4}$ |

Table 19. Winter-run steelhead egg survival summaries by spawning week at Mad River Hatchery for brood year 2022.

| Lot \# | Date | Spawning <br> Pairs | Estimated <br> Green Eggs | Estimated <br> Eyed Eggs | $\%$ <br> Eyed | Culled <br> Eggs | Total Egg <br> Production |
| :---: | :---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 1 | $1 / 4 / 22$ | 2 | 10,764 | 7,488 | $70 \%$ | 0 | 7,488 |
| 2 | $1 / 11 / 22$ | 0 | 0 | 0 | -- | 0 | 0 |
| 3 | $1 / 18 / 22$ | 4 | 28,495 | 24,924 | $94 \%$ | 0 | 24,924 |
| 4 | $1 / 25 / 22$ | 4 | 27,830 | 16,128 | $58 \%$ | 0 | 16,128 |
| 5 | $2 / 1 / 22$ | 4 | 25,252 | 16,524 | $65 \%$ | 0 | 16,524 |
| 6 | $2 / 8 / 22$ | 14 | 106,704 | 70,577 | $66 \%$ | 4,674 | 65,903 |
| 7 | $2 / 15 / 22$ | 7 | 48,816 | 41,615 | $85 \%$ | 17,255 | 24,360 |
| 8 | $2 / 22 / 22$ | 4 | 20,678 | 18,460 | $89 \%$ | 8,358 | 10,102 |
| 9 | $3 / 1 / 22$ | 6 | 33,127 | 29,046 | $88 \%$ | 16,892 | 12,154 |
| 10 | $3 / 8 / 22$ | 7 | 49,852 | 43,425 | $87 \%$ | 35,319 | 8,106 |
| 11 | $3 / 15 / 22$ | 2 | 11,700 | 10,614 | $92 \%$ | 4,941 | 5,673 |
| 12 | $3 / 22 / 22$ | 0 | 0 | 0 | -- | 0 | 0 |
| TOTALS |  | 54 | 363,218 | 278,801 | $77 \%$ | 87,439 | 191,362 |

Table 20. Winter-run steelhead egg survival summaries by spawning week at Mad River Hatchery for brood year 2023.

| Lot \# | Date | Spawning <br> Pairs | Estimated <br> Green Eggs | Estimated <br> Eyed Eggs | $\%$ <br> Eyed | Culled <br> Eggs | Total Egg <br> Production |
| :---: | :---: | :---: | ---: | ---: | ---: | ---: | ---: |
| 1 | $1 / 3 / 23$ | 0 | 0 | 0 | -- | 0 | 0 |
| 2 | $1 / 10 / 23$ | 0 | 0 | 0 | -- | 0 | 0 |
| 3 | $1 / 17 / 23$ | 1 | 5,600 | 4,692 | $84 \%$ | 1,080 | 3,612 |
| 4 | $1 / 24 / 23$ | 0 | 0 | 0 | -- | 0 | 0 |
| 5 | $1 / 31 / 23$ | 0 | 0 | 0 | -- | 0 | 0 |
| 6 | $2 / 7 / 23$ | 6 | 54,963 | 44,500 | $81 \%$ | 0 | 44,500 |
| 7 | $2 / 14 / 23$ | 5 | 31,365 | 26,970 | $86 \%$ | 0 | 26,970 |
| 8 | $2 / 21 / 23$ | 9 | 58,825 | 40,828 | $69 \%$ | 0 | 40,828 |
| 9 | $2 / 28 / 23$ | 7 | 48,514 | 44,100 | $91 \%$ | 13,320 | 30,780 |
| 10 | $3 / 7 / 23$ | 7 | 44,460 | 39,639 | $89 \%$ | 10,860 | 25,159 |
| 11 | $3 / 14 / 23$ | 5 | 31,724 | 26,352 | $83 \%$ | 9,153 | 17,199 |
| 12 | $3 / 21 / 23$ | 4 | 25,800 | 23,180 | $90 \%$ | 19,000 | 4,180 |
| TOTALS |  | $\mathbf{4 4}$ | $\mathbf{3 0 1 , 2 5 1}$ | $\mathbf{2 5 0 , 2 6 1}$ | $\mathbf{8 3 \%}$ | $\mathbf{5 3 , 4 1 3}$ | $\mathbf{1 9 3 , 2 2 8}$ |

### 2.5 Rearing

Each year hatchery staff maintain production records by estimating the total number of live individuals at each life stage (Table 21). Stage-based survival rates are calculated by family lot from fertilized egg to smolt each year to track fish rearing performance at the hatchery (Table 22). Minimum targets established in the Mad River HGMP for egg-to-fry ( $80 \%$ ) and egg-to-smolt (70\%) were met each brood year from 2019 to 2023, except for low egg-to-smolt survival that occurred in 2020 (56\%).

Table 21. Winter run steelhead egg, fry and smolt production totals at Mad River Hatchery for brood years 2019-2023. The Mad River HGMP (CDFW 2016) stipulates annual smolt releases to be no more than 150,000 adipose fin-clipped smolts.

| Brood Year | Green Eggs | Eyed Eggs | Egg Production | Fry to Pond | Smolts Released |
| :--- | :---: | :---: | :---: | :---: | :---: |
| 2019 | 210,616 | 154,391 | 142,778 | 136,197 | 134,211 |
| 2020 | 382,667 | 284,780 | 240,662 | 210,544 | 134,096 |
| 2021 | 169,142 | 129,071 | 127,844 | 113,600 | 91,249 |
| 2022 | 363,218 | 278,801 | 191,362 | 177,791 | 137,280 |
| 2023 | 301,251 | 250,261 | 193,228 | 168,835 | 141,630 |

Table 22. Stage-based survival rates for steelhead production at Mad River Hatchery for brood years 2019-2023.

| Brood Year | Survival Rates |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | Green-to- <br> Eyed Egg | Eyed Eggs culled | Egg-to-Fry | Fry-to-Smolt | Egg-to- <br> Smolt |
| 2019 | $73 \%$ | $8 \%$ | $95 \%$ | $99 \%$ | $94 \%$ |
| 2020 | $74 \%$ | $15 \%$ | $87 \%$ | $64 \%$ | $56 \%$ |
| 2021 | $76 \%$ | $1 \%$ | $89 \%$ | $80 \%$ | $71 \%$ |
| 2022 | $77 \%$ | $31 \%$ | $93 \%$ | $77 \%$ | $72 \%$ |
| 2023 | $83 \%$ | $23 \%$ | $87 \%$ | $84 \%$ | $73 \%$ |
| Minimum | -- | - | $80 \%$ | -- | $\mathbf{7 0 \%}$ |
| Target |  |  |  |  |  |

### 2.6 Disease

From 2019 to 2024, no uncommon, largescale fish disease outbreaks occurred in the rearing facilities at the hatchery. Hatchery staff monitor mortality rates in the rearing ponds each day. When mortality rates exceed ordinary expectations, samples are collected and analyzed by fish disease pathologists to determine the type and severity of disease and prescribe treatments to apply.

### 2.7 Marking

All steelhead trout produced in fish hatcheries in California receive an adipose fin clip prior to release to maintain their identity as HOR steelhead. At Mad River Hatchery, quality assurance/quality control (QAQC) measures are taken each year before release to ensure the highest possible clip rates are maintained in the hatchery population. Over 1,000 juveniles are randomly selected and examined for the presence and quality of an adipose clip. QAQC measures should indicate $\geq 99 \%$ of the smolts release should have a discernible fin clip (CDFW 2016). Since 2018, QC results show that $\geq 99 \%$ of smolts released had discernable clips ( $\geq 25 \%$ of the fin was removed) and $\geq 98 \%$ of smolts released showed $\geq 50 \%$ of the fin was removed (Figure 3). While fin clipping was completed on the smolts release from BY 2020 and BY 2021, QAQC was not completed on the smolts prior to release due to logistical constraints caused by the COVID-19 pandemic.

### 2.8 Smolt Release

Currently, hatchery smolts are targeted for release between March $15^{\text {th }}$ and April $15^{\text {th }}$ of each year during higher flow events to facilitate rapid downstream migration and decrease potential interactions with naturally produced juvenile salmon and steelhead in the Mad River basin (Table 23). However, the effects of the single batch release during elevated flows on juvenile NOR populations are not well understood, nor are its effects on HOR smolt-to-adult return rates.


Figure 3. Estimated clip rates of Mad River Hatchery smolt releases by brood year (BY) based on Quality Control measures. Clip rates are estimated by assessing a subsample of $\geq 1 \%$ (approx. 1,500 ) of the ponded juveniles for quantity and quality of fin clips.

Table 23. HOR steelhead smolt release summaries by brood year, release date and river flows.

| Brood <br> Year | Smolts <br> Released | Release <br> Date | CFS (at noon on day of release) <br> \& additional release notes |
| :---: | :---: | :---: | :--- |
| 2019 | 134,211 | $3 / 24 / 2020$ | 350 cfs , and increasing to 555 cfs the next day |
| 2020 | 134,096 | $3 / 24 / 2021$ | $1,610 \mathrm{cfs}$, descending from $2,600 \mathrm{cfs}$ four days prior |
| 2021 | 91,249 | $3 / 15 / 2022$ | 217 cfs , and increasing to 430 cfs the next day |
| 2022 | 137,280 | $3 / 22 / 2023$ | $4,540 \mathrm{cfs}$, descending from $7,000 \mathrm{cfs}$ two days prior |
| 2023 | 141,630 | $3 / 20 / 2024$ | $2,970 \mathrm{cfs}$, descending from $5,700 \mathrm{cfs}$ seven days prior |

## 3 FACILITIES MANAGEMENT

From 2019 to 2024, CDFW staff managed the facilities at MRH to comply with the standards specified in the HGMP for the hatchery water source (HGMP Section 4.0) and general facilities used for aquaculture purposes (HGMP Section 5.0). HGMP specifics relating to smolt releases, juvenile or adult steelhead predation incidents and hatchery water quality management are reported on and/or referenced in this report.

### 3.1 Smolt Release Strategy

In March of each year from 2019 to 2024, steelhead smolts reared at MRH underwent a forced release from the rearing ponds directly into the Mad River. Pond boards in the rearing pens were removed for yearlings to exit through a pipe into the river. Some fish volitionally exited through the pipe. Remaining fish were crowded into the release area at the end of each rearing pond and into the pipe that leads to the river. Release days target periods of relatively higher flows to encourage immediate downstream movement of HOR smolts and discourage competition with their NOR conspecifics. In addition, turbid river conditions associated with higher flows may reduce predation potential on released fish. Refer to Section 2.8 on Smolt Releases (Table 23) for smolt release group summaries and associated river conditions.

### 3.2 Predation Incidents

From 2019 to 2024, minimal predation on juvenile or adult fish occurred at the hatchery. The only significant incident occurred in winter of 2020 when otters periodically entered the MRH holding pen via the fish ladder and killed several adult steelhead. Given that the otters were consuming individuals, the total number of mortalities stemming from the incident is unknown. However, since NOR adults brought in from the MRSS broodstock program are tagged with spaghetti tags prior to placement in the holding pen, MRH staff were able to deduce from broodstock records that 3 NOR adults were killed by otters. Eventually, a hired trapper was able to capture and relocate the intrusive individuals.

### 3.3 Water Quality Monitoring

From 2019 to 2024, MRH self-monitored and reported water quality parameters on a monthly basis to the North Coast RWQCB (Permit Order R1-2021-0010, General NPDES No. CAG131015) to comply with their "Waste Discharge Requirements for Cold Water Concentrated Aquatic Animal Production Facility Discharges to Inland Surface Waters, Enclosed Bays and Estuaries."

Five existing effluent discharge points at MRH are monitored, which include the Fish Ladder (NPDES Permit Discharge Point \#001), the Spawning/Hatchery Building (\#002), the Settling Basins (\#003), the annual Fish Release event (\#004), and the Bio-Filter Wash Water (\#005). No reportable violations or significant events that would affect water quality parameters occurred at the facility from 2019-2024.

### 3.4 Public Relations

CDFW and MRH consider maintaining a positive relationship with the public as an essential goal of operating the hatchery facilities and maintaining the premises. MRH provides public access to a large parking area, Americans with Disabilities Act Standard compliant public restrooms, and a grassy park with picnic tables for hatchery visitation. The area is used year-round by visitors to tour the hatchery, observe spawning operations, access the Mad River for fishing, birding and other recreation, and enjoy the outdoors in small or large groups. The hatchery grounds also provide space where outdoor trainings are hosted (e.g., Wilderness First Aid). MRH is also a highly popular area for steelhead anglers during periods when the river is open to fishing. In the recent past, MRH has grown to expect very high visitation rates each year (up to 50-100 visitors/day annually).

In addition, from 2019-2024, MRH provided several local classrooms with steelhead eggs for use in the California Aquarium Education Project. Steelhead fingerlings have their adipose fin removed so they can be identified as hatchery origin. Each year, the students release their reared fish into the Mad River near the hatchery during the months of April, May, and June.

