**Rationale**

This is a mini-paper/memo/paper outline to share with coauthors and collaborators to address the following question:

*Does adaptation to natural conditions among the offspring of captive reared salmon occur as rapidly as domestication occurs in the hatchery?*

**Intro/Question**

Adaptation to captivity among hatchery reared Pacific salmon (hereafter “domestication”), can occur in a single generation (Christie et al. 2012), and has been associated with as high as a 37.5% decline in fitness in a natural setting per generation of captive breeding (Araki et al. 2007). Reintroduction programs intend to re-establish wild populations in habitats where salmon have been extirpated. These programs typically rely on supplementation with captive reared salmon (hereafter hatchery-origin (HOR) salmon) that are released into the wild first as juveniles/smolts, and then a second time in the targeted habitat (typically above a dam) when they return to freshwater as adults to spawn. These HOR salmon produce offspring in the wild that subsequently return to the target habitat as adults to spawn.

Since the long-term goal of these reintroduction programs is to establish highly productive, self-sustaining populations, the lower fitness of HOR salmon in the wild relative to locally adapted, wild salmon presents a challenge to reintroductions. Overcoming this challenge depends critically on the capacity of the naturally produced descendants of HOR salmon to rapidly adapt to natural conditions. However, this rapid re-adaptation is by no means assured, because it depends on the availability of beneficial variants in the standing genetic variation among HOR salmon.

To date, there is little empirical evidence to suggest that adaptation to natural conditions among the natural-origin (NOR) descendents of HOR salmon occurs as rapidly as domestication. This gap in the literature stems from multiple logistical and analytical challenges: (1) inferences require accurate pedigree data, necessitating sampling of a large portion of possible parents in the target habitat, (2) the variable of interest, fitness, has a negative binomial distribution and large variance, requiring large sample sizes to detect ecologically relevant difference in fitness, (3) the pedigree must include 3 or more generations, in the case of Chinook salmon this may require longitudinal sampling efforts spanning 10 or more years, and (4) confounding signals of adaptive phenotypic plasticity and genetic adaptation present experimental design challenges.

The South Fork McKenzie River spring Chinook salmon genetic pedigree includes nearly all candidate parents released above Cougar Dam from 2007-2015 and nearly all returning adult potential offspring from 2010-2020, resulting in a pedigree that spans 14 years and includes up to four generations. Importantly, releases of Chinook above Cougar Dam include not only HOR salmon and their NOR descendents, but also NOR salmon that are produced elsewhere (hereafter NOR immigrants).

The question, *does adaptation to natural conditions among the offspring of captive reared salmon occur as rapidly as domestication occurs in the hatchery,* can be addressed in one of two ways with the South Fork McKenzie River spring Chinook salmon genetic pedigree. First, we could compare fitness estimates of HOR salmon with that of their first, second and later generation NOR offspring (hereafter F1 , F2, etc). Changes in fitness with each subsequent generation would provide an estimate of the pace of adaptation to the natural conditions above the dam. However, despite the long term nature of our pedigree, the number of identifiable F2s with fitness estimates is limited (Table 1).

**Table 1:** Sample sizes for candidate parents released above the dam with fitness estimates (i.e. nearly all potential offspring have been sampled) according to their parentage.

Note that candidate parents descended from NORxHOR crosses and assigned to single parents are not included in this table.

HOR: Hatchery-Origin

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 assumed to be produced elsewhere

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

Fortunately, we may be able to take a second approach to answer our question. NOR immigrants are defined as those NOR salmon in our dataset for which nearly all of their possible parents have been sampled three to five years earlier, but no assignment has been made. To the extent to which NOR immigrants are representative of wild, locally adapted salmon, comparisons between NOR immigrant fitness and the fitness of F1s, can tell us a great deal about how the pace of *adaptation to natural conditions among the offspring of captive reared salmon.* Specifically, if the fitness of the first generation offspring of HOR salmon is indistinguishable from NOR immigrants, this suggests that re-adaptation to the wild may be as rapid as domestication.However, there are several caveats/challenges that we must address:

(1) How confident are we that can correctly identify NOR immigrants?

