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.

Today

- Genetics – So What?
  - Briefly! …

- Facility-Design Considerations
  - Wild Pop Structure; N_{ew}
  - Brood Fish Numbers; N_{eb}
  - Release magnitudes

- Lessons from Tracking Studies
  - Size-at-Release
  - Release Habitat, Season

Outline of today's presentation. I've saved the interesting stuff for last.
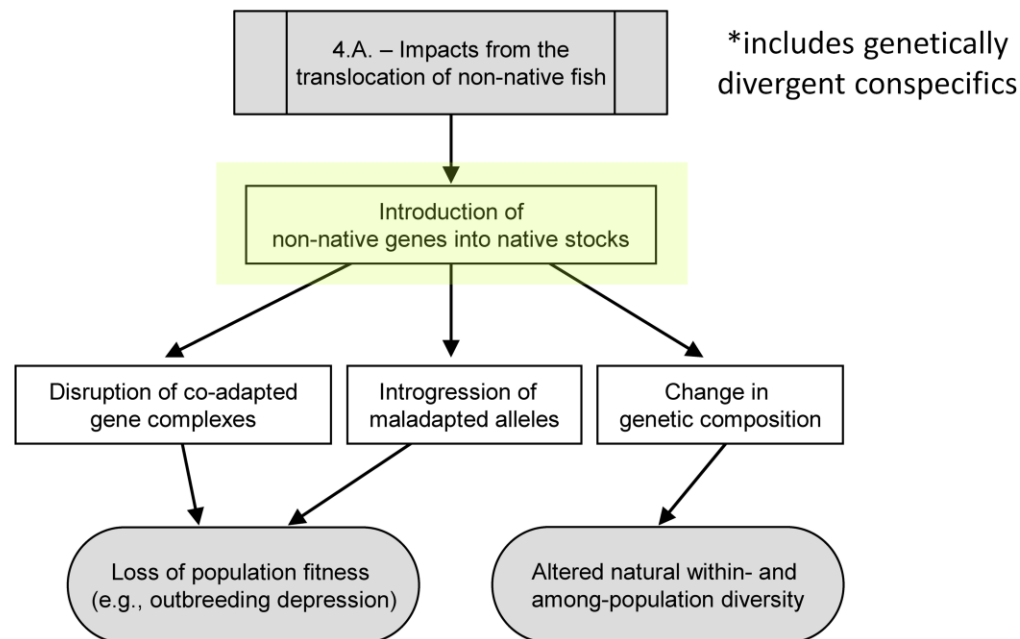
Potential Genetic Concerns

exogenous (inter-stock) gene transfers

altered selection/drift dynamic

maladapted alleles or traits

genetically modified organisms

inbreeding depression

domestication

diversity loss

introgressive hybridization

increased family-size variance

reduced adaptive potential

outbreeding depression

artificial/disruptive selection

genetic swamping

allelic replacement

reduced effective size

hybrid swarms

disrupted genomic coadaptation

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As depicted here, there are numerous potential genetic impacts when wild fish and released cultured fish interact. Despite their complexity, these concerns can be grouped into three basic categories: brood-source considerations, propagation-related considerations, and release-magnitude considerations. This form of categorization facilitates the straightforward planning and implementation of risk-adverse genetic management procedures.

Type I – Brood Fish Source

4.A. – Impacts from the translocation of non-native fish

*includes genetically divergent conspecifics

Introduction of non-native genes into native stocks

Disruption of co-adapted gene complexes

Introgression of maladapted alleles

Change in genetic composition

Loss of population fitness (e.g., outbreeding depression)

Altered natural within- and among-population diversity

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.