### 3.5 Facilities Maintenance and Upgrades

Several changes or upgrades to hatchery facilities and equipment were completed from 20192024 to improve various aspects of broodstock collection, spawning, and rearing at the facility.

- A new hatchery truck was purchased to replace the old truck. It will also be outfitted with a new water tank to provide more room for NOR fish during transport to the hatchery
- Three main pumps were rebuilt and new pipes were installed that carry water up to the aeration towers to maintain the biological filtration system and continue recycling rearing pond water through the oyster shell ponds.
- New predator exclusion caging installed to protect rearing ponds including upgraded avian predator prevention wires, extended chain-link fencing and automatic gates
- In addition to the ultraviolet (UV) light sterilization systems in place at the headworks of each raceway, a new $24^{\prime}$ UV system was installed at the oyster shell beds
- Roofs were replaced on hatchery buildings


## 4 BIOLOGICAL MONITORING

### 4.1 Genomic validation of pNOB

Pedigree reconstructions utilizing broodstock genotypes were used to estimate pNOB and compared to the pNOB observed from adipose fin clip status. Dried tissue samples from 20182022 broodstock were genotyped at the CDFW Fisheries Genetics Laboratory. Genomic DNA was extracted using the Omega Biotek Magbind Blood and Tissue Kits on a Hamilton Nimbus liquid handling unit. Genotyping was done using TaqMan (Applied Biosystems, Inc.) assays on 96.96 Dynamic Arrays (Fluidigm Corp.). PCR amplification was conducted using the Fluidigm Juno and the arrays were imaged using the Fluidigm Biomark system. Scoring and genotyping was done using Fluidigm's SNP Genotyping Analysis software (version 4.5.1). All samples were genotyped at the 96 single nucleotide polymorphism (SNP) loci described by Abadía-Cardoso et al. (2013) except for locus Omy_121006-131, which was replaced with the proximate locus Omy_R04944 in the Omy05 inversion area (Pearse et al. 2019).

Genotypes for the MRH broodstock from 2013-2017 were obtained from prior work done by Kinziger et al. (2022) and combined with genotypes from 2018-2022 broodstock. Following Kinziger et al. (2022), parentage assignments were done using SNPPIT (i.e. SNP Program for Intergenerational Taging) (https://github.com/eriqande/snppit) (Anderson 2012).

### 4.1.1 Parentage Analysis Results

Forty-two Individuals missing data at 12 or more SNP loci were excluded from analysis (i.e. KidMiss $\geq 12$, [Anderson 2010]). We used a False Discovery Rate (FDR) of $<0.005$ to control the rate of false discoveries (i.e., parentage assignments likely having one or two incorrectly identified parents). Applying these constraints reduced the set of animals included in the analysis to 438 of a possible 499 (Table 24). All returning adults that were assigned to a parent pair were identified as hatchery origin and conversely, all returning adults that were not assigned to a parent pair were considered as natural origin. Proportion of NOR broodstock from pedigree analysis ranged from 0.25 to 0.50 from spawning years 2018-2022 whereas proportion of NOR broodstock observed from adipose fin clips ranged from 0.45 to 0.51 over the same period (Table 25).

Table 24. Summary of winter-run steelhead Mad River Hatchery broodstock genotyped from 2018-2022 returns including the number of samples missing genotypes at more than 12 loci, and the number of genetically unique male and female individuals used in the parentage analysis.

| Year | Total <br> broodstock | Number <br> tissues <br> genotyped | Number <br> missing loci <br> KidMiss $\mathbf{\geq 1 2}$ | Number in analysis |  |  |
| :--- | ---: | :---: | ---: | ---: | ---: | ---: |
|  |  | Males | Females | Total |  |  |
| 2018 | 122 | 122 | 25 | 38 | 42 | 80 |
| 2019 | 84 | 84 | 8 | 35 | 40 | 75 |
| 2020 | 133 | 133 | 3 | 63 | 66 | 129 |
| 2021 | 52 | 52 | 1 | 27 | 24 | 51 |
| 2022 | 108 | 108 | 5 | 53 | 50 | 103 |

Table 25. Estimates of the proportion of NOR steelhead used as broodstock at Mad River Hatchery 2013-2022 from pedigree reconstruction and from adipose fin clip status.

| Spawn <br> Year | Number of <br> broodstock | Pedigree Reconstruction <br> Number <br> HOR |  | Number <br> parents <br> found | Proportion <br> NOR | Number <br> HOR | Number <br> NOR |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  | 229 | 201 | 28 | 0.12 | 215 | 13 | 0.06 |
| 2014* | 28 | 24 | 4 | 0.14 | 17 | 11 | 0.39 |
| 2015* | 181 | 156 | 25 | 0.14 | 87 | 94 | 0.52 |
| 2016* | 229 | 209 | 20 | 0.09 | 104 | 125 | 0.55 |
| $2017^{*}$ | 240 | 201 | 39 | 0.16 | 104 | 136 | 0.57 |
| 2018 | 80 | 56 | 24 | 0.30 | 41 | 39 | 0.49 |
| 2019 | 75 | 56 | 19 | 0.25 | 41 | 34 | 0.45 |
| 2020 | 129 | 68 | 61 | 0.47 | 65 | 64 | 0.50 |
| 2021 | 51 | 31 | 20 | 0.39 | 26 | 25 | 0.49 |
| 2022 | 103 | 51 | 52 | 0.50 | 50 | 53 | 0.51 |

*Data reproduced from Kinziger et al. (2022)

### 4.2 Population Monitoring Methods

The biological monitoring of the HGMP seeks to estimate steelhead population abundances and MRH breeding system parameters necessary to approximate PNI, including pHOS and pNOB (Ford 2002, HSRG 2009) (Figure 4). The former is attempted by monitoring steelhead run size in the Mad River to estimate both natural origin (NOR) and hatchery origin (HOR) adult returns. Under the ideal situation where all parameters are estimable, pHOS can be calculated as:

$$
p H O S=\frac{(\mathrm{HOR}-\mathrm{H}-\mathrm{HOB})}{(\mathrm{HOR}-\mathrm{H}-\mathrm{HOB})+(\mathrm{NOR}-\mathrm{NOB})}
$$

where $H$ represents sport harvest of hatchery origin fish, and HOB and NOB are the number of respective HOR and NOR used in the hatchery as broodstock. pNOB can be estimated from both adipose fin status and scale verification methods or by parentage analysis (See section 2.3.1) using genotype data. The following sections describe the approach, results, assumptions, and discussion of the biological monitoring for the MRH HGMP.


Figure 4. Diagram of the breeding system for anadromous steelhead (Oncorhynchus mykiss) in the Mad River basin. The hatchery origin (HOR) sub-population reared and released at the hatchery is made up of a proportion of hatchery-origin broodstock ( pHOB ) mixed with naturalorigin broodstock ( pNOB ) brought into the hatchery from the wild. The natural origin (NOR) subpopulation that emerges and rears in the wild is made up of a proportion of natural-origin spawners (pNOS) mixed with hatchery-origin spawners that "stray" from the hatchery. Proportionate Natural Influence (PNI), originally described as the 'approximate PNI index' by HSRG (2009) as an approximation of the proportional genetic influence of the natural environment on a population.

### 4.2.1 Sonar-based run size estimation

### 4.2.1.1 SONAR operation

We deployed a long-range ARIS 1200 ( $\mathrm{n}=48$ beams, Adaptive Resolution Imaging Sonar, Sound Metrics, 11010 Northup Way, Bellevue, WA 98004) sonar camera at RM 7.0 in the Mad River on in early September of each year. (A short-range ARIS 1800 unit was used as needed when troubleshooting the ARIS 1200 was necessary.) The units continuously recorded fish passing the site 24 hours per day through April of the following year, less a limited number of short duration periods when the unit was pulled from the river during high river discharge events. The ARIS sonar was placed in a glide habitat unit just upstream of a low gradient riffle in an area with a stream bottom profile that allowed near complete ensonification with little shadowed areas (Maxwell 2007). We used ARIScope software (Sound Metrics) with updated firmware to run the sonar. Within the software program, we selected 'auto' for frequency, 'max' for transmit, 'auto' for pulse, "'max' for frame rate, 'auto' for detail, and 'continuous' for timing mode. We aimed the ARIS sonar perpendicular to the current, and esonifed the majority of the channel width which ranged from 48 to 70 m wide depending upon stream flow. The tilt or pitch of the sonar was manually set between $-0.4^{\circ}$ to $-3^{\circ}$, to allow near complete ensonification of the channel from stream bottom to stream surface at various stream flows and water depths. Fish exclusion panels were placed immediately downstream of the sonar and extended from the stream bank 1-2 m beyond the sonar to prevent fish from swimming behind the sonar camera or too close to the sonars' image start ( 0.7 m ). The sonar was checked daily to ensure esonification and pitch, and manually adjusted as necessary. We recorded files in 20-minute time increments throughout the survey periods to an external 4 tb hard drive.

### 4.2.1.2 Species apportionment

Images generated by the sonar camera are normally of high enough quality such that trained technicians can both discriminate salmonids from other families of fishes, wildlife, or floating debris and allow measurement of individual fish lengths with roughly 5 cm precision. The species of Salmonidae, however, cannot be distinguished from the sonar imagery alone. We collected an auxiliary set of data to characterize the species composition of salmonids in the river and use these data to apportion our un-speciated sonar imagery to species. Beginning with sonar installation in September of each year, two technicians snorkeled pool and run habitats from Blue Lake to highway 101 bridge approximately bi-weekly recording the number and size class of salmonid species observed. Snorkel surveys continued until the first significant fall rains elevated river discharges and water turbidities precluding effective visual observations.

Beginning in the 2023-2024 season, tangle nets were deployed by Department staff within the lower river to capture adult salmonids throughout the year to continue collecting species apportionment data once high flows and turbidity make snorkel surveys ineffective. Tangle netting techniques were used from 7-November-2023 to 12-April-2024 and deployment ranged
from the Hwy 101 Bridge near Arcata upstream to the Hatchery Rd bridge in Blue Lake. Tangle nets have been used effectively on the Elwa River (Peters et al. 2021) and in mark-selective fisheries along the west coast to non-lethally capture adult anadromous fish for scientific means, and subsistence or commercial harvest. Tangle nets are much more versatile than traditional seines; they are lighter and thus more portable and easier to work with, they are less detectable by fish and thus less likely to be avoided, and they can be used in a wide variety of habitats and deployment strategies. They were fished both statically, by anchoring and deploying the net across a fish passage corridor, and dynamically, by drifting with the river's current and/or with the assistance of boats through runs and pools. We used tangle-nets that are 96 feet long and 12 feet deep with a 2 -inch square mesh of $8 \#$ monofilament. Each animal captured with the tangle net was recorded by species and measured by total length. Tangle nets were also used to apportion steelhead origins (HOR vs NOR) based on the presence (NOR) or absence (HOR) of an adipose fin. Aside from their use for species- and origin-based apportionment, tangle-net-captured HOR steelhead were used to conduct a mark-recapture study with reward value spaghetti tags (see Section 4.2.2), and NOR steelhead deemed mature enough to fully ripen in captivity were sent to the Mad River Hatchery for broodstock (see Section 2.2). Additional information and results from tangle net sampling are presented later in this report and describe the specific netting techniques used (Section 4.2.3) and their effectiveness for the 2023-24 study year (Section 4.3.1.5).

### 4.2.1.3 Temporal stratification

For surveys beginning in years 2019-2022, we calculate the total number of net upstream migrating fish for each of four temporal strata labeled A, B, C, and D (Figure 5, Figure 6, Figure 7, and Figure 8). Strata A encompassed the early season from September $5^{\text {th }}$ to the onset of first rains within which auxiliary species apportionment data was available. In strata A we estimate the total net upstream abundance of salmonids by species in $42-53 \mathrm{~cm}$ and $\geq 54 \mathrm{~cm}$ size categories that relate to anadromous life histories of 1 ocean season (i.e. grilse) and 2 or more ocean years (i.e. adult) respectively. Strata B represents the period when Chinook Salmon, Coho Salmon and Steelhead are assumed to have significant overlap in run timing but no auxiliary species apportionment data was available, and therefor estimates of only total salmonids in each size class can be made. Strata C includes the period beginning January $9^{\text {th }}$ and ending March $15^{\text {th }}$ of each year when we assumed all salmonid passage was composed of pre-spawn (i.e. no post spawn down running kelt) steelhead. We further assumed all images in strata D from March $15^{\text {th }}$ to March $31^{\text {st }}$ were post spawn down running kelt steelhead. We used CDFW Steelhead Report Card reported natural and wild catches to apportion fish $\geq 42 \mathrm{~cm}$ into hatchery and natural stocks in both stratum C and D.