(2) Are NOR immigrants representative of wild, locally-adapted salmon?

(3) Is the broodstock integrated?

(4) Modeling Challenges

I believe we may be in a position to use accurately identify NOR immigrants and use them as stand-ins for wild, locally adapted salmon (see **Caveats/Questions** section at end of this document)and are able to address our main question.

**Methods/Results**

*Definitions and Data*

We define F1s as the NOR offspring of HOR salmon released above the dam. F1s are identified in the pedigree as offspring assigned to two HOR parents. Offspring assigned to a single parent are excluded from the analysis. We define NOR immigrants as NOR salmon that do not assign to any candidate parent. Importantly, because we do not sample candidate parents prior to 2007 and most (98%) returning adults are ages 3 to 5, we can only confidently identify NOR immigrants that return in 2012 and later years.

We estimate fitness using total lifetime fitness (TLF) defined as the total number of adult offspring assigned to a parent. Because most (98%) returning adults are ages 3 to 5, we cannot accurately estimate TLF for an individual until 5 years have passed since it was released above the dam. Our latest year of offspring sampling is 2020. Therefore, our TLF estimates only extend for salmon released above the dam in 2015 and earlier years.

Together, accurate identification of NOR immigrants and estimation of TLF limits our dataset to salmon released above the dam from 2012 to 2015. The number of HORs, F1s and NOR immigrants released above the dam these years is presented in table 1.

*Hypothesis Testing*

To evaluate if F1s have different fitness from HOR salmon and NOR immigrants, we fit a generalized linear model on TLF using a negative binomial distribution, log link function and a predictor termed *generation. Generation* was a factor and reflected whether an individual salmon was HOR, an F1 or an NOR immigrant. We also explored several covariates with model selection including length, sex, and release day. *Year* was the year of release and was fit as a fixed factor instead of a random effect because only 4 levels were available (2012 – 2015). *Length* was fork length in cm. *Sex* used the sex as determined at the genetic sex marker, if available, if not available sex was determined from gross morphology. *Release day* was the centered Julian day of release. Model fit used the glm.nb function of the *MASS* package*.* Model selection used backwards stepwise selection based on likelihood ratio tests and a critical p-value of 0.05. Multicollinearity was evaluated using GVIFs and a cutoff of GVIF or GVIF(1/2\*DF) < 2. Model validation on the final model relied on simulated residuals using the *DHARMA* package in R. Post-hoc testing for different predicted fitness between levels of the predictor *generation* was conducted using Tukey’s HSD in the *emmeans* package, and *emmeans* was also used to present the estimated marginal mean predicted TLF values for each level of *generation,* after controlling for the effect of other significant predictors

The final model included an effect of *generation, length* and *year.* Model fit is presented in table 2. *Generation* significantly improved fit to the data relative to a null model that only included the effect of *year* and *length* according to both AIC (delta AIC = 23) and a likelihood ratio test (p-value = 1.36e-06). Estimated marginal means for different levels of *generation* are presented in Figure 1. Significance testing for differences in fitness between all pairwise comparisons of levels of *generation* are presented in table 3.

**Table 2:** Model Fit

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Fixed effects** | **β** | **s.e** | **LRT p-value** | **Wald**  **p-value** |
| (Intercept) | -6.96 | 0.57 |  | 2.00E-16 |
|  |  |  |  |  |
| generation[F1] | 0.51 | 0.12 |  | 1.70E-05 |
| generation[NORimmigrant] | 0.63 | 0.15 |  | 3.14E-05 |
|  |  |  |  |  |
| length[cm] | 0.07 | 0.01 |  | 2.00E-16 |
|  |  |  |  |  |
| year[2013] | 0.69 | 0.12 |  | 7.43E-09 |
| year[2014] | -0.03 | 0.14 |  | 0.826 |
| year[2015] | -0.03 | 0.15 |  | 0.861 |

