**Summary**

This document summarizes some analyses conducted using the 2007-2020 South Fork McKenzie River genetic data that are not included in the 2022 report. These are mostly incomplete and still need some discussion and follow up work.

1. **Mate Pair TLF**
2. **Mate Pair Frequencies**
3. **F1 vs F2 vs NOR Immigrant Fitness**
4. **Genetic Diversity**

**Mate Pair TLF**

*Question*

Is there evidence that spawning between NORs and HORs reduces NOR fitness? To address this question, we’ll compare the number of returning adults (fitness) that are produced by NORxNOR, NORxHOR, HORxNOR and HORxHOR (female x male) mate pairs. Lower fitness of NORxHOR or HORxNOR pairs than NORxNOR suggests a deleterious effect of HORs on NOR productivity above the dam.

*Data / Methods*

We only consider the years where both NOR and HOR salmon are released above the dam (i.e. 2010 and later), and only the years where at least age 5 offspring have returned (2015 and earlier). We also only examine the small subset of the pedigree where both parents are known. This excludes the majority of parents where TLF = 0 (80% of parents) and the small number of single parent assignments. We will attempt to solve this problem in a different analysis below (Mate Pair Frequency).

For now, we use a “simple” negative-binomial GLMM with *cross type* (NORxNOR, NORxHOR, HORxNOR and HORxHOR) as the sole explanatory variable and year as a random effect. Something to think about in the future is how to integrate other variables that we know are predictive of TLF at the individual level (e.g. release date) into a model for mate pairs.

Significance of the effect of *cross type* as a predictor of fitness for mate pairs is evaluated using delta AIC, and a likelihood ratio test against a null model that only includes the random effect of year.

Effects are summarized using the estimated marginal means for each level of the predictor.

*Results*

Including cross type significantly improved the model fit to the data compared to a null model (delta AIC = ~4, LRT p-value = 0.021). There are three big takeaways from table 1 and figure 1 below.

1. HORxHOR mate pairs were significantly less fit than NORxNOR mate pairs.
2. There was a trend towards HOR males reducing fitness of NOR females, but this is not significant, and there are fewer of this cross type than others in the pedigree.
3. There was no evidence that HOR females reduce fitness of NOR males.

**Table 1.** GLMMmodel fit. Results of generalized linear mixed model examining the influence of *cross type* on the number of offspring produced by different mate pairs. Estimated effect (β) and standard error (s.e.) of each fixed predictor on the link (logit) scale for predictors that were retained in the final model are presented. Variance (σ2) and standard deviation (s.d.) for random intercepts for year are also presented. The null hypothesis that each predictor did not significantly improve the model effect was tested with a likelihood ratio test (LRT p-value). The null hypothesis that each level of predictor has an effect significantly different than the baseline level (NOR x NOR) was evaluated with the Wald test (Wald p-value).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Fixed effects** | **β** | **s.e** | **LRT**  **p-value** | **Wald**  **p-value** |
| (Intercept) | 0.542 | 0.103 |  | **1.5 x 10-7** |
| *HORxNOR* | 0.070 | 0.075 | **0.021** | 0.348 |
| *NORxHOR* | -0.068 | 0.111 | **0.021** | 0.543 |
| *HORxHOR* | -0.209 | 0.101 | **0.021** | **0.039** |
| **Random effects** | **σ2** | **s.d.** |  |  |
| *year*(Intercept) | 0.039 | 0.197 |  |  |
|  |  |  |  |  |

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**Figure 1:** Estimated pair fitness for the different cross types. The number of mate pairs for each cross type is also provided.

*Summary*

HORxHOR mate pairs have lower fitness than NORxNOR. This is not a surprising result given that HOR fitness is lower than NOR fitness at the individual level.

Interestingly there was a trend towards HOR salmon lowering the fitness of NOR salmon, but only when the HOR mate was male. The challenge here is that while the fitness of these mate pairs was lower, there are substantially fewer of them than any other mate pair type, suggesting that there may be sexual selection against HOR males by both NOR and HOR females. In other words, the fact that we are not able to incorporate the majority of parents into this analysis because they have zero fitness is reducing our sample size, and this may particularly effect the HOR males.