Beginning in survey year 2023-2024, we did not need to stratify our SONAR estimates prior to March $15^{\text {th }}$ (when all fish are assumed to be upstream migrating and unspawned). During the period between September 5th to the onset of the first rains, auxiliary species apportionment
data was collected via snorkel visual observation. The presence or absence of an adipose fin on a subset of observed steelhead was recorded. Once river conditions precluded snorkel-based species and steelhead stock (i.e. HOR vs NOR) apportionment, tangle net captures were used for apportionment.

### 4.2.1.4 Run size estimation

We divided the sonar imagery into 20-minute Primary Sample Unit (PSU) segments. A systematic sample of either every twelfth (seasons 2019-2020 and 2022-2023) or every third (seasons 2020-2021, 2021-2022, 2023-2024) PSU beginning at the top of the hour at midnight was reviewed. For each 20-minute sample, a trained technician counted and measured each salmonid image $\geq 42 \mathrm{~cm}$ and indicated if the fish was moving upstream or downstream through the visual field. Net directional movement was calculated for each PSU as the number of upstream moving fish minus the number of downstream moving fish in each size class. Any missing data sample counts (e.g. when the SONAR camera was removed from the river or otherwise inoperable) were imputed using a moving average of 10 samples before and after the missing sample. In the case of long missing gaps where all values next to the missing value were also missing, the window size is increased incrementally until at least 2 non-missing values are included in the average (Moritz and Bartz-Beielstein 2017).

Species apportionment data needed to produce salmon and steelhead abundance estimates by species and stock for the Mad River include (1) early season (i.e., fall, sometimes early winter) snorkel survey observations, and (2) steelhead angler report card data. With snorkeling observations limited by water visibility, abundance estimates for the 2019-20 through 2022-23 monitoring seasons could only be produce in discrete temporal "chunks" (i.e., strata). For survey years beginning in 2019-2022, estimates of run size abundance for each stratum ( $A, B, C$, and $D$ ) in each year were constructed using a non-parametric bootstrap superpopulation resampling routine for finite populations (Davison and Hinkley 2009). Approximate 95 percent confidence limits are constructed as the $2.5 \%$ and $97.5 \%$ quantiles of the generated bootstrap distribution (Efron 1979). For the early portion of the season when auxiliary species apportionment observations were available (Strata A), we extended the resampling routine to first make species assignments, then produce species and size class specific estimates of total upstream fish passage. During the late season when steelhead hatchery and wild catches were reported via the Steelhead Report Card (Strata C and D) we extend the bootstrap routine to estimate hatchery and wild steelhead abundance for fish $\geq 42 \mathrm{~cm}$. Pseudo-code for the bootstrap procedure used to estimate run size abundance is:

1. For each 20 min SONAR count $j$ in week $i$
a. Simulate River Sampling $\leftarrow$ Randomly resample with replacement the auxiliary species proportion (strata A) or hatchery/wild report card data (strata C and D) in
closest week to week i, n' times; where $n^{\prime}=$ sample size of species proportion data.
b. Assign Species $\leftarrow$ Randomly sample species with replacement from Simulated River Sample for each fish in count $j$
2. Simulate Population $\leftarrow$ Randomly sample with replacement speciated 20 min SONAR counts $N$ times, where $N$ is the total number of twenty minute samples in the season
a. Randomly sample Simulated Population without replacement $n$ times, where $n=$ sample size of 20-minute sonar review PSUs
3. $\theta \leftarrow$ Calculate fish passage by species as mean count over all PSUs * $N$
4. $\theta^{*} \leftarrow$ Repeat steps $1: 31000 x$ ( $k$ sims)
5. Construct Estimate of Total Fish passage by species as mean $\Theta^{*}$
6. Approximate $95 \% \mathrm{Cl}$ constructed as $2.5 \%$ and $97.5 \%$ quantiles of $\Theta^{*}$

The bootstrap procedure composed of steps 1-6 is used for stratum A, C and D. Only steps 2-6 are used for strata B.

For survey year 2023-24, species apportionment data was extended to cover the entire migration season using both snorkeling (when possible) and tangle net sampling (as needed). As such, the bootstrap procedure was adapted to accommodate the extended species- and originapportionment (via fin clip observations) data, replacing the use of Steelhead Report Card reported NOR/HOR catches in the previous years. The consistency in the apportionment data collected throughout the season allowed for the estimation of species and stock abundances without parsing into strata, yielding a single estimate per species/stock. Pseudo-code for the bootstrap procedure used to estimate run size abundance in 2023-2024 with the addition of step 1c, is:

1. For each 20 min SONAR count $j$ in week $i$
a. Simulate River Sampling $\leftarrow$ Randomly resample with replacement the auxiliary species proportion data in closest week to week $i$, $n^{\prime}$ times; where $n^{\prime}=$ sample size of species proportion data.
b. Assign Species $\leftarrow$ Randomly sample species with replacement from Simulated River Sample for each fish in count $j$
c. Assign HOR or NOR to assigned steelhead $\leftarrow$ Randomly sample with replacement from steelhead NOR/HOR apportionment Sample for each fish in steelhead Assigned Species fish in count j
2. Simulate Population $\leftarrow$ Randomly sample with replacement speciated and NOR/HOR 20 min SONAR counts $N$ times; where $N=$ total number twenty minute samples in the season
d. Randomly sample Simulated Population without replacement $n$ times, where $n=$ sample size of 20 min sonar review PSUs
3. $\theta \leftarrow$ Calculate fish passage by species as mean count over all PSUs *N
4. $\theta^{*} \leftarrow$ Repeat steps $1: 31000 x$ ( $k$ sims)

## 5. Construct Estimate of Total Fish passage by species as mean $\theta^{*}$

6. Approximate $95 \% \mathrm{Cl}$ constructed as $2.5 \%$ and $97.5 \%$ quantiles of $\Theta^{*}$

### 4.2.2 All calculations were executed in program $R$ ( $R$ Core Team 2023).Capture-mark-recapture run size estimation of steelhead stock abundance

We initiated a capture-mark-recapture experiment in study year 2023-2024 to estimate steelhead HOR, NOR, and angler harvest independently of the sonar monitoring. We marked hatchery origin steelhead captured in the lower river during our tangle netting operations with spaghetti reward tags valued at $\$ 20, \$ 50$ and $\$ 100$. We then widely advertised the opportunity for anglers to redeem rewards for any captured and/or harvested steelhead on social media, and with posters at all common fishing accesses (figure $x$ poster) including a smart phone QR code to facilitate angler response. Steelhead ascending the ladder at Mad River Hatchery served as the second sampling period where all animals were inspected for the presence of reward tags.

We estimate the anadromous adult steelhead return to the Mad River utilizing a LincolnPetersen change in ratio estimator with the bias adjustment of Chapman (1951). The hatchery origin steelhead run size (HOR) is estimated using the following equation:

$$
H O R=\left(\left(M_{H O R}-H t\right)+1 *(S+1)\right) /(R+1)
$$

Where $M_{H O R}$ is the number of hatchery origin steelhead captured and tagged during tangle net sampling in the lower river, Ht is the number of tagged hatchery origin steelhead harvested by anglers, $S$ is the total number of hatchery origin steelhead returning to the hatchery, and $R$ is the number of tagged hatchery origin steelhead recaptured at the hatchery.

The number of natural origin steelhead run size (NOR) is estimated by assuming equal capture probability of NOR and HOR fish during tangle net sampling as:

$$
N O R=\left(M_{N O R}+1\right) *(S+1) /(R+1)
$$

Where $M_{N O R}$ is the number of natural origin steelhead captured (no tag applied) during tangle net sampling.

Total harvest of hatchery origin stock $(H)$ is estimated by:

$$
H=\left(H t / M_{H O R}\right) * H O R
$$

Estimates of hatchery origin steelhead potentially spawning in natural areas can then be estimated by subtracting the number of steelhead used as broodstock at the hatchery (HOB) and the number of steelhead harvested (H) from the estimate of HOR. Similarly, the number of NOR fish potentially spawning in natural areas is adjusted by subtracting the number of Natural Origin Breeders (NOB) from estimates of NOR. We then estimate pHOS as:

$$
p H O S=(H O R-H-H O B) /((H O R-H-H O B)+(N O R-N O B))
$$

and finally, using the adipose fin status and scale analysis of all hatchery-spawned steelhead to determine the proportion of NOR used as broodstock ( pNOB ), we estimate PNI as in Figure 4.

We apply a non-parametric bootstrap approach to calculate confidence intervals around our estimated parameters. Three period capture histories were constructed for individual fish as; (1) tagged in the lower river below the majority of angling effort, (2) tagged fish harvested by anglers and (3) unique hatchery fish ascending the ladder at MRH for the first time. A fish tagged, not harvested, and recaptured at the hatchery has a capture history of '1-0-1', an untagged HOR fish that was not harvested but recovered at MRH would have a capture history of ' $0-0-1$ '. Because a fish must have been tagged to have the tag returned by an angler, and a harvested fish cannot return to MRH, capture histories ' $0-1-1$ ' and ' $0-1-0$ ' are impossible. $M_{N O R}$ was set constant for the estimation of NOR steelhead because under our assumptions of equal capture probability of HOR and NOR steelhead during tangle netting operations, estimator uncertainty arises only from the resampling of HOR S and $R$ (capture histories ' $1-0-1$ ' and ' $0-0$ $1^{\prime}$ ). Generating capture histories with these constraints allowed us to bootstrap one set of 3 period capture histories to calculate abundance estimates and associated confidence intervals of all parameters with only two sample estimators. Within each of 10,000 bootstrap replicates, we simulate "new" data by sampling with replacement over the set of observed capture histories and calculate the associated estimates using the equations above. We use a percentile bootstrap confidence interval by taking the lower and upper $2.5 \%$ quantiles of the associated simulated estimates obtained over the 10,000 bootstrap replicates.

### 4.2.3 Tangle Netting on the Mad River

The 2023-24 season was the first in which tangle nets were used to capture fish on the Mad River. With limited tangle net training opportunities available before the season began, field crews spent a significant amount of time learning how to use the nets safely and efficiently, using a few techniques and across a wide range of flow conditions. This trial-and-error approach limited fish capture rates at times, especially early in the season. However, field staff learned quickly enough to ensure enough data was collected throughout the season to use for apportionment.

For tangle net sampling on the Mad River, we developed some general guidelines for safe and efficient sampling based on each tangle netting technique and river flows at the time of sampling (Table 26). Our tangle netting efforts can be simplified into two main techniques: drift netting and fixed net sets. Drifting tangle nets was the method used early in the season when flows were lower and field staff had a good understanding of the under-water snags or other areas that present problems for drifted nets. Sticks, logs and metal debris embedded in the substrate were common obstacles encountered that reduced effectiveness of sampling by preventing fish capture, snagging, and damaging nets. Snagging nets in faster moving water also can be hazardous for field staff and any animals entangled in the nets. To avoid snagging objects and damaging nets, lead lines were dragged through sampling units any time they were visited for the first time, or if the unit had experienced a significant flow event since the previous sampling event. All "snaggy" sampling sites were generally avoided.

On the Mad River, drifted tangle nets were effective from summer base flows up to approximately 2,000 cfs. As flows continue to increase beyond 2,000 cfs, the levels of risk to people, fish, data and equipment also increase. Tangle nets were commonly drifted between 2,000 and 3,000 cfs after January 1 with some success but catches generally ranged from just 0 2 fish per day. Overall, main advantages for the drift net method are that it does not require fish migration or movement, and it functions well at lowest water levels. Its disadvantages are that it requires more people and equipment, it is not practical at high flows, and has greater risks associated with snags in the water (Table 26).

When flows were too high for drifting nets, fixed tangle net sets became a viable option for capturing migrating fish. For this technique, nets were anchored in carefully selected slack water areas using anchors and rope. Selecting sites for fixed nets was often difficult, as the nets require a particular size, shape, speed and depth of eddy currents to hold nets far enough into the channel to transect fish migration lanes. However, when quality sites are found (such as in the Kadle Hole or near Lindsay Creek mouth) fixed nets were an excellent way to catch fish when flows are up-most fish encountered in February were captured using fixed sets. See Table 26 for a list of advantages and disadvantages of each tangle net method.