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

**Table 3:** *Post-hoc* significance testing between different levels of *generation*. Ratios and standard errors (SE) are presented on the response (TLF) scale, hypothesis testing is conducted on the log-scale using Tukey method for comparing a family of 4 estimates and results averaged over the levels of *year.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Contrast** | **ratio** | **SE** | **z.ratio** | **p.value** |
| F0 / F1 | 0.464 | 0.0549 | **-6.484** | **<.0001** |
| F0 / F2 | 0.615 | 0.2816 | -1.061 | 0.713 |
| F0 / NORimmigrant | 0.425 | 0.0634 | **-5.735** | **<.0001** |
| F1 / F2 | 1.326 | 0.6233 | 0.599 | 0.9322 |
| F1 / NORimmigrant | 0.915 | 0.1452 | -0.558 | 0.9443 |
| F2 / NORimmigrant | 0.69 | 0.3302 | -0.775 | 0.866 |

Together these results suggest that both F1s and NOR immigrants have significantly greater fitness than HORs (p-value = <0.0001). HORs are estimated to have 46% and 42% of the returning adult offspring of F1s and NOR immigrants respectively from 2012 to 2015. While F1 fitness was somewhat lower than NOR immigrant fitness (F1 fitness / NOR immigrant fitness \*100 = 92%), this difference was not significant (p-value = 0.94).

**Caveats/Questions**

*(1) How confident are we that can correctly identify NOR immigrants?*

We are primarily focused on “non-exclusion probabilities” when we examine our assignment protocol, which describe type I error rates, e.g. how likely is a given offspring to be assigned spuriously to a parent. However, we do not carefully examine type II error, which would falsely identify NOR descendants of HOR salmon released above the dam as NOR immigrants. While type II error rates for Colony are expected to be low given the information content of our dataset (Harrison et al. 2013), our requirement of no more than a single mismatch per parent-offspring pair in addition to the assignments cutoffs determined by Colony and Cervus may inflate our type II error rate.

While our ability to identify trends in the proportion of NOR immigrant over time at the Cougar trap suggests that many NOR immigrants are correctly identified, any F1s/F2s falsely called NOR immigrants would lead to type II error in our main question by reducing the apparent difference in fitness between F1s and NOR immigrants. We would need to take some time to explore type II error rates in the final pedigree before being confident in our results.

Another challenge is the presence of adfluvial females and precocial males. I think we are okay here. While the small numbers of adfluvials and precocials are relevant to broader questions about the productivity of the above dam population, to falsely identify offspring of these fish as an NOR immigrant because we don’t sample their adfluvial/precocial parent would require both parents to be unsampled. For example, even in the generous case where 10% of successful female spawners are adfluvial and 10% of successful male spawners are precocial, only 1% (e.g. 10% x 10%) of offspring would descend from adfluvial x precocial mate pairs and be falsely identified as NOR immigrants because of unsampled parents.

*(2) Are NOR immigrants representative of wild, locally-adapted salmon?*

Assuming NOR immigrants are correctly identified (see above), are they a good stand-in for wild, locally adapted salmon? And, importantly, is the NOR immigrant vs F1 comparison appropriate? I’m inclined to believe that they are about as good as one can get given the history of hatchery influence in the region, and the limits of experimental design but I think this is up for debate.

Hatchery Influence:

Is there even such a thing as wild, locally-adapted salmon on the McKenzie? To what extent has hatchery influence and stock transfers eroded local adaptation?

The percent of hatchery origin spawners in recent years in the upper (above Leaburg Dam) McKenzie is low (pHOS ~27% (range 15-45%)) (Bowerman et al. 2018). Furthermore, the McKenzie sub-basin is the only Willamette tributary that is known to have sustained natural production throughout the twentieth century (NMFS 2006) and the majority of salmon that return to the McKenzie have been naturally produced since at least 2002, when marking of returning HOR salmon became reliable (Johnson and Friesen 2010).