In any case, given the negative binomial distribution and substantial variation of fitness in this dataset, my suspicion is 68 is a sufficient sample size to provide reasonable power to find a significant effect at this effect size.

Put another way, there is likely insufficient sample size to find that when HOR males do successfully spawn with NOR females, these mate pairs have lower fitness than NORxNOR mate pairs, but this lower sample size may be due to sexual selection against HOR males.

**Mate Pair Frequencies**

*Question*

Given the relative numbers of HOR and NOR males and females above the dam in each year, what frequencies of cross types do we expect under random mating, and does the pedigree conform to these expectations?

This question helps us understand why are there so few NORxHOR (female x male) crosses in the pedigree. It also provides a different perspective than the section above (Mate Pair TLF) about the relative fitness of the different cross types by taking into account individuals with zero fitness.

*Data/Methods*

We filter the pedigree to include only mate pairs from 2010 – 2015, just as we did in the section above (Mate Pair TLF). Then we will count the number of mate pairs in the pedigree under each cross type and compare these counts to the expected number if there was random mating using a chi-squared test.

One unresolved question here is whether or not to include only unique mate pairs, or to count mate pairs multiple times if they have more than one offspring in the pedigree. By counting a single pair multiple times (e.g. TLF of a given mate pair), we may be able to use these results to try to address the question from the section above titled mate pairs 1: do HOR spawners reduce the fitness of the NORs that they spawn with? In effect, we are incorporating the TLF = 0 samples into our expected/null frequencies.

Counting only unique mate pairs instead of counting only unique mate pairs may be more appropriate if we are attempting to use these results to explore if there is sexual selection against HORs. Why should it matter if a given mate produced one or ten offspring if our goal is to is simply use the pedigree to determine if they spawned? However, this perspective has a serious false-zero problem; we can only detect mate pairs if their offspring survive to adulthood and are sampled! In other words, the pedigree integrates prespawn mortality, sexual selection and selection that occurs over the life of the offspring, so it is not the best piece of information to evaluate sexual selection alone.

We consider both approaches, but take this problems into account.

*Results*

The patterns are similar whether we count unique mate pairs multiple times according to the number of offspring they produce (figure 2), or only once (figure 3). All subsequent results presented here are based on figure 2.

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**Figure 2:** Observed and expected frequencies of different *cross types* (female x male, H = HOR, N = NOR). Mate pairs are counted multiple times if they have multiple offspring.

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**Figure 3:** Observed and expected frequencies of different *cross types* (female x male, H = HOR, N = NOR). Only unique mate pairs are counted.

The chi squared test was significant in all years (table 2), indicating a significant departure from the expected number of cross types given the relative frequencies of NOR and HOR males and females above the dam.

**Table 2:** Uncorrected p-values for Chi-squared goodness of fit test for the counts of HORxHOR, HORxNOR, NORxHOR, and NORxNOR crosses in the pedigree, given the frequencies of NOR and HOR males and females.

|  |  |
| --- | --- |
| **Year** | **P-value** |
| 2010 | 5.32E-05 |
| 2011 | 2.81E-12 |
| 2012 | 2.86E-04 |
| 2013 | 9.54E-30 |
| 2014 | 1.47E-01 |
| 2015 | 1.96E-05 |

As expected given the finding that HORxHOR (female x male) mate pairs have lower fitness than NORxNOR, there are more offspring descended from NORxNOR mate pairs and fewer offspring descended from HORxHOR mate pairs than expected in all years.

There are consistently more offspring descended from HORxNOR mate pairs than expected across all years.

There is no consistent pattern for NORxHOR mate pairs.

*Summary*

I think we will all need to put our heads together to carefully interpret these results and consider whether this analysis is appropriate or not.

Since the hypothesis testing here only examines whether the total distribution between the mate pair types fits the null expectation or not, it is not surprising that such we see small p-values for all years; they are likely driven by the higher and lower (respectively) observed frequencies of NORxNOR and HORxHOR crosses.

The main pattern that we need to consider is why there should be more offspring descended from HORxNOR crosses than expected by chance, but no consistent pattern for NORxHOR.