Table 26. Comparison of drifted and fixed tangle netting techniques based on 2023-24 sampling.

| Tangle Net Method | Mad River Flow range | Staffing | Equipment | Advantages | Disadvantages |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Drift set (active) | <2,000 cfs is ideal; possible between 2,000 and $3,000 \mathrm{cfs}$ | 2-4 people depending on location (3 is ideal) | - Kayak(s) <br> - Raft (a <br> raft with <br> trailer is <br> ideal) | - Useful under low flow conditions <br> - Does not require fish migration for capture <br> - Can sample anywhere without obstacles <br> - Can be used to target holding fish | - Requires more people <br> - Requires more equipment <br> - Greater safety risks for people and equipment |
| Fixed set (passive) | $>3,500 \mathrm{cfs}$ is typical (or when \& wherever large eddies are present) | 2 people | - Kayak(s) <br> - Anchor(s) | - Less equipment required (e.g., raft not needed) <br> - Functional during higher flows <br> - Requires fewer people to operate <br> - Can be used to target migrating fish | - Requires higher flows <br> - Only works in particular sampling locations (e.g., slack water, eddies) <br> - Passive technique; fish must move to be captured |

### 4.3 Population Monitoring Results

### 4.3.1 SONAR-based Run Size Estimates

### 4.3.1.1 Study Year 2019-2020

CDFW field crews observed 279 Coho Salmon, 640 Chinook Salmon and 410 steelhead in the 4253 cm length range and 542 Coho Salmon, 1,632 Chinook Salmon and 268 steelhead $\geq 54 \mathrm{~cm}$ in total length during the species apportionment snorkel dives from 5-September 2019 through 4December 2020 in temporal strata A (Figure 5). A total of 4,108 Steelhead Report Card catches were reported in strata C and D, of which 1,416 (34\%) were natural origin, and 2,692 (66\%) hatchery origin (Figure 5). A total of 6,516 salmonids greater than or equal to 42 cm in total length were estimated to have passed the SONAR camera moving upstream in stratum A-C (Table 27). We estimate a total run size of steelhead greater than or equal to 42 cm in total length in strata C to be 3,907 composed of $34.5 \%$ natural origin (adipose intact) fish. We estimate 1,939 downstream moving steelhead in strata $D$, presumably post-spawn kelts, $47 \%$ of which were NOR. All stratum estimates of net upstream passing salmonids are presented in Table 27.




Figure 5. Top panel depicts daily expanded net movement of salmonids greater than 54 cm total length for the 2019-2020 season derived from SONAR images (left primary Y axis) and Mad River discharge (right secondary Y axis). Vertical lines separate the time series into temporal stratum A, B, C, and D. Middle panel depicts daily snorkel counts of salmonid species (CO=Coho Salmon, KS=Chinook Salmon, $\mathrm{SH}=$ steelhead) greater than 54 cm . Bottom panel displays daily hatchery and natural steelhead catches reported on the CDFW Steelhead Report Card from completed trips.

Table 27. SONAR derived estimates of total net upstream passage of salmonids in temporal strata A, B, C, D of survey year 2019-20 at river mile 7 on the Mad River, Humboldt County, CA.

| Strata | Dates of estimation | Size Class | Stock |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| A | 15 Sept-4 Dec 19 | $\begin{gathered} \geq 54 \mathrm{~cm} \\ 42-53 \mathrm{~cm} \end{gathered}$ | Chinook | Coho | Steelhead |
|  |  |  | $\begin{gathered} 532(264,840) \\ 254(76,461) \\ \hline \end{gathered}$ | $\begin{aligned} & \hline 215(63,413) \\ & 163(24,336) \\ & \hline \end{aligned}$ | $\begin{aligned} & 58(0,259) \\ & 327(158,509) \\ & \hline \end{aligned}$ |
| B | 5 Dec - 8 Jan 20 | $\begin{gathered} \geq 54 \mathrm{~cm} \\ 42-53 \mathrm{~cm} \end{gathered}$ | Total Salmonids |  |  |
|  |  |  |  | $\begin{gathered} 963(648,1296 \\ 97(0,254) \\ \hline \end{gathered}$ |  |
| c | 9 Jan-15 Mar 20 | $\geq 42 \mathrm{~cm}$ | Steelhead Hatchery S |  | Steelhead Natural |
|  |  |  | 2558 (1848,3 |  | $9(839,1848)$ |
| D | 16 Mar - 31 Mar 20 | $\geq 42 \mathrm{~cm}$ | Steelhead Hatchery S |  | Steelhead Natural |
|  |  |  | -1031 (-1443,-683) |  | -908 (-1274,-588) |

### 4.3.1.2 Study Year 2020-2021

CDFW field crews observed 159 Coho Salmon, 665 Chinook Salmon and 215 steelhead in the 4253 cm total length size class and 248 Coho Salmon, 658 Chinook Salmon and 80 steelhead $\geq 54$ cm during the species apportionment snorkel dives from 5-September 2020 through 4-December-2021 in temporal strata A (Figure 6). A total of 2,214 Steelhead Report Card catches were reported in strata C and D, of which 692 (28\%) were natural origin, and 1,522 (72\%) hatchery origin (Figure 6). A total of 2,442 salmonids greater than or equal to 42 cm in total length were estimated to have passed the SONAR camera moving upstream in strata A-C (Table 28). We estimate a total run size of steelhead greater than or equal to 42 cm in total length in strata C to be 740 fish composed of $37 \%$ natural origin (adipose intact) fish. We estimate 233 downstream moving steelhead in strata $D$, presumably post-spawn kelts, $13 \%$ of which were NOR. All strata estimates of net upstream passing salmonids are presented in Table 28.


Figure 6. Daily expanded net movement of salmonids greater than 54 cm for the 2020-2021 season derived from SONAR images (left primary Y axis) and Mad River discharge (right secondary Y axis) (Top Panel). Vertical lines separate the time series into temporal stratum A-D for used for estimates of total net movement. Daily snorkel counts of salmonid species greater than 53 cm (Middle panel). Bottom panel displays daily hatchery and wild steelhead catches reported on the CDFW Steelhead Report Card from completed trips.

Table 28. SONAR derived estimates of total net upstream passage of salmonids in temporal strata A, B, C, D of survey year 2020-21 at river mile 7 on the Mad River, Humboldt County, CA.

| Strata | Dates of estimation | Size Class | Stock |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 15 Sept - 4 Dec 20 | $\begin{aligned} & \geq 54 \mathrm{~cm} \\ & 42-53 \mathrm{~cm} \end{aligned}$ | Chinook | Coho |  | Steelhead |
|  |  |  | 932 (706,1179) | $100(36,171)$ |  | $26(0,67)$ |
|  |  |  | $778(522,1044)$ | $146(57,237)$ |  | $99(0,279)$ |
| B | 5 Dec - 8 Jan 21 | $\begin{gathered} \geq 54 \mathrm{~cm} \\ 42-53 \mathrm{~cm} \end{gathered}$ | Total Salmonids |  |  |  |
|  |  |  |  | 2 (3 | 84) |  |
|  |  |  |  | 30 (27 |  |  |
| C | 9 Jan - 15 Mar 21 | $\geq 42 \mathrm{~cm}$ | Steelhead Hatchery |  | Steelhead Natural |  |
|  |  |  | 454 (174,7 |  |  | $(100,465)$ |
| D | 16 Mar - 31 Mar 21 | $\geq 42 \mathrm{~cm}$ | Steelhead Hatchery |  | Steelhead Natural |  |
|  |  |  | -202 (-365, |  |  | (-117,51) |

### 4.3.1.3 Study Year 2021-2022

CDFW field crews observed 7 Coho Salmon, 1120 Chinook Salmon and 256 steelhead 42-53 cm and 19 Coho Salmon, 1368 Chinook Salmon and 201 steelhead $\geq 54 \mathrm{~cm}$ during the species apportionment snorkel dives from 5-September-2021 through 22-October-2022 in temporal strata A (Figure 7). A total of 1,359 Steelhead Report Card catches were reported in strata C and D, of which 363 (27\%) were natural origin, and 996 (73\%) hatchery origin (Figure 7). A total of 5,337 salmonids greater than or equal to 42 cm in total length were estimated to have passed the SONAR camera moving upstream in stratum A-C (Table 29). We estimate a total run size of steelhead greater than or equal to 42 cm in total length in strata $C$ to be 2,081 fish composed of $514(25 \%)$ natural origin (adipose intact) fish. We estimate 999 downstream moving steelhead in strata D, presumably post-spawn kelts, $31 \%$ of which were NOR. All stratum estimates of net upstream passing salmonids are presented in Table 29.


Figure 7. Top panel depicts daily expanded net movement of salmonids greater than 54 cm for the 2021-2022 season derived from SONAR images (left primary $Y$ axis) and Mad River discharge (right secondary Y axis). Vertical lines separate the time series into temporal stratum A-D for used for estimates of total net movement. Middle panel depicts daily snorkel counts of salmonid species greater than 54 cm . Bottom panel displays daily hatchery and wild steelhead catches $\geq 42 \mathrm{~cm}$ reported on the CDFW Steelhead Report Card from completed trips.

Table 29. SONAR derived estimates of total net upstream passage of salmonids in temporal strata A, B, C, D of survey year 2021-22 at river mile 7 on the Mad River, Humboldt County, CA.

| Strata | Dates of estimation | Size Class | Stock |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| A | 5 Sept - 22 Oct 21 | $\begin{gathered} \geq 54 \mathrm{~cm} \\ 42-53 \mathrm{~cm} \end{gathered}$ | Chinook | Coho | Steelhead |
|  |  |  | $\begin{aligned} & 550(334,787) \\ & 430(171,705) \end{aligned}$ | $\begin{gathered} 14(0,36) \\ 5(0,27) \end{gathered}$ | $\begin{gathered} 42(0,93) \\ 19(0,123) \end{gathered}$ |
|  | 22 Oct - 8 Jan 22 | $\begin{gathered} \geq 54 \mathrm{~cm} \\ 42-53 \mathrm{~cm} \end{gathered}$ | Total Salmonids |  |  |
| B |  |  | 2435 (2090,2787) |  |  |
| C | 9 Jan - 15 Mar 22 | $\geq 42 \mathrm{~cm}$ | Steelhead Hatchery |  | Steelhead Natural |
|  |  |  | 1567 (1190,1910) |  | $514(348,693)$ |
| D | 16 Mar - 31 Mar 22 | $\geq 42 \mathrm{~cm}$ | Steelhead Hatchery |  | Steelhead Natural |
|  |  |  | -690 (-1026,-384) |  | -309 (-493,-138) |

### 4.3.1.4 Study Year 2022-2023

CDFW field crews observed 37 Coho Salmon, 1102 Chinook Salmon and 101 steelhead 42-53 cm and 52 Coho Salmon, 1714 Chinook Salmon and 56 steelhead $\geq 54 \mathrm{~cm}$ total length during the species apportionment snorkel dives from 5-September-2022 through 30-November-2023 in temporal strata A (Figure 8). Steelhead Report Card data are not available until they are returned and collated and therefore estimates of assumed steelhead in stratum $C$ and $D$ cannot be apportioned to hatchery or natural origin at this time. A total of 4,012 salmonids greater than or equal to 42 cm in total length were estimated to have passed the SONAR camera moving upstream in stratum A-C (Table 30). We estimated total steelhead in strata C to be 1,297 fish, and 81 downstream moving steelhead in strata D, presumably post-spawn kelts. All stratum estimates of net upstream passing salmonids are presented in Table 30.


Figure 8. Top panel depicts daily expanded net movement of salmonids greater than 54 cm for the 2022-2023 season derived from SONAR images (left hand $Y$ axis) and Mad River discharge (right hand $Y$ axis). Vertical lines separate the time series into temporal stratum A-D for used for estimates of total net movement. Middle panel depicts daily snorkel counts of salmonid species greater than 54 cm . Steelhead Report Card data is not yet available for this year.

Table 30. SONAR derived estimates of total net upstream passage of salmonids in temporal strata A, B, C, D of survey year 2022-23 at river mile 7 on the Mad River, Humboldt County, CA.

| Strata | Dates of estimation | Size Class | Stock |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| A | 5 Sept-29 Nov 22 | $\begin{gathered} \geq 54 \mathrm{~cm} \\ 42-53 \mathrm{~cm} \end{gathered}$ | Chinook | Coho | Steelhead |
|  |  |  | $\begin{aligned} & 1132(602,1764) \\ & 725(361,1127) \\ & \hline \end{aligned}$ | $\begin{gathered} 34(0,132) \\ 0(0,100) \\ \hline \end{gathered}$ | $\begin{aligned} & 101(0,212) \\ & 138(0,324) \end{aligned}$ |
| B | 30 Nov - 8 Jan 23 | $\begin{gathered} \geq 54 \mathrm{~cm} \\ 42-53 \mathrm{~cm} \end{gathered}$ | Total Salmonids |  |  |
|  |  |  | $\begin{gathered} \hline 380(162,606) \\ 205(24,396) \end{gathered}$ |  |  |
| C | 9 Jan - 15 Mar 23 | $\geq 42 \mathrm{~cm}$ | Steelhead |  |  |
|  |  |  | 1297 (900,1692) |  |  |
| D | 16 Mar - 31 Mar 23 | $\geq 42 \mathrm{~cm}$ | Steelhead |  |  |
|  |  |  | -81 (-216,48) |  |  |

### 4.3.1.5 Study year 2023-2024

CDFW field crews observed 137 Coho Salmon, 1,784 Chinook Salmon and 181 steelhead $\geq 42$ cm in total length during the species apportionment snorkel dives from 7-September-2023 through 29-November-2023. The adipose clip status of 100 steelhead were observed during snorkel surveys, of which 86 were intact (assumed to be NOR) and 14 removed (HOR). Tangle nets captured 25 NOR and 26 HOR steelhead between 12-December-2023 and 4-March-2024. Snorkeling results, tangle net sampling results and the HOR/NOR steelhead catch summaries are presented in Appendix B, C and D, respectively. A total of 7,211 salmonids $\geq 42 \mathrm{~cm}$ in total length were estimated to have passed the SONAR camera moving upstream from 6-September2023 to 15-March-2024 (Figure 9, Table 31). Using the extended temporal coverage of species apportionment samples provided by the tangle net sampling, we estimate total escapement of anadromous salmonids for the 2023-24 season through 15-March-2024 to include 1,548 total Chinook Salmon between 42 and 53 cm total length, 2,737 Chinook Salmon $\geq 54 \mathrm{~cm}$ total length, 957 Coho Salmon, 940 HOR steelhead and 2,100 NOR steelhead (Table 31).