I think the extent to which stock transfers (see Broodstock Integration section below), high hatchery production and low natural production has eliminated locally adaptative genetic variants among naturally produced fish at the sub basin level in the Willamette, and how much differentiation has accumulated since these stock transfers stopped (either through selection or drift) are still open questions. Differentiation at microsatellites suggest that there is structure among Upper Willamette tributaries (Johnson and Friesen 2014), but no differentiation between hatchery stocks and NOR fish within a sub-basin. However, non-significant Fst at neutral markers and high gene flow does not always preclude differentiation at genomic regions under selection, a hard fought lesson learned by ecologists over the past two decades (Tigano and Friesen 2016).

In the case that there are no locally adaptive genetic variants among naturally produced fish at the sub-basin level, then NOR immigrants are arguably still the best group against which to compare HOR and F1 fitness: (1) since pHOS is certainly lower in the upper McKenzie than above Cougar, they are likely to be much later generation descendants of HORs than F1s and (2) if there are no such thing as wild fish, then late generation NOR descendants of HORs are the closest thing we have.

Experimental Design:

To draw meaningful comparisons, we must compare fitness between groups of individuals spawning in the same location at the same time, and we must sample nearly all candidate parents for a given potential offspring. Therefore we are limited to comparing F1s and HORs to NOR immigrants released above the dam. If the tendency to disperse for NORs not produced above the dam (i.e. chance of being sampled at the Cougar Trap) is associated with lower fitness, then NOR immigrants may be a poor stand in for wild, locally-adapted salmon.

There is a tradeoff between the quality of spawning sites and the costs associated with competing for it (Adkinson 2014 is one example of this). Therefore, it is not difficult to imagine evolution of a reaction norm between competitive ability and tendency to disperse: less competitive individuals may tolerate increased risks of dispersing farther from natal spawning grounds to reduce intra-sexual competition (although I’m not aware of any empirical study supporting this in salmon). Since size at maturity is closely associated with fitness and competitive ability, I checked to see if NOR immigrants were smaller than NOR sampled during carcass surveys in the mainstem McKenzie and on the South Fork below the dam. NOR immigrants did not differ in size from NOR salmon sampled during spawning ground surveys throughout the McKenzie and on the South Fork below the dam.

*(3) Is the broodstock integrated?*

Since hatchery operations began, incorporation of non-Willamette basin salmon into broodstock is thought to be rare, but there has been substantial stock transfers within the Willamette Basin (Johnson and Friesen 2010). For much of the 20th century, hatchery operations on the McKenzie used a mixed broodstock consisting of Middle Fork Willamette and McKenzie stocks. Since 1990, only McKenzie basin fish have been used as broodstock and the proportion of NORs integrated into broodstock has varied between years. Currently (I think – best to confirm this with folks at ODFW, I can’t find the data or the HGMPs, just references to them), the goal is 10% NOR, and there may be (according to the 2019 EIS) a long term goal to increase this to up 50%. I did find that pNOB was zero in 2015 and 2016. Understanding this is not critical to addressing the question but provides important context for the results.

*(4) Modeling Challenges*

We only have 4 years of data and the sample sizes are not balanced well between years. We also need to think about covariates and how to incorporate them into the model.

LSDR was implemented in 2013 and 2014, and downstream recycling was implemented in all subsequent years. Because both of these programs selectively limit the number NOR immigrants released upstream relative to NOR salmon produced above the dam, the majority of NOR immigrants in the dataset come from just one year, 2012. While GLM parameter estimates and hypothesis testing will not be biased by the lack of balance between levels of a predictor (this would just be reflected in large standard errors in the parameter estimates), it does warrant a closer examination of the pattern within each year. Here are the mean + sd for TLF across generations in each year.

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