**F1 vs F2 vs NOR Immigrant Fitness**

*Question*

We have already established that NOR salmon have greater fitness than HOR salmon, and that NORxNOR mate pairs have greater fitness than HORxHOR mate pairs, but we have not tried to parse these effects across successive generations of descendants of HOR salmon, or parse the fitness of NOR offspring of HOR salmon from that of NOR immigrants.

Specifically, what is the TLF of F1s (defined as NOR offspring of HOR parents), F2s (defined as NOR offspring of F1s) and NOR immigrants (defined as NOR salmon that did not assign to any candidate parents and presumed to be wild, locally adapted salmon produced elsewhere)

These results provide some insight into the relative influence of plastic and genetic hatchery effects and whether reintroduced populations can adapt to natural conditions as rapidly as genetic adaptation to captivity occurs in hatchery populations. However sample sizes are likely to be challenging because identification of F2s requires assignment to both parents in the pedigree for two generations.

*Data/Methods*

For F1s and F2s we only consider individuals for which we know the following:

(1) TLF : This limits us to candidate parents (e.g. released above the dam) released in 2015 or earlier.

(2) Both parents assigned (in the case of F1s)

(3) All four grandparents (in the case of F2s)

For NOR immigrants, F1s and F2s, we also need to exclude any individuals released 2011 or earlier, because not all possible parents above the dam are sampled (using ages 3-5, since only 2% are age-6) This leaves just four years (2012, 2013, 2014 and 2015) to draw individuals from to make our comparison.

For hypothesis testing, we fit a negative binomial GLM on TLF with two predictors: *generation* (F0/F1/F2/NOR immigrant) and *year* (4 levels is too few to include as random effect), and evaluated individual predictors with AIC, LRTs, and Wald Tests. In the future, we may consider exploring additional covariates using model selection if there is evidence of overdispersion in the model fit.

*Results*

First let’s look at sample sizes (table 3)

**Table 3:** Sample sizes for candidate parents according to their parentage. Note that candidate parents descended from NORxHOR crosses and assigned to single parents are not included in this table.

F0: Hatchery Outplants

F1: Candidate parents with HORxHOR parents

HORxNOR: Candidate parents with one HOR and one NOR parent.

F2: Candidate parents with F1 parents

NOR Immigrant: Candidate parents with no assigned to parents

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Year** | **F0** | **F1** | **F2** | **NOR immigrant** |
| 2012 | 447 | 276 |  | 174 |
| 2013 | 455 | 127 |  | 26 |
| 2014 | 506 | 48 | 8 | 37 |
| 2015 | 619 | 15 | 22 | 12 |

Sample sizes are definitely insufficient to draw any meaningful comparisons involving F2s (just 30 across 2 years).

Model validation looked fine. Including *generation* (F0/F1/F2/NOR immigrant) significantly improved the fit to the data over a null model with only year (delta AIC = 52, LRT p-value = 6.814e-13). Using F0 as the focal level, F1s and NOR immigrants had greater fitness than F0s, while F2s did not (table 4). These comparisons were also the only significant pairwise comparisons (Tukey’s HSD < 0.05) between any levels of *generation* (table 5)

**Table 4:** GLM model fit of TLF using *generation* (F0/F1/F2/NOR immigrant) and *year* as predictors for candidate parents from 2013 to 2015.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Fixed effects** | **β** | **s.e** | **LRT**  **p-value** | **Wald**  **p-value** |
| (Intercept) | -1.6636 | 0.1016 |  | **2.00E-16** |
| generation[F1] | 0.7677 | 0.1184 | **6.814e-13** | **8.93E-11** |
| generation[F2] | 0.4858 | 0.4577 | **6.814e-13** | 0.288483 |
| generation[NORimmigrant] | 0.8562 | 0.1493 | **6.814e-13** | **9.78E-09** |
| year[2013] | 0.4287 | 0.1205 | **6.438e-07** | **0.000374** |
| year[2014] | -0.1464 | 0.14 | **6.438e-07** | 2.96E-01 |
| year[2015] | -0.3181 | 0.1506 | **6.438e-07** | 0.034631 |

Let’s also translate these βs into something humans can understand. We’ll convert the parameters in table 4 into the response scale (TLF) (figure 4).