Although fish capture rates using tangle nets were fewer than observation rates when snorkeling by orders of magnitude, the assemblage of fish species observed were in line with general expectations based on time of year, with mostly Chinook Salmon in early November, more Coho Salmon in late November/early December, and only steelhead after 1-January-2024 (Plot C, Figure 9). Total catch per unit effort (CPUE) using tangle nets fell from 6 fish per day and 1.3 fish per hour in November to just 0.75 fish per day and 0.14 fish per hour across January (Table 32, Figure 10). When flows dropped back down in April, tangle net CPUE rebounded to 1.0 fish per hour when catches were predominately post-spawn steelhead kelts migrating back to sea.


Figure 9. Time series of data used to estimate the net number of upstream migrating salmonid species and hatchery and natural steelhead $\geq 42 \mathrm{~cm}$ total length in the Mad River 2023-2024 season. (A) Net daily passage of unknown salmonid fish species $\geq 42 \mathrm{~cm}$ total length imaged by SONAR (green bars left y axis) and average daily Mad River discharge (blue line, right y axis), (B) daily counts of salmonid species $\geq 42 \mathrm{~cm}$ fork length observed during snorkel surveys and, (C) tangle net captures of Coho and Chinook Salmon and steelhead in the Mad River below Blue Lake bridge used for species apportionment, (D) the number of hatchery and natural steelhead observed during both snorkeling and tangle net surveys used to apportion steelhead into hatchery or natural run components, and (E) resulting daily SONAR net upstream moving salmonids apportioned to both species and steelhead hatchery and natural run components.

Table 31. SONAR derived estimates with upper (UCI) and lower (LCI) confidence intervals of total net upstream passage of salmonids from 5 Sept 2023 to 15 March 2024 at river mile 7 on the Mad River, Humboldt County, CA.

| Stock | Sub-group | Size Range | Estimate | LCI | UCI |
| :---: | :---: | :---: | ---: | ---: | ---: |
| Chinook | Grilse | $42-53 \mathrm{~cm}$ | 1,548 | 1,206 | 1,911 |
|  | Adult | $\geq 54 \mathrm{~cm}$ | 2,736 | 2,163 | 3,333 |
|  | TOTAL |  | 4,284 | 3,369 | 5,244 |
| Coho |  | $\geq 42 \mathrm{~cm}$ | 957 | 526 | 1,479 |
| Salmon |  | $\geq 42 \mathrm{~cm}$ | 2,100 | 1,545 | 2,674 |
| Steelhead | Natural | Hatchery | $\geq 42 \mathrm{~cm}$ | 940 | 606 |

Table 32. Catch per unit effort using tangle nets per hour, day and month for each species throughout the 2023-24 monitoring season.

| Month | Chinook Salmon |  | Coho Salmon |  | Steelhead |  | Total Fish |  | Mean <br> Monthly CFS |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | per Day | per Hr | per Day | per Hr | per Day | per Hr | per Day | per Hr |  |
| Nov '23 | 1.9 | 0.41 | 4.1 | 0.91 | 0 | 0 | 6.0 | 1.31 | 208 |
| Dec '23 | 0.3 | 0.07 | 1.0 | 0.20 | 0.4 | 0.09 | 1.8 | 0.35 | 1,587 |
| Jan '24 | 0 | 0 | 0 | 0 | 0.8 | 0.14 | 0.8 | 0.14 | 5,798 |
| Feb '24 | 0 | 0 | 0 | 0 | 2.5 | 0.53 | 2.5 | 0.53 | 4,739 |
| Mar '24 | 0 | 0 | 0 | 0 | 0.5 | 0.13 | 0.5 | 0.13 | 5,040 |
| Apr '24* | 0 | 0 | 0 | 0 | 5.3 | 1.00 | 5.3 | 1.00 | 1,797 |

[^0]

Figure 10. Stacked bars presenting catch per unit effort using tangle nets per hour and month for adult salmonids throughout the 2023-24 Mad River monitoring season.

### 4.3.2 Steelhead Capture-Mark-Recapture estimates

We tangle netted and reward tagged 21 HOR steelhead between 11-December-2023 and 24-February-2024. Two reward tags were returned from angler harvested fish. Of the remaining 19 tagged fish, 11 returned to the hatchery, and a total of 719 individual untagged steelhead ascended the ladder at Mad River Hatchery. Using the capture-mark-recapture methods (opposed to the SONAR-derived estimates), we estimate 1,373 ( $\pm 1021-2423$ ) HOR and 1,766 $( \pm 1,085-3,833)$ NOR steelhead returned to the Mad River. Of the returning HORs, we estimate 123 ( $\pm 2-371$ ) animals were harvested by anglers (Figure 11). Our capture-mark-recapture estimate of pHOS is 0.38 ( $\pm 0.26-0.49$ ). Using adipose intact status and scale reading we assume pNOB to be 0.078 and estimate PNI to be 0.17 ( $\pm 0.14-0.23$ ). Figure 11 depicts the capture-mark-recapture (CMR) estimates of HOR and NOR steelhead, and harvest of HOR steelhead together with SONAR derived estimates of NOR and HOR steelhead for comparison.


Figure 11. Estimated abundance of HOR, NOR and harvested HOR steelhead generated with Capture-Mark-Recapture (CMR) and SONAR derived estimates of NOR and HOR steelhead in the Mad River, 2023-2024.

## 5 DISCUSSION

Since the publication of the HGMP for the Mad River Hatchery (CDFW 2016), the stated goal of the program has been to provide enhanced angling and harvest opportunities in the Mad River, while operating MRH as an integrated hatchery through continued inclusion of a minimum 50\% NOR breeders into hatchery stock. Achieving production goals has been successful, with annual hatchery smolt releases ranging from approximately 91,000 to 142,000 smolts and averaging 125,000 smolts per brood year since 2017. However, over the same period, achieving a consistent minimum $50 \%$ pNOB of MRH broodstock has seen variable results. The majority of NOR steelhead incorporated into pNOB were captured by volunteer anglers in brood years 2020 to 2022. Volunteer anglers were successful at supplying adipose fin-intact, presumably NOR steelhead, to MRH for use as broodstock. Once staff were experienced in identifying scale characteristics specific to natural- and hatchery-reared juveniles, pedigree reconstruction based on SNP genotypes indicate that real-time scale pattern analysis at the hatchery was effective at discerning mis-clipped (adipose fin-intact) fish from true NOR fish. This resulted in 30-50\% of the target breeders at MRH during these spawn years validated as NOR. Difficulties remained, however, at successfully ripening in-river captured NOR females in the hatchery holding tanks to spawning maturity. While a nearly $1: 1$ sex ratio of NOR steelhead were brought into the hatchery from 2020-2022, the sex ratio of NOB ranged from 2:1 to 3:1, male to female, with most females released back to the river unspawned after a period of holding due to undermaturity. Spawning seasons 2023 and 2024 saw a marked drop in pNOB at MRH. The high discharge and turbidity of the Mad River during the steelhead NOR collection and spawning period severely influenced the effort and fish caught by anglers in the volunteer program. The volunteer angler program was the only mechanism consistently used to capture fish for MRH broodstock during the 2020-2023 spawning years.

In response to the low steelhead pNOB in spawning year 2023, CDFW staff implemented a new in-river tangle netting project in the winter of 2023-24 as part of an adaptive management strategy. Tangle-nets have been used effectively on the Elwa River (Denton et al. 2021) and in mark-selective fisheries along the west coast to non-lethally capture adult anadromous fish for scientific means, and subsistence or commercial harvest. Tangle nets are much more versatile than traditional seines; they are lighter and thus more portable and easier to work with, they are less detectable by fish and thus less likely to be avoided, and they can be used in a wide variety of habitats and deployment strategies. They can be fished statically by deploying and anchoring the net across a fish passage corridor or fished dynamically by drifting with the river's current and/or with the assistance of boats through runs and pools. We implemented tangle netting on the Mad River with three main objectives: (1) capture NOR steelhead for use in MRH broodstock, (2) collect in-river species/stock composition information across the season for use in apportioning sonar counts to species, and (3) apply angler reward tags to HOR steelhead to estimate angler harvest and HOR/NOR steelhead abundance via capture-mark-recapture modeling.

Incorporating tangle nets into the program was partially successful at supplying NOR steelhead to MRH for use as broodstock. Thirty-two NOR steelhead were captured in the lower river, but only 8 ( 2 female, 6 male) steelhead were mature enough to be ripened and used for spawning. While the difficulties in maturation of steelhead brought into MRH for broodstock is not specific to the collection method, location and timing of capture may influence maturity to spawning. Consequently, when steelhead captured with tangle nets in the lower river appeared bright with hard gonads and ovaries, most were not delivered to MRH for potential spawning to reduce unnecessary impacts on NOR steelhead that would likely fail to mature in holding pens.

Tangle netting allowed species apportionment sampling throughout the winter, augmenting the early season snorkeling observations to provide estimates of total escapements by species and stock from the sonar data, vastly improving our ability to monitor stock abundances. The success tangle netting achieved did not, however, come without challenges. The 2023-24 water year (January, in particular) left poor conditions for fish sampling on the Mad River in any manner. While low flows from Sept-Nov provided good visibility for snorkel counts, extremely wet weather leading up to 13 -January-2024 brought Mad River discharge up to 53,900 cfs, registering as the 5th largest peak discharge on record since 1950. Discharge did not fall back into consistent good-quality sampling conditions (e.g., <2,000 cfs) until April 5. River conditions did, however, force a steep learning curve on the effective deployment of tangle nets in the Mad River. By the end of the season, staff gained valuable experience in both effective sampling site selection and gear deployment strategies at variable flows.

In study year 2023-24, we incorporated a full season of species-apportionment and clipapportionment (i.e., HOR/NOR apportionment for steelhead) data, and propagated the uncertainty in these proportions into our estimate of stock abundances derived from SONAR counts. While this accounting of uncertainty is useful in evaluating the precision of estimates, the relative bias of our estimates relies upon adherence to some critical assumptions. For our estimates to remain unbiased, our species and HOR/NOR apportionment auxiliary data (i.e., snorkel and tangle net sampling) must be a random sample of the true proportion passing the sonar site at RM 7.0. We used snorkel observations from multiple sites from the highway 101 bridge at RM 4.0 in the lower river to the Blue Lake bridge at RM 11.5 during the early portion of the season. Once turbidity in the river precluded snorkel observation, tangle net sampling focused near the sonar camera, but suitable sampling sites at variable flows similarly extended both upstream and downstream of the sonar site. Whether these proportions of species observed in the sampled segments of river represent a random sample of the proportions of species passing the sonar site at RM 7.0 is not known. However, adjustments were made to our sampling space if certain observations were expected to violate model assumptions. For example, adult Coho Salmon showed a tendency to hold in pools near the mouth of Lindsay Creek-Mad River's prominent Coho Salmon spawning tributary-which increased their relative observation rates in those areas compared to the remaining sampling space. To minimize bias in species apportionment towards Coho Salmon, we excluded observations between RM 7.9 and RM 8.5 before 1-December-2023 from our analysis (Lindsay Creek mouth at RM 8.48).

While we attempt to capture the uncertainty related to sample sizes of apportionment data in our bootstrapping procedure, we also out of necessity bin the apportionment data to the closest week of sonar counts without pre-determined sample size (i.e. any week of apportionment data with at least 1 fish is used to apportion sonar counts). This algorithm is sensitive to small sample sizes and completely misses the intended accounting of apportionment uncertainty, and undoubtedly introduces bias during weeks with samples sizes of one.

Tangle netting supplied a suitable method of capture for reward tagging of HOR steelhead necessary to estimate angler harvest and abundance when paired with MRH recovery and capture-mark-recapture models. The relatively simple two sample estimators we employ come with several restrictive assumptions that must be met for the estimates to remain unbiased. First, this model assumes that the populations is closed (geographically and demographically). Given we are focused on anadromous fish entering the river to spawn, and sampling was largely restricted to the lower 10 miles of river, we feel confident that the vast majority of fish were on an upstream migration, and it is unlikely that fish spawned in the lower river below our sampling sites.