FLORIDA'S NEW SALTWATER FISH
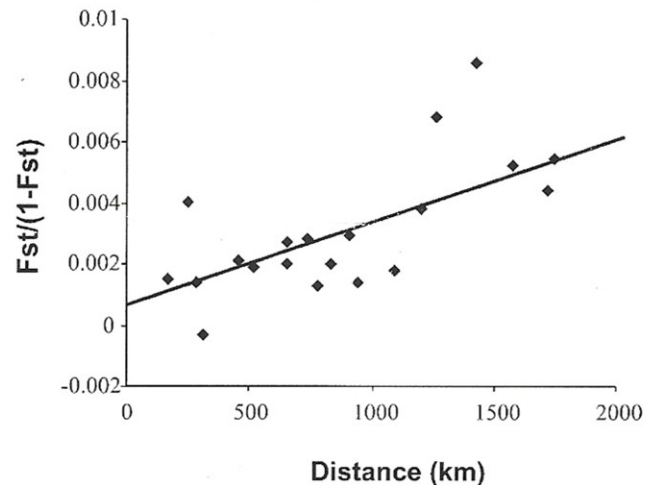HATCHERY NETWORK
Partners and Supporters:

Fig. 4 Relationship between genetic distance ($F_{ST}/(1\text{-}F_{ST})$, where F_{ST} is the θ measure of Weir and Cockerham (1984) and geographic distance (km) for samples of red drum. Geographic distance between sample localities followed the coastline of the northern Gulf of Mexico

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.

FLORIDA'S NEW SALTWATER FISH
HATCHERY NETWORK
Partners and Supporters:

Genetic Neighborhood Size

GNS = 700-900 km (2D; Gold and Turner 2002)

Isolation by Distance Slope-Tuning Model
for Mean S-G Dispersal Distance

MSGD = 470 km (1D)

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.

Who can go where?
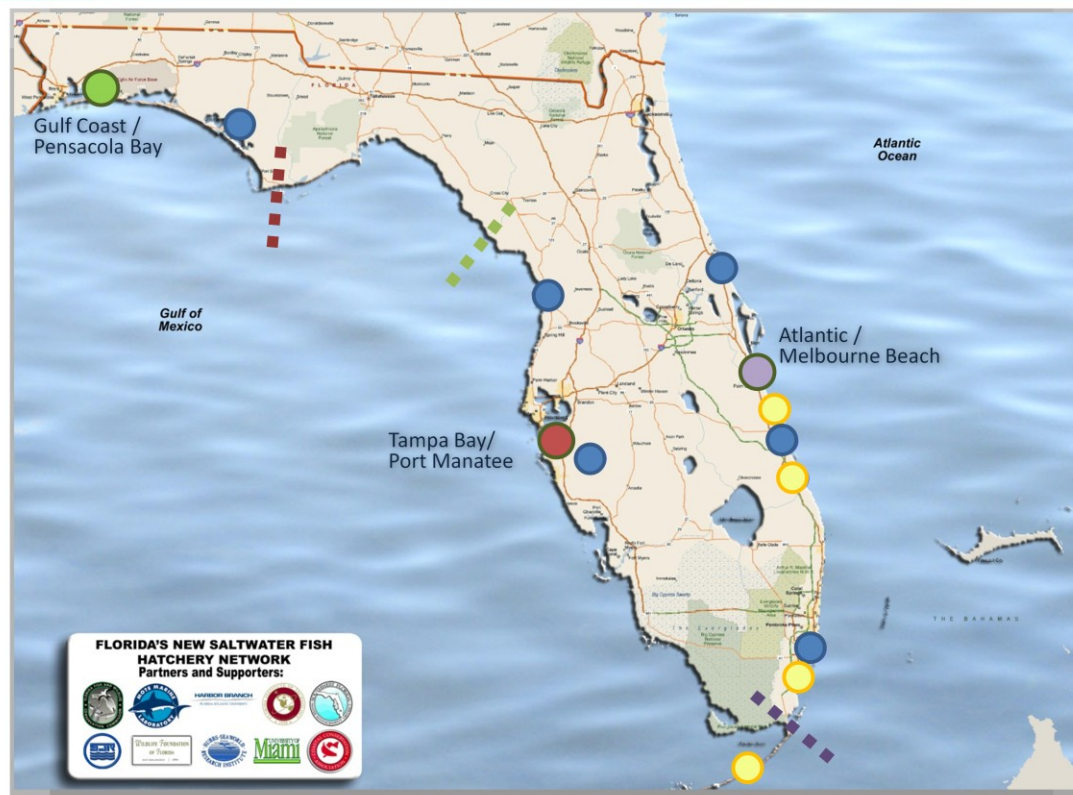
For example, for brood fish collected off of Tampa Bay, progeny could safely be stocked southward to Florida Bay and northward to the Gulf county-Franklin county border. Progeny of brood fish collected in Pensacola could be stocked eastward to Dixie county. GOM and Atlantic red drum stocks should not be intermixed.

