Many marine fish species are currently under threat from habitat degradation or over-exploitation, such as overfishing. Fisheries managers combat the over-exploitation of species in a variety of ways including controlling fishing efforts, habitat protection or restoration, and aquaculture-based enhancement. Aquaculture-based enhancements, like stock enhancement, can aid in the conservation of depleted, threatened, and endangered populations. Stock enhancement specifically focuses on the continual release of hatchery fish into the wild with the intent of sustaining fisheries. In California waters, overfished white seabass stocks in the 1920s prompted multiple conservation and management actions, culminating in the Ocean Resources Enhancement and Hatchery Program (OREHP) which began stocking white seabass in 1986 in collaboration with Hubbs SeaWorld Research Institute (HSWRI) and tracking the hatchery contribution to the wild using coded wire tags (CWT). In accordance with the responsible approach to marine stock enhancement, the program has implemented several genetic components such as hatchery breeding groups, hatchery effective population size estimates, parentage assessments, and genetic population structure studies. Previous work on population structure identified three distinct white seabass populations: a northern population (Southern California Bight), a southern population (Pacific Baja), and a Gulf of California population. However, there is need of a more powerful marker set to assess fine-scale genetic flow and diversity.

Adult samples from fisheries-dependent collections (2018-2019) will be used to assess hatchery contribution to the wild adult population (n=50). To put fine-scale patterns of gene flow and diversity into context, samples from 1986 to 2006 along the southern California coast from six collection locations will be used to assess hatchery contribution to the wild subadult population as well as wild population's genetic health (n=300 per location) (Figure 1). As part of a larger effort, hatchery breeding groups, hatchery effective population size estimates, parentage assigned to most likely candidate parent or left unassigned, and GENEPOP inbreeding coefficient, and GW statistic will be calculated with ARLEQUIN. The survey targets ages 0-3; therefore provides a collection spanning multiple year classes across approximately a generation for this species and will be used to assess hatchery contribution to the wild subadult population as well as wild population’s genetic health (n=300 per location) (Figure 1). Early experimental results indicate sufficient polymorphism.

**Microsatellite marker development and validation**
- White seabass whole genome sequenced by Applied Biosystems and microsatellite primers designed by MSAT commander
- Over 70 microsatellites screened
- Selected labelled primers assembled into multiplex groups and visualized with capillary gel electrophoresis
- All loci tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD)
- All project samples will be genotyped with optimized microsatellite suite and chromatograms will be scored by two independent readers

Parentage Model Development
- Allele frequency file constructed from 300 wild caught fish
- Cervus used to assign parents to offspring with likelihood method (n=492)
- Parentage assigned to most likely candidate parent or left unassigned
- Hatchery contribution to wild population calculated by hatchery fish/total fish = % contribution
- Verified hatchery fish (n=100) will be used to determine if there is reliability in identifying hatchery fish with both CWT and genetic methods

Population Structure and Genetic Characterization
- STRUCTURE used to evaluate population structure based on clustering method (hatchery fish removed from analysis)
- SPAGeDi used to determine if allele-identity (Fst) or allele-size (Rst) performs better
- Basic genetic diversity metrics including number of alleles per locus (N_a), allelic size range (A), observed heterozygosity (H_o), gene diversity, inbreeding coefficient, and GW statistic will be calculated with ARLEQUIN and GENEPOP
- Effective population size (N_e) determined with LDNe method

**Objective**
A. Design and optimize a microsatellite marker panel for white seabass
B. Use developed microsatellite markers to develop a genetic parentage model
  - What contribution are hatchery fish making to the wild population(s)?
  - Are there differences between adult and subadult hatchery contribution estimates?
C. Characterize wild populations of white seabass
  - Do white seabass along the southern California coast form a single genetic population?
  - Do current estimates of diversity, N_e, and Fst indicate sufficient adaptive potential for long-term sustainability of fisheries?
  - Has the population experienced a bottleneck in the recent past?

**Sample Collection**
- Samples were collected by HSWRI in a fisheries-independent gillnet survey from 1986 to 2006 along the southern California coast from six collection locations
- Since stocking began, 492 genetic broodstock samples have been archived
- The survey targets ages 0-3; therefore provides a collection spanning multiple year classes across approximately a generation for this species and will be used to assess hatchery contribution to the wild subadult population as well as wild population’s genetic health (n=300 per location) (Figure 1)
- To put fine-scale patterns of gene flow and diversity into context, samples from Mexico were included (Figure 1)
- Adult samples from fisheries-dependent collections (2018-2019) will be used to assess hatchery contribution to the wild adult population (n=50)

**Methods**

**Raw sequence data yielded about 2 million paired-end reads**
- 17,000 microsatellites initially found
- 1,333 primers were designed by MSAT commander
  - 1198 di-nucleotides
  - 85 tri-nucleotides
  - 43 tetra-nucleotides
  - 7 penta-nucleotides
- Over 70 microsatellites screened
  - Temperature gradient PCR (Figure 2)
  - Multiple individual PCR (Figure 3)
- Two multiplex groups of 6 microsatellites each have been selected and optimized (Figure 4)

**Acknowledgements**
Dr. Tanya Darden, SCDNR; Dr. Mike Denson, SCDNR; Dr. Tracery Smart, SCDNR
Dr. Andy Shedlock, CofC; Dr. Mark Drawbridge, HSWRI; Mike Shane, HSWRI