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**Figure 4:** Predicted TLF according to *generation* (F0/F1/F2/NOR immigrant) after controlling for year. Error bars are 95% confidence limits for the predicted TLF.

As we can see in figure 4, the fitness of F1s and NOR immigrants is greater than that of HORs (F0). Another important takeaway here is that there’s no evidence that F1 fitness differs from that of NOR immigrants. NOR immigrants are predicted to have ~10% higher fitness than F1s (predicted TLF 0.44 vs 0.40), but this difference is not significant. Finally, given the low sample sizes, the confidence limits are too wide to draw any conclusions about F2s.

What about future years, we will have more F2s then? Let’s count the F2s from 2016 – 2020.

**Table 5:** Number of F1, NORimmigrant, and F2s released above the dam from 2016 – 2020.

|  |  |  |  |
| --- | --- | --- | --- |
| **year** | **F1** | **NORimmigrant** | **F2** |
| 2016 | 14 | 16 | 34 |
| 2017 | 13 | 39 | 22 |
| 2018 | 17 | 2 | 19 |
| 2019 | 17 | 12 | 3 |
| 2020 | 42 | 1 | 11 |

*Summary*

Using 2012 – 2015 candidate parents, we observe increased fitness of NOR salmon with HOR parents (F1s) and NOR immigrants relative to HOR salmon (F0s). F1s had slightly lower fitness than NOR immigrants, but this difference was not significant, suggesting that just as adaptation to captivity is rapid and can occur in a single generation, the strong selection imposed on HOR spawners and their offspring in natural settings may lead to just as rapid adaptation to the wild. However, there are a lot of assumptions baked in here and we will need to think more before drawing this conclusion.

Finally, there is insufficient sample size to draw any conclusions about F2s. The number of F2s in later return years is higher, but since their potential offspring have yet to return, we do not have TLF estimates for these individuals yet. The overall lower number of F1s and NOR immigrants released above the dam in this period (2016 – 2020) may present other problems for these evaluating fitness in these years though.

**Genetic Diversity**

*Question*

Do HORs, F1s (NOR offspring of HORs), or NOR immigrants vary in genetic diversity?

We are most interested in two comparisons:

1. Do HORs have less diversity than NOR immigrants?
2. Do F1s have lower diversity than HORs?

*Data/Methods*

We use all available individuals 2012 and later (when NOR releases began, and NOR immigrants can be identified with some confidence) and compare genetic diversity (as measured by expected heterozygosity (He)) between HORs, F1s (NOR offspring of HORs), or NOR immigrants. Hypothesis testing is by Monte-Carlo Simulation in adegenet.

*Results*

Given that these are microsatellites chosen for high information content, He was high across all groups (table 6).

**Table 6:** Mean expected heterozygosity for HORs, NORs with HOR parents, and NOR immigrants from 2012-2020.

|  |  |
| --- | --- |
| **Group** | **He** |
| HOR | 0.9293 |
| F1 | 0.9233 |
| NORimmigrant | 0.9259 |

F1s have lower genetic diversity than both HORs (p-value = 0.001, 1000 simulations) and NOR immigrants (p-value = 0.003, 1000 simulations). NOR immigrants have lower diversity than HORs, but this comparison was only marginally significant ((p-value = 0.04, 1000 simulations).

*Summary*

There is evidence of reduced diversity among F1s relative to HORs, suggestive of strong selection. This isn’t exactly surprising because we already know most NORs are F1s and there are way fewer returning NORs than outplanted HORs.

The finding that NOR immigrants do not have greater diversity than HORs is novel. If we assume NOR immigrants are reflective of “wild” fish, this is a good indicator that the hatchery genetic management plan is successful in creating a genetically diverse hatchery stock. See (Johnson and Friesen 2014)

Johnson MA, Friesen TA (2014) Genetic Diversity and Population Structure of Spring Chinook Salmon from the Upper Willamette River, Oregon. North American Journal of Fisheries Management, 34, 853-862.