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

Waples, R.S. 2006. *Conservation Genetics* 7:167-184

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.

Effective Size of Wild Red Drum

Data = <u>23,232</u> genotypes from fish ID'd as wild

- collected in PTB assessment area from 1999-2005

- not progeny of stocked fish

- 4 loci (screening assay); fully genotyped

- confidence in the analytical method

LdNe

From our Project Tampa Bay (PTB) genetic library, we have data from more that 23,000 wild red drum. We have used this huge database to estimate N_e as described.
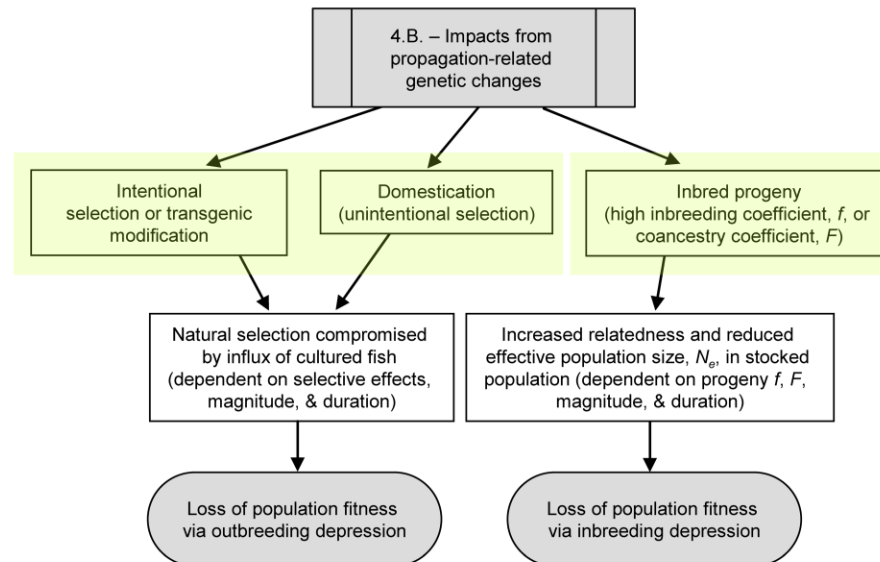
Result

Mating Model is random
Lowest Allele Frequency Used = 0.00100

Overall r^2 = 0.00005
Expected r^2 Sample = 0.00004

Estimated N_e = **48,581**

95% CIs for N_e
* Parametric 32,720
86,828

* Jackknife on Loci 32,595
87,564

LdNe

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.

Type II – Propagation-related

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.