Next and most importantly, we must assume that the probability of capture of hatchery origin and natural origin steelhead are the same and that every sample is random. Robson and Reiger (1964) concluded that for a two-period Lincoln-Petersen mark-recapture estimator to be unbiased, four times the total recovery sample size should be greater than the total population size. While we meet this criterion in this experiment, our estimate may be biased due to violation of the underlying assumptions of the model, potentially magnified by low recovery sample sizes. If certain subgroups of the population are more or less likely to be recaptured than others, the population estimate may be biased. In this study, most hatchery origin steelhead were captured in the later portion of the experiment during February, and recapture timing at the hatchery suggests that on average fish took three weeks to enter the hatchery from tagging in the lower river. With the Hatchery ladder closing on 19-March-2024, some marked individuals may not have had sufficient time to enter the hatchery, eliminating their chances of being recaptured. We used a non-parametric bootstrap approach to estimate the sampling error from our CMR data for several reasons, including (1) the bootstrap approach does not rely on the asymptotic normality assumption which is perhaps questionable in this case given the amount of available data, and (2) again, due to sample sizes, the estimates of variance using the delta method may lead to unreliable confidence intervals (King and McCrea 2019, Roff 1973). While we adopt these statistical methods to more appropriately estimate sampling error given small sample sizes, this approach does not overcome biases arising from heterogeneity in capture rates amongst individuals or groups in the sample.

Third, we assume capturing and marking does not affect catchability or otherwise affect fish behavior and that marks are not lost. Marking fish using spaghetti tags is a standard method that has been used widely for the study of anadromous fish for its reliability in tag retention and
lack of adverse effects to fish welfare (Runde et al. 2022). Spaghetti tags are relatively inconspicuous and should not be a visual que for predators (including fisherman) to target. Tangle net capture is relatively non-invasive and involves very little time handling and holding fish. Captured animals required little to no recovery time and appeared vigorous upon release. However, Any disruption in spawning migration may alter fish behavior. In at least one study evaluating the effects of capture and handling on run timing, it was found that capture by drifted gill nets of a similar size may have increased transit times by 12\%-24\% compared to unhandled fish (Sethi et al. 2018).

Lastly, the recording and reporting of tag numbers at the time of capture and at recapture events is assumed complete and accurate. Department staff had substantial training and data quality and control procedures to ensure that tag numbers are accurately recorded during all Department capture and recapture events. Spaghetti tags were given $\$ 20, \$ 50$, and $\$ 100$ cash reward values to assess tag return rates and incentivize anglers to report tags consistently and accurately. Tagging efforts were advertised broadly to the fishing community, and tag return made simple with a mobile app accessed via QR code.

We were able to estimate the parameters of the integrated steelhead breeding system in the 2023-24 season with CMR modeling and use full-season in-river sampling to extend our species apportionment sampling enough to estimate both multi-species and HOR/NOR steelhead stock abundances from SONAR monitoring. Estimates of steelhead abundances derived from the two approaches are reasonably consistent with one another. It should be noted, however, that the two approaches are not entirely independent. Both CMR and SONAR models rely heavily upon the NOR/HOR proportions derived from the same tangle net sample.

PNI cannot be estimated directly for study years 2019 to 2023 presented in this report. Deficiencies include insufficient auxiliary data to fully apportion SONAR counts to both species and steelhead origin, and unknown harvest rates of hatchery fish taken in the in-river fishery. Estimated harvest rates for the 2022-2023 and 2023-2024 seasons will become available as CDFW creel survey data for these years is analyzed and reported. Without these data, estimates of pHOS and therefore PNI cannot be reasonably made without conjecture. With the inclusion of tangle net sampling, reward tagging and recapture sampling at MRH in study year 2023-24, we were able to estimate pHOS to be 0.38 ( $\pm 0.23-0.49$ ). Using adipose intact status and scale reading validation we assume pNOB to be 0.078 and provide the first true estimate of PNI of the Mad River steelhead breeding system to be 0.17 ( $\pm 0.14-0.23$ ) for the 2024 spawning year.

Both the abundance of hatchery and natural steelhead returns to Mad River appear to be larger in 2023-24 than the previous 4 years reported here. During brood years 2019-2022, the proportion of HORs to NORs derived using Steelhead Report Card Catch data (estimated in strata C from Jan 9-March 15) range from 2:1 to 3:1 HOR dominant while those proportions are transposed to 2:1 in favor of NOR in study year 2023-24. This result is likely in part due to the use of steelhead Report Card catch data to apportion hatchery and natural components 20192022. When compared to on-the-ground creel censuses, these data have been found to be
biased toward hatchery catch reporting (Zuspan 2018). Incomplete species and hatchery/natural apportionment of SONAR counts for strata B in 2019-2022 clearly make direct comparisons of abundance difficult. Even with these caveats to the multi-year comparison, we believe run sizes in 2023-24 were above the prior two years, and further that the natural stock appears to have been a larger fraction of the total steelhead run size. Annual HOR adult returns to the hatchery have been mixed, averaging 1,410 adults and ranging from a high of 2,619 in 2020 to a low of 278 in 2023. Steelhead report card data are not yet available for 2023 and 2024 to compare annual returns to catch rates, however, reported catches of HOR steelhead from 2019 to 2022 generally track hatchery returns when fishing conditions are good (Figure 12). Reported HOR landings from 2019 to 2022 ranged from 578 to 1,205 fish caught. Poor weather and angling conditions aside, anglers on the Mad River have reported success catching and harvesting hatchery-raised steelhead.

Results of the genetic parentage analysis indicate that the identification of returning steelhead as NOR (and thereby estimates of pNOB) based upon adipose fin clip status were vastly improved upon by using scale verification methods, where scale patterns of each individual's juvenile life history were inspected in real-time before spawning. The reduction in the number of corrections made to fish origin assignments after 2021 is likely due to (1) higher quality fin clipping of steelhead as juveniles and/or (2) more accurate assessments of adult steelhead origin when retrieving fish from MRSS volunteers. For example, a fish with an intact adipose fin may still be identified as HOR based on subtle qualities of the adipose fin or the condition of its other fins. We hope to further test the use of scale analysis as a tool to identify true fish origin in the future by directly comparing results of individual scale analysis and genetic testing.


Figure 12. Total counts of first-pass HOR steelhead at the MRH fish ladder, reported HOR steelhead landings from steelhead report cards, and NOR steelhead brought to MRH for broodstock (with origin verified by scale inspection). Report card data from 2017, 2018, 2023 and 2024 were not available at the time of this reporting. *NOR origin assignments for steelhead brought to MRH in 2017-18 were not substantiated by scale pattern verification.

### 5.1 Recommendations

### 5.1.1 Strategies to Increase pNOB

### 5.1.1.1 Shift spawning at MRH Iater

Mad River Hatchery normally opens the fish ladder to accept upstream migrating steelhead beginning in January and closes the gates in mid-March. This time period and coincident spawn timing was established to mirror the in-river run timing of steelhead in the lower Mad River. This time period is not, however, consistent with spawn timing of NOR steelhead resulting in difficulties maturing and ripening NOR steelhead brought into the hatchery for spawning too early. Deibner-Hanson (2021) found similar passage timing of wild steelhead into lower Redwood Creek, just to the north of Mad River, while Ricker et al. (2014) and Anderson and Ward (2016) found peak redd deposition date on the spawning grounds a month later indicating lower river run timing does not represent the inherent spawn timing of natural stocks. NOR steelhead appear to require a longer period of in-river residency to mature to spawning condition than HOR fish, and the environmental cues that initiate maturation of NOR steelhead are not duplicated in the hatchery holding facilities. Operating the hatchery holding facilities longer and shifting spawning operations later may allow the in-river sampling to intercept more NOR fish in the river at later stages of maturation allowing integration into the broodstock.

It is understood that spawn timing is a heritable trait in steelhead (Abadía-Cardoso et al. 2013, Beulke et al. 2023) and simply shifting spawning operations to use later entry fish can inadvertently shift run timing. This recommendation is therefore not to shift spawning operations to later entry fish, but rather to hold off spawning fish until later to allow NOR fish to mature in-river before capture, allowing integration into the broodstock. This could be accomplished by tagging fish as they enter the hatchery allowing the tracking of entry date of individuals and matching the spawning date to reflect entry date (i.e. spawn animals consistent with their entry data later in the calendar). This will require holding fish longer at MRH potentially impacting the MRH capacity. Coupling this approach with activities to reduce pHOS (5.1.2.1) may mitigate overloading the hatchery holding facility.

### 5.1.1.2 Target NOR broodstock collection higher in the watershed

In-river sampling needs to balance two competing objectives. The first objective is related to the biologic monitoring for the HGMP. Both species apportionment for the SONAR-based abundance and tagging for the CMR-based abundance estimates require sampling in the lower river. However, steelhead sampled in the lower river are not mature enough to successfully ripen to spawning condition in the holding facilities in the hatchery. We therefore recommend balancing the in-river sampling program to incorporate more effort sampling higher in the watershed later in the run to capture steelhead that are in later stages of maturation and higher likelihood of integration into the broodstock. This recommendation coupled with shifting spawning operations later (see 5.1.1.1) have the potential to increase pNOB at MRH.

### 5.1.1.3 Evaluate the Development of broodstock from juveniles collected from natural areas

Supplementation programs designed to raise individuals in captivity for later release are a tool typically used by hatcheries to reach conservation goals and are largely considered for only imperiled stocks (Van Doornik 2010). Here we present the concept of collecting juvenile Oncorhynchus mykiss from natural areas and rearing these fish to maturity in the hatchery for use as broodstock, thereby allowing control of the number of NOR spawners at MRH. Naturalorigin juvenile $O$. mykiss are abundant and ubiquitous occurring in both the mainstem and most tributaries of the Mad River. Collection of animals at age 0 during summer months would not be difficult compared to the collection of adults in winter. The location of the collections could be controlled to both represent multiple areas of the watershed and avoid areas known to be highly impacted by hatchery strays. O. mykiss juveniles could be transported to MRH and reared in segregated ponds for 2-3 years until maturity, then paired with anadromous steelhead (either NOR or HOR) collected as adult returns. This would ensure a minimum of $50 \% \mathrm{pNOB}$ and, if paired with anadromous NOR adults, may approach $100 \%$ pNOB, thereby supporting both recreational fishing and conservation objectives.

This approach was used in Abernathy Creek, Washington, where juveniles collected from natural areas were successfully reared and used as broodstock for both conservation and production objectives (Bingham et al. 2014). In this study the single juvenile collection well-represented the genetic makeup of the wild population but diverged rapidly within two generations. Here we propose continued juvenile collections each year with each collection used only once as breeders to avoid domestication effects and ensure the avoidance of potential inbreeding (Bingham, pers. comm. 2024; Waples and Do 1994).

Careful consideration of potential unintended genetic consequences should be made prior to adopting such a novel but enormously beneficial approach to broodstock management. For example, since anadromy has been shown to be a female sex-linked trait (Ohms et al. 2014, Kendall et al. 2015), female parents selected for breeding should be sourced from anadromous returns (i.e., not residents) to best produce anadromous stocks. In this case, drawing male (instead of female) parents from the pool of juvenile-derived breeders may limit the potential selection for resident O. mykiss phenotypes (Ohms et al. 2014, Kendall et al. 2015). Careful consideration should also be made regarding where in the watershed juveniles are collected to strategically represent the genetic diversity of the population by representing the spatial diversity of a large watershed (Fong 2020). Genetic monitoring of allele frequencies should continue throughout the program to ensure risks are mitigated, goals are achieved, and the program can be adaptively managed. Cost benefit analysis of this approach will also be a consideration as this option would entail additions to MRH operation budgets that currently do not exist.

### 5.1.1.4 Evaluate exogeneous gonadotropin treatment of NOR steelhead

Many NOR steelhead captured in-river and transported to MRH for use as broodstock do not mature in the holding facilities and must be released unspawned. Reproductive dysfunction in fish held in captivity is a common phenomenon and is usually the result of deficiencies in environmental stimuli failing to trigger hormones necessary for maturation. We recommend the evaluation of exogenous compounds to expedite final maturation of oocytes. In natural spawning, nerve cells in the brain delivers sGnRH to the pituitary. The sGnRH serves to free gonadotropins from pituitary cells. The gonadotropins then direct maturation of the gonads through gonadal steroid hormones. The introduction of exogeneous gonadotropins has been shown to significantly reduce maturation times and predictably expedite maturation. This approach should be done within an experimental framework to ensure gonadotropin-releasing hormone or analogs thereof (e.g. GnRH) do not reduce egg weight, eyeing rate, and hatching rate (Svinger 2013).

### 5.1.2 Strategies to Reduce pHOS <br> 5.1.2.1 HOR Egg evacuation, euthenization and kelt reconditioning

MRH currently releases HOR fish not used as broodstock back to the river to increase angling opportunities. Consequently, these HOR animals are 'forced' to spawn in the natural setting to complete their life cycle and contribute significantly to pHOS, driving down PNI of the breeding system. We estimate well over $50 \%$ of HOR fish returning to the Mad River enter MRH at least once and there were more HOR ascending the ladder than were necessary to reach egg collection goals in BY 2024. We recommend evacuating HOR females' eggs before returning fish to the river, as well as euthanizing or holding HOR males until after most of the natural area spawning has occurred. This recommendation is intended to balance the need to avoid selection against iteroparity (i.e., rather than complete euthanasia of all HOR not used for broodstock), enhancing angling opportunity and reduction of pHOS.

