**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. **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 |  |  |
|  |  |  |  |  |

Chart, box and whisker chart

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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 not 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.

**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 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.