The Project-Tampa-Bay Way

Tank	Mom 1	Mom 2	Mom 3
Dad 1	33	33	33
Dad 2	33	33	33
Dad 3	33	33	33
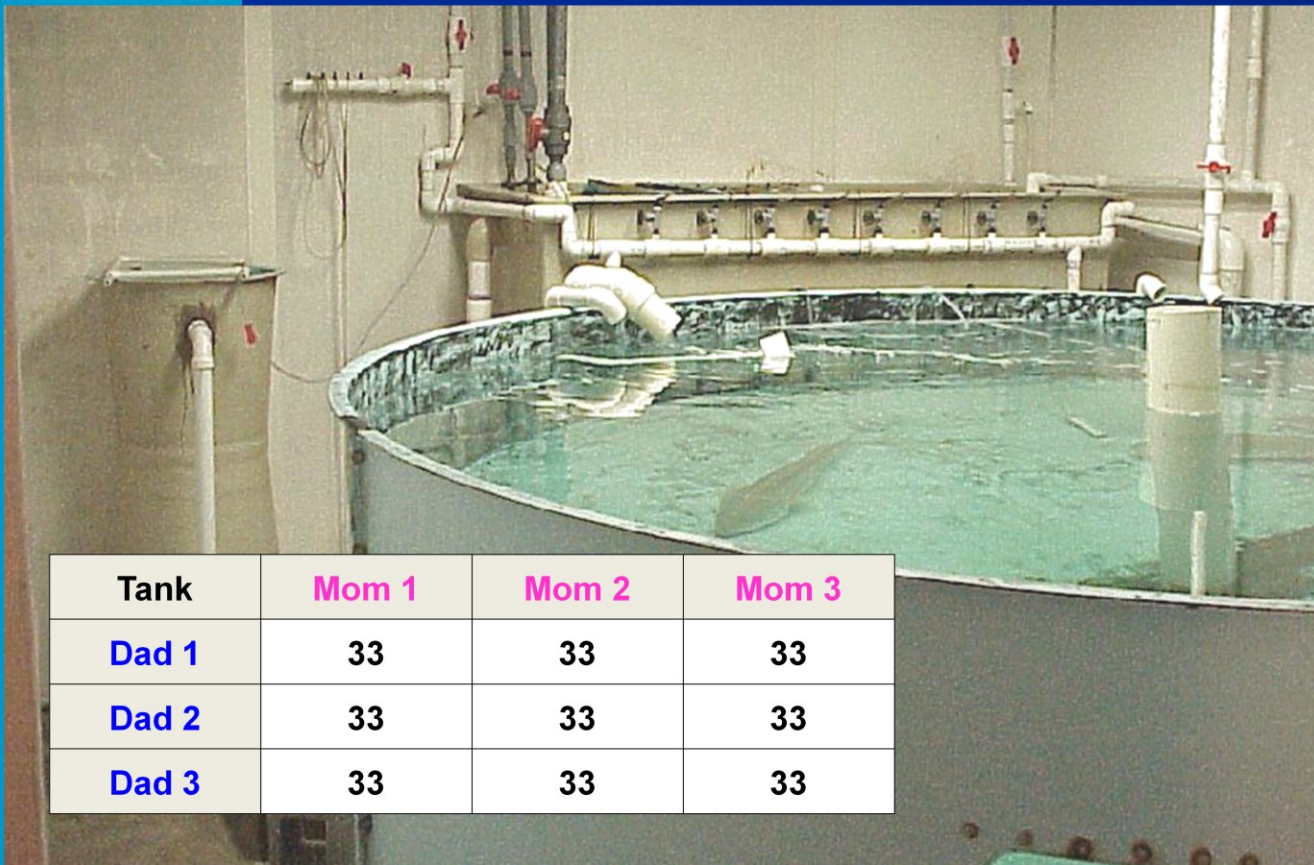
In PTB, male and female parents for mated in small breeding groups (6 fish). The spawns from several such groups were reared and released each season. Breeders were completely replaced each season. In a perfect world, the individual success of any one breeder would have been equal to that of the other breeders.

PTB 'Genetic Efficiency'

Data = Post-release recaps (≥2 months at-large; disease, stress, feeding)

F04-1	052 615 321	053 318 519	053 006 837
053 375 048	7	1	3
053 376 517	15	32	21
053 315 833	8	8	2

F04-3	051 613 114	051 637 016	052 264 076
052 087 276	15		20
051 818 777			
052 290 117	16		29

F04-8	041 615 863	053 016 566	052 337 609
051 620 542	3		87
053 365 018	6	31	1
053 302 577	3		

F04-9	052 032 534	052 341 879	052 884 540
051 773 354		14	37
051 893 548		43	11
053 800 810		8	18

Overall:

34 Tanks
79 Females
90 Males

2,225 Recaps

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.

Effective Size – standard estimate

$$N_{eI,C} = \frac{4}{\left[\dfrac{\sigma^2_{k,C} + k_C(k_C - 1)}{k_C(k_C n_C - 1)} \right] + \left[\dfrac{\sigma^2_{h,C} + h_C(h_C - 1)}{h_C(h_C m_C - 1)} \right]}$$

k_C	=	average number of progeny per female
$\sigma^2_{k,C}$	=	variance in female progeny production
n_C	=	number of females
Term	=	probability of shared maternity

This slide shows a commonly used method to <u>directly</u> estimate N_e (in this case, from a hatchery cohort, C) using parent-offspring data.