### 5.1.2.2 Initiate and engage in watershed restoration

The driving concept of the Mad River Hatchery HGMP is that hatchery operations and natural origin stocks do not and should not function in isolation (i.e., integration and conservation, not segregation). Watershed processes operate over multiple time and spatial scales. Habitat decline and climate change are the key threats for most natural populations in California. Links between climate and landscape processes, land use and habitat conditions, and the biologic response to these functions are the conceptual framework within which sound management and recovery actions are based. Therefore, we must include hatchery management, natural habitats, salmonids and their food webs together in a cohesive manner. We recommend that CDFW focus on engagement with federal and non-governmental organizations and the public to not only plan and implement recovery strategies in the Mad River watershed, but also recognize and adjust MRH's role in population recovery. This recommendation is listed in this section because increased abundance and resilience of NOR steelhead directly affect pHOS, but a
resilient steelhead population functioning in balance with a recreational fishery that invigorates future generations of a conservation minded public has wide ranging benefits to both salmonids and society.

### 5.1.3 Balance socio-economic benefits and HGMP performance criteria

5.1.3.1 Evaluate targets outlined in current HGMP

CDFW management of MRH steelhead broodstock attempts to meet targets for the integration of natural origin steelhead into the broodstock at a minimum of $50 \%$. This report summarizes the adaptive management process whereby new methods are implemented, and results evaluated with research and monitoring. This is an ongoing process that has achieved some successes and will continually face challenges. (1) The implementation of the MRSS volunteer angler program to collect NOR steelhead from the river and (2) the initiation of real-time scale analysis and genomic assays to validate natural origin steelhead for use as broodstock are examples of this learning process. While the high-water, high-turbidity seasons of 2023 and 2024 hampered MRSS angling that was effective in collecting NOB in the prior 3 seasons, the implementation of novel in-river sampling techniques shows promise to augment angler efforts moving forward. This iterative process of informed trial and error is the engine of the scientific process. As management of MRH moves forward within the adaptive management framework, a level of flexibility is necessary to accommodate both success and failure. Therefore, we recommend $100 \%$ pNOB be a target for MRH broodstock integration, but the floor of 50\% pNOB include flexibility such that MRH can still function in its goals to enhance recreational angling while the approaches to managing broodstock composition for conservation goals are evaluated and improved. From an economy of scale perspective, MRH cannot operate as a facility if juvenile production is highly unpredictable and unstable, being governed strictly by $50 \%$ pNOB criteria. Simply put, CDFW is invested in both conservation objectives and supporting the socioeconomic benefits that sport angling provides for the community and future generations. We recommend reviewing and renewing the MRH HGMP to include either the floor pNOB to be adjusted based upon real time monitoring of NOR steelhead (i.e., adult return-based targets) and/or placing an appropriate floor on juvenile production that allows MRH to operate through years of difficult NOR collection. We must consider a longer-term future for conservation efforts of salmon and steelhead not only within Mad River but across coastal California. If MRH is closed due to inability to meet HGMP targets, the likelihood of operating it again in any capacity is severely diminished, threatening our collective objectives and loss of a potentially critical infrastructure needed for species conservation at a time when we face predicted climateinduced changes across California.

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## 7 APPENDICES

## Appendix A. Annual MRH HGMP reporting requirements.

(From MR HGMP, Appendix 6, page 138-9)

1) Hatchery Name
a) production period for report - mm/dd/yr through mm/dd/yr.
b) hatchery production goals and objectives
c) miscellaneous data/information
2) Hatchery Trapping Operations
A. Hatchery Trapping
a) date, location
b) the number of Chinook and coho salmon trapped and released
c) the number of mortalities, by species, sex, mark and cause of take
d) the number and disposal method for incidental mortalities
B. Alternative procurement methods
a) the number of Chinook and coho salmon trapped and released
b) the number of mortalities, by species, sex, mark and cause of take
c) the number and disposal method for incidental mortalities
3) Production Summary
a) the number of steelhead spawned
b) the number of steelhead eggs taken
c) the number and sex of natural spawners used for broodstock
d) the number of grilse spawned for broodstock
4) Hatchery Operations
A. Spawning
a) the number of broodstock
b) description of spawning protocol i.e 1:1, split egg-lots
c) fecundity
d) fertility
e) green to eyed-egg survival
f) report the number of tissue samples taken and their disposition
B. Incubation
a) survival by family lot from fertilized egg to fingerling
b) rate of integrated production (wild $x$ hatchery prior to ponding)
C. Rearing
a) pond survival rates from fingerling to smolt
b) feed rate, growth rate
c) rearing density
d) phenotype quality control
e) alternative or natural rearing conditions
D. Disease
a) disease outbreaks, number of fish affected, mortality
b) treatment
c) prophylactic treatments
E. Marking
a) the number of steelhead
b) report the percent marked/unmarked steelhead
F. Release
a) date(s) of release
b) type of release (volitional, forced)
c) river flow during release
G. Facilities Management
a) water temperature
b) type of release (volitional, forced)
c) river flow during release
d) predator losses
e) NPDES report(s)
H. Public Relations Facilities Management
a) water temperature
b) list visitors, tours, educational opportunities

I Scientific Studies
a) experimental rearing methods
b) river and hatchery steelhead studies
5) Restoration
a) summary of all-in-river habitat
6) Steelhead Report Card Results for Mad Restoration
a) estimated number of natural and steelhead caught
b) estimated number of natural and steelhead released
c) comparison of angler catch (1+2) pre and post production goal change
d) estimate trend in hatchery/natural ratio
e) estimate trend in MRH strays to adjacent basin within NC DPS
f) evaluate changes in angling regulations for trend in (1-5)

Appendix B. Species apportionment snorkel survey counts of total species observed by date and reach on the Mad River during the 2023-24 season.

| Date | Survey Reach | Chinook Salmon |  | Coho Salmon | Steelhead |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Adult | Grilse |  | Adult | Half-pounder |
| 7-Sep-23 | 4 | 2 | 1 |  | 1 | 2 |
| 12-Sep-23 | 4 | 3 | 4 |  | 1 | 5 |
| 15-Sep-23 | 3 | 8 | 7 |  | 25 | 174 |
| 19-Sep-23 | 4 | 2 | 2 |  |  | 2 |
| 21-Sep-23 | 3 | 16 | 20 | 1 | 13 | 95 |
| 22-Sep-23 | 5 | 13 | 36 |  | 5 | 27 |
| 27-Sep-23 | 4 | 9 | 3 |  |  | 9 |
| 28-Sep-23 | 3 | 70 | 41 | 2 | 5 | 102 |
| 28-Sep-23 | 5 | 33 | 41 | 1 | 7 | 23 |
| 3-Oct-23 | 4 | 13 | 11 |  | 3 | 26 |
| 6-Oct-23 | 3 | 111 | 73 | 7 | 21 | 224 |
| 6-Oct-23 | 5 | 14 | 30 |  | 2 | 10 |
| 9-Oct-23 | 4 | 1 | 6 |  | 1 | 8 |
| 13-Oct-23 | 3 | 148 | 67 | 4 | 2 | 240 |
| 13-Oct-23 | 5 | 36 | 62 |  | 4 | 22 |
| 16-Oct-23 | 4 | 6 | 12 |  | 1 | 6 |
| 18-Oct-23 | 5 | 47 | 28 |  | 3 | 25 |
| 24-Oct-23 | 4 | 6 | 20 |  | 1 | 9 |
| 27-Oct-23 | 3 | 84 | 94 | 22 | 14 | 115 |
| 27-Oct-23 | 5 | 40 | 82 |  | 7 | 24 |
| 1-Nov-23 | 3 | 190 | 142 | 29 | 34 | 194 |
| 1-Nov-23 | 4 | 24 | 82 | 1 | 2 | 15 |
| 13-Nov-23 | 3 | 7 |  | 1 | 3 | 40 |
| 13-Nov-23 | 4 | 1 | 3 | 43 | 6 | 63 |
| 13-Nov-23 | 5 | 87 | 15 | 19 | 9 | 50 |
| 17-Nov-23 | 3 | 1 |  |  | 5 | 5 |
| 17-Nov-23 | 4 |  |  |  |  | 1 |
| 28-Nov-23 | 3 |  |  | 40 | 5 | 45 |
| 28-Nov-23 | 5 | 42 | 15 | 19 | 2 | 25 |
| 29-Nov-23 | 4 | 2 |  | 59 | 3 | 21 |

Appendix C. Species captured by tangle net date and reach on the Mad River during the 2023-24 season.

| Date | Effort Type | Hours | Sample Units | Total Sets | CH | CO | $\begin{gathered} \text { SH } \\ \text { (NOR) } \end{gathered}$ | $\begin{gathered} \mathrm{SH} \\ (\mathrm{HOR}) \end{gathered}$ | $\begin{aligned} & \text { SH } 1 / 2 \mathrm{lb} \\ & (<41 \mathrm{~cm}) \end{aligned}$ | Total Adults |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 6-Nov-23 | Tangle Drift | 4 | 1 | 3 | 3 |  |  |  |  | 3 |
| 7-Nov-23 | Tangle Drift | 3 | 1 | 2 | 1 |  |  |  |  | 1 |
| 8-Nov-23 | Tangle Drift | 3 | 1 | 3 |  | 1 |  |  |  | 1 |
| 9-Nov-23 | Tangle Drift | 7 | 4 | 9 | 6 | 6 |  |  |  | 12 |
| 20-Nov-23 | Tangle Drift | 3 | 1 | 2 |  | 4 |  |  |  | 4 |
| 21-Nov-23 | Tangle Drift | 7 | 4 | 6 |  | 16 |  |  | 1 | 16 |
| 22-Nov-23 | Tangle Drift | 5 | 2 | 8 | 3 | 2 |  |  |  | 5 |
| 5-Dec-23 | Tangle Drift | 7 | 4 | 8 | 2 | 1 |  |  |  | 3 |
| 8-Dec-23 | Tangle Drift | 3 | 1 | 3 | 1 |  |  |  |  | 1 |
| 11-Dec-23 | Tangle Drift | 3 | 1 | 3 |  | 1 |  |  | 1 | 1 |
| 12-Dec-23 | Tangle Drift | 8 | 6 | 9 |  | 1 | 1 | 1 |  | 3 |
| 18-Dec-23 | Tangle Drift | 3 | 1 | 3 |  | 1 |  |  |  | 1 |
| 22-Dec-23 | Tangle Drift | 7 | 7 | 11 |  |  |  | 1 |  | 1 |
| 23-Dec-23 | Tangle Drift | 4 | 2 | 4 |  |  |  | 1 |  | 1 |
| 26-Dec-23 | Tangle Drift | 7 | 7 | 14 |  | 4 |  |  | 3 | 4 |
| 28-Dec-23 | Tangle Drift | 4 | 5 | 10 |  | 1 |  |  | 1 | 1 |
| 2-Jan-24 | Tangle Drift | 6 | 6 | 7 |  |  |  |  |  | 0 |
| 4-Jan-24 | Tangle Drift | 5 | 3 | 7 |  |  |  |  | 1 | 0 |
| 5-Jan-24 | Tangle Drift | 6 | 4 | 9 |  |  |  | 1 |  | 1 |
| 8-Jan-24 | Tangle Drift | 6 | 4 | 11 |  |  |  | 2 | 2 | 2 |
| 19-Jan-24 | Tangle Drift | 4 | 4 | 5 |  |  |  |  |  | 0 |
| 25-Jan-24 | Tangle Drift | 3 | 1 | 11 |  |  |  |  |  | 0 |
| 29-Jan-24 | Tangle Drift | 6 | 3 | 10 |  |  | 1 |  |  | 1 |
| 29-Jan-24 | Tangle Fixed | 2 | 1 | 1 |  |  | 1 |  |  | 1 |
| 30-Jan-24 | Tangle Drift | 6 | 4 | 11 |  |  | 1 |  |  | 1 |
| 7-Feb-24 | Tangle Drift | 4 | 2 | 4 |  |  |  | 1 |  | 1 |
| 8-Feb-24 | Tangle Drift | 4 | 1 | 12 |  |  |  |  |  | 0 |
| 8-Feb-24 | Tangle Fixed | 5 | 1 | 1 |  |  | 5 | 1 |  | 6 |
| 9-Feb-24 | Tangle Fixed | 5 | 2 | 2 |  |  | 1 | 1 |  | 2 |
| 9-Feb-24 | Tangle Fixed | 4 | 1 | 1 |  |  | 2 | 2 |  | 4 |
| 10-Feb-24 | Tangle Fixed | 5 | 1 | 1 |  |  | 3 | 4 |  | 7 |
| 11-Feb-24 | Tangle Fixed | 3 | 1 | 1 |  |  |  | 1 |  | 1 |
| 12-Feb-24 | Tangle Fixed | 5 | 1 | 1 |  |  |  |  |  | 0 |
| 13-Feb-24 | Tangle Net | 6 | 4 | 13 |  |  | 2 | 3 |  | 5 |
| 16-Feb-24 | Tangle Fixed | 2 | 2 | 2 |  |  |  |  |  | 0 |
| 16-Feb-24 | Tangle Fixed | 4 | 1 | 1 |  |  |  |  |  | 0 |


