Genetic Management Components of Facility Design and Operation

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Much of what will be discussed relates to lessons learned from the Project Tampa Bay red drum stock enhancement program. The comprehensive research effort in Tampa Bay has involved the staffs of six separate, but integrated, research groups at FWRI and Mote Marine Lab. The people noted here, along with the 12-member Marine Stock Enhancement Advisory Board (MSEAB), were the leaders of that effort.

Acknowledgements

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F.D.M. folks (FWRI)

Ken Leber & Carole Neidig – Angler Fin-clip Program
Mote Marine Lab Center for Fisheries Enhancement

Luiz Barbieri and the MSEAB
Outline of today’s presentation. I’ve saved the interesting stuff for last.
Potential Genetic Concerns

| exogenous (inter-stock) gene transfers | reduced adaptive potential |
| altered selection/drift dynamic       | outbreeding depression     |
| maladapted alleles or traits          | artificial/disruptive selection |
| genetically modified organisms        | genetic swamping           |
| inbreeding depression                 | allelic replacement        |
| domestication                         | reduced effective size     |
| diversity loss                        | hybrid swarms              |
| introgressive hybridization           | disrupted genomic coadaptation |
| increased family-size variance        |                              |
The first category of genetic risk relates to brood fish source. The easiest way to prevent possible impacts from non-native fishes/non-indigenous genes is to utilize brood fish from an appropriate spatial/temporal source. The decision as to what is appropriate should be guided by empirical study of stock structure and population connectivity in natural populations.
There has been a lot of genetic data collected for red drum. This figure from Gold and Turner (2002) perhaps most clearly depicts the first-order genetic dynamics of this species in the Gulf. Generally speaking, genetic differences accumulate among red drum as a function of geographic distance.
The genetic “isolation-by-distance” pattern for red drum is used to guide brood-source decisions. Gold and Turner noted that the genetic neighborhood size of GOM red drum likely ranges from 700-900 km. Using a slope-tuning approach to compute the average, single-generation dispersal distance for individual red drum, we have confirmed that red drum progeny should be stocked within ~470 KM (in either direction) of their brood source.
For example, for brood fish collected off of Tampa Bay, progeny could safely be stocked southward to Florida Bay and northward to the Gulf county-Franklin county border. Progeny of brood fish collected in Pensacola could be stocked eastward to Dixie county. GOM and Atlantic red drum stocks should not be intermixed.
In addition to spatial considerations for brood fish source, we also consider the level of diversity within and among subpopulations. The “effective population size” ($N_e$) is an important parameter for genetic risk assessment. It is basically a statistical indicator of variation in individual reproductive success. For geneticists, low values of $N_e$ signal that a species may be susceptible to certain forms of adverse genetic impact. Theory suggests that impacts may be expected when $N_e$ dips below 500 in the short term. There are several ways to estimate this metric. For demographically abundant, long-lived, broadly structured species, a robust approach to the estimation of $N_e$ is to examine statistical associations among alleles across genetic loci. To do so, we need reliable genotype data from a LOT of specimens.

**Estimate of Effective Size**

Linkage disequilibrium ($D$) – non-random association among alleles at different gene loci.

$D$ is inversely proportional to $N_e$ in a predictable way

**LdNe** Version 1.31

“A program to estimate effective population size from genotypic data based on linkage disequilibrium”

Robin Waples and Chi Do

From our Project Tampa Bay (PTB) genetic library, we have data from more than 23,000 wild red drum. We have used this huge database to estimate $N_e$ as described.
We have found that the effective size of the tested red drum subpopulation is on the order of $10^4$. This is suitably high value.

<table>
<thead>
<tr>
<th>Mating Model is random</th>
<th>Lowest Allele Frequency Used = 0.00100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall $r^2 =$</td>
<td>0.00005</td>
</tr>
<tr>
<td>Expected $r^2$ Sample =</td>
<td>0.00004</td>
</tr>
<tr>
<td>Estimated $N_e =$</td>
<td>48,581</td>
</tr>
<tr>
<td>95% CIs for $N_e$</td>
<td></td>
</tr>
<tr>
<td>* Parametric</td>
<td>32,720</td>
</tr>
<tr>
<td></td>
<td>86,828</td>
</tr>
<tr>
<td>* Jackknife on Loci</td>
<td>32,595</td>
</tr>
<tr>
<td></td>
<td>87,564</td>
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</table>
The second category of genetic risk is associated with genetic changes that could occur during the course of fish propagation. These include intentional selection, domestication and inbreeding and can ultimately lead to a decrease in population fitness (i.e., higher overall mortality rates and/or lower birth rates) in the recipient stock. We carefully guard against these changes via genetic management practices implemented during production. Perhaps our biggest concern is associated with the use of sufficient numbers of breeders to mitigate against future increases in levels of relatedness and reduced effective sizes in the recipient stock.
In PTB, male and female parents for mated in small breeding groups (6 fish). The spawns from several such groups were reared and released each season. Breeders were completely replaced each season. In a perfect world, the individual success of any one breeder would have been equal to that of the other breeders.
For the overall PTB program, 79 females and 90 males were used, divided into 34 different breeding groups. The pink numbers refer to moms; blue numbers to dads. Our parent-offspring data, obtained from our PTB genetic tracking study (based on 2,225 recaptures) indicate that variance in individual reproductive success was fairly high. In this slide, an example of the varying reproductive success is given for four breeding groups spawned in the Fall of 2004.
This slide shows a commonly used method to directly estimate $N_e$ (in this case, from a hatchery cohort, C) using parent-offspring data.
This slide shows our improved method to estimate $N_e$, again using parent-offspring data, but taking into account that the parents will have some degree of inbreeding and relatedness themselves.
For the 169 parents used in hatchling production for the PTB program, only 32-34 were “genetically effective” breeders.