Effective size - adjusted

$$N_{eI,t} = 4 \div \left[\left[\frac{\sigma^2_{k,t-1} + k_{t-1}(k_{t-1}-1)}{k_{t-1}(N_{t-1}-1)} \right] \cdot [1 + F_{t-2} - 2\theta_{t-2}] + \left[\frac{\sigma^2_{h,t-1} + h_{t-1}(h_{t-1}-1)}{h_{t-1}(N_{t-1}-1)} \right] \cdot [1 + F_{t-2} - 2\theta_{t-2}] + 8\theta_{t-2} \right]$$

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.

Result

k_C	=	28.20
$\sigma^2_{k,C}$	=	3330.86
n_C	=	79
$P_{\text{shared maternity}}$	=	0.065

$$N_{eI,H} = 34.78$$

$$N_{eI,H*} = 32.24$$

h_C	=	24.76
$\sigma^2_{h,C}$	=	2154.50
m_C	=	90
$P_{\text{shared paternity}}$	=	0.050

Effective : Actual = 21%

For the 169 parents used in hatchling production for the PTB program, only 32-34 were "genetically effective" breeders.

Result

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

LdNe

This same result ($N_e \sim 34$) was generated using the 'linkage-disequilibrium' method described earlier.

Reason for the Result

012 068 287	16	052 297 348	3	052 770 089	1
015 077 301	12	052 278 544	1	051 852 529	1
014 852 078	4	041 358 592	23	052 015 374	0
012 541 268	0	012 376 890	3	052 262 082	245
014 562 026	2	041 340 526	4	052 615 321	30
7f7e bf7 b39	67	041 260 269	7	053 318 519	41
014 269 875	1	041 312 639	0	053 006 837	26
014 620 357	0	052 125 365	12	051 613 114	31
014 619 312	0	052 797 354	48	051 637 016	0
013 114 095	20	052 296 588	0	052 264 076	49
Crystal R. 4	1	052 853 854	0	041 615 863	12
014 116 795	0	053 304 608	4	053 016 566	31
013 542 532	2	053 359 089	0	052 337 609	88
041 260 269	0	053 627 007	0	052 032 534	0
041 371 301	33	052 345 558	1	052 341 879	65
016 613 112	328	052 297 620	0	052 884 540	66
041 383 529	58	052 581 828	0	017 008 634	0
015 099 560	111	052 859 367	0	014 375 357	0
041 344 125	45	051 879 319	11	015 430 008	0
052 125 788	122	052 063 597	131	015 620 578	0
041 528 637	0	052 023 558	0	013 769 020	0
041 513 892	222	053 371 799	0	016 049 773	0
041 324 068	0	052 049 020	0	011 871 809	1
041 377 838	86	052 781 797	0	014 768 807	0
040 886 334	0	053 321 795	114	015 372 373	44
052 273 310	1	041 332 071	1	041 326 595	0
017 258 539	3				

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.

New Design

$N_B = 60 \times 6.5$

$N_{eI,H*} = 185$

Effective : Actual = 50%
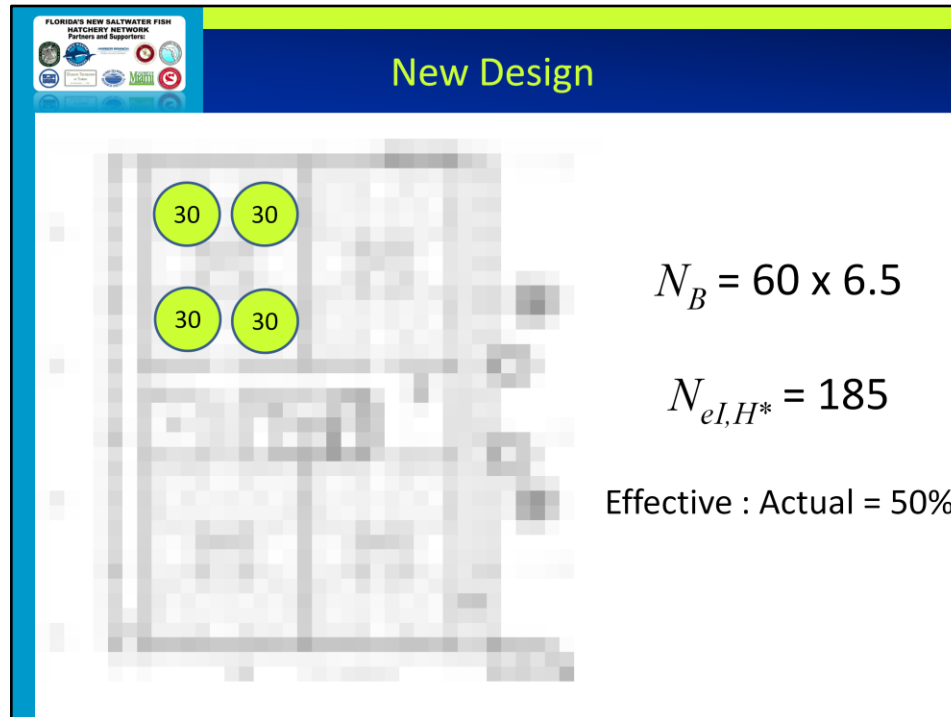
In the new brood-fish facility design, we expect that the genetic efficiency will be improved (from 21% to >50%). Because of larger tank sizes, we also have the capacity to use more brood fish (60+) each year.