| Date | Effort Type | Hours | Sample Units | Total Sets | CH | CO | $\begin{gathered} \mathrm{SH} \\ (\mathrm{NOR}) \end{gathered}$ | $\begin{gathered} \mathrm{SH} \\ (\mathrm{HOR}) \end{gathered}$ | $\begin{aligned} & \text { SH } 1 / 2 \mathrm{lb} \\ & (<41 \mathrm{~cm}) \end{aligned}$ | Total Adults |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 22-Feb-24 | Tangle Fixed | 4 | 1 | 1 |  |  | 1 | 1 |  | 2 |
| 23-Feb-24 | Tangle Fixed | 5 | 1 | 1 |  |  | 1 | 2 |  | 3 |
| 26-Feb-24 | Tangle Drift | 4 | 3 | 8 |  |  | 1 |  |  | 1 |
| 27-Feb-24 | Tangle Drift | 5 | 3 | 10 |  |  | 2 | 1 |  | 3 |
| 28-Feb-24 | Tangle Drift | 6 | 6 | 9 |  |  | 2 |  | 1 | 2 |
| 29-Feb-24 | Tangle Drift | 4 | 3 | 8 |  |  | 2 | 1 |  | 3 |
| 4-Mar-24 | Tangle Fixed | 4 | 1 | 1 |  |  |  | 1 |  | 1 |
| 14-Mar-24 | Tangle Drift | 2 | 1 | 2 |  |  |  |  |  | 0 |
| 14-Mar-24 | Tangle Fixed | 1 | 1 | 1 |  |  |  |  |  | 0 |
| 18-Mar-24 | Tangle Drift | 5 | 5 | 7 |  |  |  |  |  | 0 |
| 27-Mar-24 | Tangle Fixed | 3 | 1 | 1 |  |  |  | 1* |  | 1 |
| 3-Apr-24 | Tangle Drift | 4 | 3 | 6 |  |  | 1* |  |  | 1 |
| 9-Apr-24 | Tangle Drift | 6 | 9 | 18 |  |  | 5* | 3* |  | 8 |
| TOTAL |  | 222 | 133 | 282 | 16 | 38 | 32 | 29 | 10 | 115 |

*Most steelhead captured after 14-Mar-24 were identified as post-spawn, downstream moving kelts.

Appendix D. Total hatchery-origin (adipose fin absent) and natural-origin (adipose fin present) steelhead $\geq 42 \mathrm{~cm}$ total length observed during snorkel surveys and tangle netting by Julian week for the 2023-24 monitoring season.

| Year | Julian Week | Hatchery | Natural |
| :---: | :---: | ---: | ---: |
| 2023 | 36 | 0 | 3 |
| 2023 | 37 | 0 | 15 |
| 2023 | 38 | 3 | 9 |
| 2023 | 39 | 1 | 9 |
| 2023 | 40 | 1 | 7 |
| 2023 | 41 | 0 | 5 |
| 2023 | 42 | 0 | 2 |
| 2023 | 43 | 0 | 9 |
| 2023 | 44 | 1 | 10 |
| 2023 | 46 | 6 | 12 |
| 2023 | 48 | 2 | 5 |
| 2023 | 50 | 1 | 1 |
| 2023 | 51 | 2 | 0 |
| 2024 | 1 | 1 | 0 |
| 2024 | 2 | 2 | 0 |
| 2024 | 5 | 0 | 3 |
| 2024 | 6 | 10 | 11 |
| 2024 | 7 | 3 | 2 |
| 2024 | 8 | 3 | 2 |
| 2024 | 9 | 2 | 7 |
| 2024 | 10 | 1 | 0 |
| Total |  | 39 | 112 |

Appendix E. Number of fish $\geq 42 \mathrm{~cm}$ total length by species and Julian week observed during snorkel surveys and tangle netting throughout the 2023-24 monitoring season.

| Year | Julian Week | Chinook | Coho | Steelhead |
| :---: | :---: | :---: | :---: | :---: |
| 2023 | 36 | 8 | 0 | 4 |
| 2023 | 37 | 20 | 0 | 26 |
| 2023 | 38 | 87 | 1 | 18 |
| 2023 | 39 | 197 | 3 | 12 |
| 2023 | 40 | 251 | 7 | 25 |
| 2023 | 41 | 318 | 4 | 7 |
| 2023 | 42 | 76 | 0 | 3 |
| 2023 | 43 | 305 | 19 | 22 |
| 2023 | 44 | 353 | 21 | 35 |
| 2023 | 45 | 7 | 1 | 0 |
| 2023 | 46 | 111 | 21 | 21 |
| 2023 | 47 | 3 | 8 | 0 |
| 2023 | 48 | 58 | 61 | 8 |
| 2023 | 49 | 3 | 1 | 0 |
| 2023 | 50 | 0 | 2 | 2 |
| 2023 | 51 | 0 | 1 | 2 |
| 2023 | 52 | 0 | 1 | 0 |
| 2024 | 1 | 0 | 0 | 1 |
| 2024 | 2 | 0 | 0 | 2 |
| 2024 | 5 | 0 | 0 | 3 |
| 2024 | 6 | 0 | 0 | 20 |
| 2024 | 7 | 0 | 0 | 5 |
| 2024 | 8 | 0 | 0 | 5 |
| 2024 | 9 | 0 | 0 | 9 |
| 2024 | 10 | 0 | 0 | 1 |
| Total |  | 1797 | 151 | 231 |

Appendix F. Weekly comparison of HOR broodstock, NOR broodstock, and the proportion of NOR steelhead used as broodstock at Mad River Hatchery 2013-2022 by visual inspection of adipose fin and scale sample pattern (HOR ${ }_{v}$, NOR ${ }_{v}$, $\mathrm{pNOB}_{v}$ ), and from genetic pedigree reconstruction (HOR $\left.{ }_{\mathrm{g}}, \mathrm{NOR}_{\mathrm{g}}, \mathrm{pNOB}_{\mathrm{g}}\right)$.

| Spawn Date | Total Broodstock | $\mathrm{HOR}_{v}$ | NOR ${ }_{v}$ | $\mathrm{pNOB}_{v}$ | HORg | NORg | $\mathrm{pNOB}_{\text {g }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1/6/2018 | 4 | 2 | 2 | 0.500 | 2 | 2 | 0.500 |
| 1/13/2018 | 14 | 7 | 7 | 0.500 | 10 | 4 | 0.286 |
| 1/20/2018 | 8 | 5 | 3 | 0.375 | 8 | 0 | 0.000 |
| 1/27/2018 | 10 | 5 | 5 | 0.500 | 8 | 2 | 0.200 |
| 2/3/2018 | 4 | 2 | 2 | 0.500 | 3 | 1 | 0.250 |
| 2/10/2018 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 2/17/2018 | 8 | 4 | 4 | 0.500 | 5 | 3 | 0.375 |
| 2/24/2018 | 18 | 9 | 9 | 0.500 | 9 | 9 | 0.500 |
| 3/3/2018 | 4 | 2 | 2 | 0.500 | 3 | 1 | 0.250 |
| 3/10/2018 | 8 | 4 | 4 | 0.500 | 7 | 1 | 0.125 |
| 3/17/2018 | 2 | 1 | 1 | 0.500 | 1 | 1 | 0.500 |
| 3/24/2018 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 2018 Total | 80 | 41 | 39 | 0.488 | 56 | 24 | 0.300 |
| 1/2/2019 | 2 | 1 | 1 | 0.500 | 2 | 0 | 0.000 |
| 1/8/2019 | 9 | 5 | 4 | 0.444 | 6 | 3 | 0.333 |
| 1/15/2019 | 14 | 7 | 7 | 0.500 | 12 | 2 | 0.143 |
| 1/22/2019 | 6 | 3 | 3 | 0.500 | 6 | 0 | 0.000 |
| 1/29/2019 | 5 | 3 | 2 | 0.400 | 4 | 1 | 0.200 |
| 2/5/2019 | 13 | 7 | 6 | 0.462 | 8 | 5 | 0.385 |
| 2/12/2019 | 9 | 5 | 4 | 0.444 | 6 | 3 | 0.333 |
| 2/19/2019 | 7 | 4 | 3 | 0.429 | 5 | 2 | 0.286 |
| 2/26/2019 | 5 | 3 | 2 | 0.400 | 4 | 1 | 0.200 |
| 3/5/2019 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 3/12/2019 | 2 | 1 | 1 | 0.500 | 1 | 1 | 0.500 |
| 3/19/2019 | 3 | 2 | 1 | 0.333 | 2 | 1 | 0.333 |
| 2019 Total | 75 | 41 | 34 | 0.453 | 56 | 19 | 0.253 |
| 1/7/2020 | 2 | 1 | 1 | 0.500 | 1 | 1 | 0.500 |
| 1/14/2020 | 5 | 3 | 2 | 0.400 | 3 | 2 | 0.400 |
| 1/21/2020 | 4 | 2 | 2 | 0.500 | 2 | 2 | 0.500 |
| 1/28/2020 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 2/4/2020 | 5 | 3 | 2 | 0.400 | 3 | 2 | 0.400 |
| 2/11/2020 | 17 | 8 | 9 | 0.529 | 9 | 8 | 0.471 |
| 2/18/2020 | 29 | 15 | 14 | 0.483 | 16 | 13 | 0.448 |
| 2/25/2020 | 25 | 13 | 12 | 0.480 | 14 | 11 | 0.440 |
| 3/3/2020 | 12 | 6 | 6 | 0.500 | 6 | 6 | 0.500 |


| Spawn Date | Total Broodstock | HOR ${ }_{v}$ | NOR ${ }_{v}$ | pNOB ${ }^{\text {v }}$ | $\mathrm{HOR}_{\mathrm{g}}$ | NORg | $\mathrm{pNOB}_{\mathrm{g}}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 3/10/2020 | 21 | 9 | 12 | 0.571 | 9 | 12 | 0.571 |
| 3/17/2020 | 9 | 5 | 4 | 0.444 | 5 | 4 | 0.444 |
| 3/24/2020 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 2020 Total | 129 | 65 | 64 | 0.496 | 68 | 61 | 0.473 |
| 1/5/2021 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 1/12/2021 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 1/19/2021 | 6 | 3 | 3 | 0.500 | 3 | 3 | 0.500 |
| 1/26/2021 | 2 | 1 | 1 | 0.500 | 1 | 1 | 0.500 |
| 2/2/2021 | 6 | 3 | 3 | 0.500 | 5 | 1 | 0.167 |
| 2/9/2021 | 6 | 3 | 3 | 0.500 | 5 | 1 | 0.167 |
| 2/16/2021 | 9 | 5 | 4 | 0.444 | 5 | 4 | 0.444 |
| 2/23/2021 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 3/2/2021 | 8 | 4 | 4 | 0.500 | 4 | 4 | 0.500 |
| 3/9/2021 | 12 | 6 | 6 | 0.500 | 7 | 5 | 0.417 |
| 3/16/2021 | 2 | 1 | 1 | 0.500 | 1 | 1 | 0.500 |
| 3/23/2021 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 2021 Total | 51 | 26 | 25 | 0.490 | 31 | 20 | 0.392 |
| 1/4/2022 | 4 | 2 | 2 | 0.500 | 2 | 2 | 0.500 |
| 1/11/2022 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 1/18/2022 | 8 | 4 | 4 | 0.500 | 4 | 4 | 0.500 |
| 1/25/2022 | 8 | 4 | 4 | 0.500 | 4 | 4 | 0.500 |
| 2/1/2022 | 8 | 4 | 4 | 0.500 | 4 | 4 | 0.500 |
| 2/8/2022 | 26 | 13 | 13 | 0.500 | 13 | 13 | 0.500 |
| 2/15/2022 | 14 | 7 | 7 | 0.500 | 7 | 7 | 0.500 |
| 2/22/2022 | 8 | 4 | 4 | 0.500 | 4 | 4 | 0.500 |
| 3/1/2022 | 12 | 6 | 6 | 0.500 | 6 | 6 | 0.500 |
| 3/8/2022 | 11 | 4 | 7 | 0.636 | 5 | 6 | 0.545 |
| 3/15/2022 | 4 | 2 | 2 | 0.500 | 2 | 2 | 0.500 |
| 3/22/2022 | 0 | 0 | 0 | -- | 0 | 0 | -- |
| 2022 Total | 103 | 50 | 53 | 0.515 | 51 | 52 | 0.505 |


[^0]:    *April sampling and mean CFS covers the period from April 1 - April 15