### Result

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<table>
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<tbody>
<tr>
<td>$k_C$</td>
<td>28.20</td>
</tr>
<tr>
<td>$\sigma^2_{k,C}$</td>
<td>3330.86</td>
</tr>
<tr>
<td>$n_C$</td>
<td>79</td>
</tr>
<tr>
<td>$P_{\text{shared maternity}}$</td>
<td>0.065</td>
</tr>
</tbody>
</table>

\[
N_{el,H} = 34.78
\]

\[
N_{el,H^*} = 32.24
\]

Effective : Actual = 21%
Mating Model is random
Lowest Allele Frequency Used = 0.01000

Overall $r^2$ = 0.00972
Expected $r^2$ Sample = 0.00046

Estimated $N_e$ = 34

95% CIs for $N_e$
* Parametric
  31.9
  35.8

* Jackknife on Loci
  30.6
  37.2

This same result ($N_e \sim 34$) was generated using the ‘linkage-disequilibrium’ method described earlier.
This chart shows the individual contributions of female parents to the 2,225 recaptures. It is quite apparent that these contributions varied greatly among individuals.
In the new brood-fish facility design, we expect that the genetic efficiency will be improved (from 21% to >50%). Because of larger tank sizes, we also have the capacity to use more brood fish (60+) each year.

\[ N_B = 60 \times 6.5 \]

\[ N_{eI,H^*} = 185 \]

Effective : Actual = 50%
Finally, we consider the third type of genetic risk – i.e., those that stem from the release of too many fish. Notably these risk can accrue under some circumstances even when the brood fish source is appropriate and when selection and inbreeding has been avoided at the propagation stage.
From our PTB genetic tracking of fish larger than 200 mm, we learned that ~2.6% of the sub-adult red drum in were of hatchery origin in the years immediately following stocking.

<table>
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<th>Recapture Probabilities</th>
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<td>( \omega_j ) = probability that two randomly chosen individuals were both derived from the ( j )th spawning group</td>
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- 2 spawning groups ~ hatchery and wild
- Genetic ID of fin clips from fish \( \geq 200 \) mm SL (low mortality; recruited to fishery)
- 11,231 of our 26K clips were from fish \( \geq 200 \) mm SL
- Percentage of hatchery red drum was 2.6%

Thus, \( \omega_H = 0.026^2 \); \( \omega_w = 0.974^2 \)
Using the PTB effective number of breeders, a 2.6% hatchery contribution, and an initial $N_e$ of 48,500 for the wild stock, the $N_e$ in the admixture would be expected to decline to $\sim 24,500$. When making this estimate, we assumed, very conservatively, that the offshore subpopulation is ‘fed’ only by recruitment from Tampa Bay. However, we are reasonably certain that the adult subpopulation is comprised of recruits from a much larger geographic base. If so, the expected decline in $N_e$ after stocking would be considerably lower.
Here we consider a hypothetically large-scale program that results in a (first-generation) hatchery-derived component of 25% in the entire west FL subpopulation (Apalachicola to Florida Bay). We assume 185 effective breeders and used the conservative estimate of 24,500 for the contemporaneous $N_e$ of the wild stock. Under these conditions, this would likely result in a post-supplementation $N_e$ of $\sim 2,800$. Even though the estimated value is considerably lower compared to the pre-stocking value, it is a risk-adverse level over evolutionarily short timescales. Post-release genetic monitoring should be used to ensure that this is the case.

$$N_{el} = \left[ \frac{\omega_H}{N_{el,H}} + \frac{\omega_W}{N_{el,W}} \right]^{-1}$$

$$= \left[ \frac{(0.25)^2}{185} + \frac{(0.75)^2}{24,500} \right]^{-1} = 2,770$$
We have talked at length about how data from PTB have been used to inform genetic management of future stocking programs in FL. We have also learned a great deal from these data about where and how to stock red drum. The results from our empirical testing as they relate to release location/habitat/season/size are described in Tringali et al. 2008. In the few remaining slides, these results are summarized.
A total of 1,340,098 phase-1 fish were released in the Alafia River (AR). All AR fish were released in sync with natural production. A total of 2,386,879 phase-1 fish were released in the Little Manatee River (LMR). LMR fish were released either in sync (n = 738,226) or out of sync (n = 1,648,953) with natural production. Phase-1 fish = 25–45 mm standard length (SL) (~1 month old). The recapture rate for a given test group denotes the number of fish recaptured ÷ the number of fish released. Recapture data clearly indicate that phase-1 survival was significantly higher the AR compared to the LMR.
The effect of size-at-release on recapture rate was evaluated for releases in the Alafia River. Phase-1 fish = 25–45 mm standard length (SL) (~1 month old); phase-2 fish = 60–110 mm SL (~5 months old); phase-3 fish = 130–180 mm SL (~8 months old). Recapture rates for phase three fish were 6-7 times higher than those of the smaller size classes. If LMR phase-1 releases are included in the assessment, the phase-3 recapture rate is ~ 23 times higher than the phase-1 and -2 rates.
Spatial (microhabitat) trends evident in recapture rate and these varied by release size. Phase-1 fish survival was highest for those fish released in the 4th river mile of the AR. In contrast, phase-3 survival was highest for those released close to the river mouth. Habitat suitability and availability, as they relate to specific release-size classes, are important factors and should be included in research/assessment components of future efforts.


Tringali, M. D. 2006. A Bayesian approach for the genetic tracking of cultured and released individuals. Fish. Res. 77:159-172.

Tringali, M. D. 2003. Genetic changes in natural populations caused by the release of cultured fishes. Department of Biology, University of South Florida, Tampa, FL.


Thanks