Type III – Release Magnitude
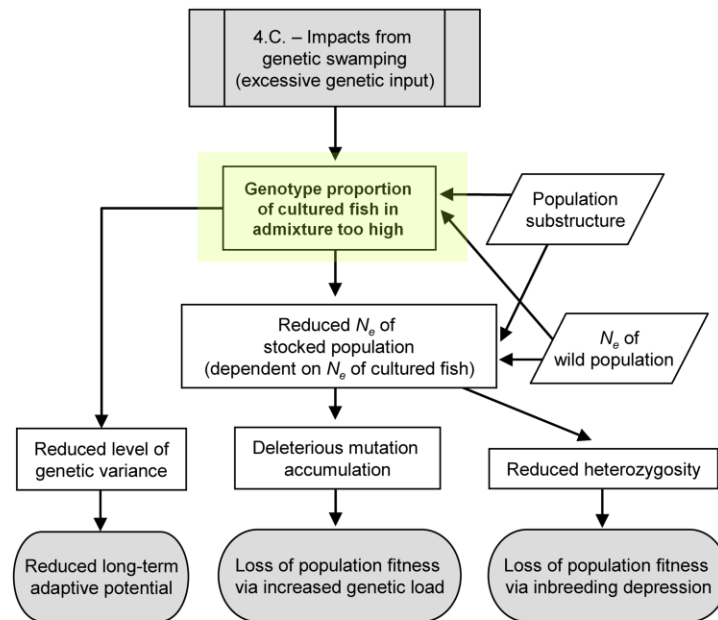
Finally, we consider the third type of genetic risk – i.e., those that stem from the release of too many fish. Notably these risk can accrue under some circumstances even when the brood fish source is appropriate and when selection and inbreeding has been avoided at the propagation stage.

Recapture Probabilities

ω_j = probability that two randomly chosen individuals were both derived from the *j*th spawning group

- 2 spawning groups ~ hatchery and wild

- Genetic ID of fin clips from fish ≥ 200 mm SL (low mortality; recruited to fishery)

- 11,231 of our 26K clips were from fish ≥ 200 mm SL

- Percentage of hatchery red drum was 2.6%

- Thus, $\omega_H = 0.026^2$; $\omega_w = 0.974^2$

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.

Mixture Model

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

$$= \left[\frac{(0.026)^2}{32} + \frac{(0.974)^2}{48,500} \right]^{-1} = 24,579$$

PTB expectation

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 ~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.

Mixture Model

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

$$= \left[\frac{(0.25)^2}{185} + \frac{(0.75)^2}{24,500} \right]^{-1} = 2,770$$

FMFEI expectation

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 Ne of ~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.

Project Tampa Bay

- **Empirically assess stocking strategies**

    - Release Location (among-river variability)

    - Release Habitat (within-river variability)

    - Release Season (in and out of sync with natural production)

    - Size-at-Release

Tringali et al. 2008. Marine Stock Enhancement in Florida. *Reviews in Fisheries Science* 16: 51-57

3rd ISSESR –Seattle, Sept 2006

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.
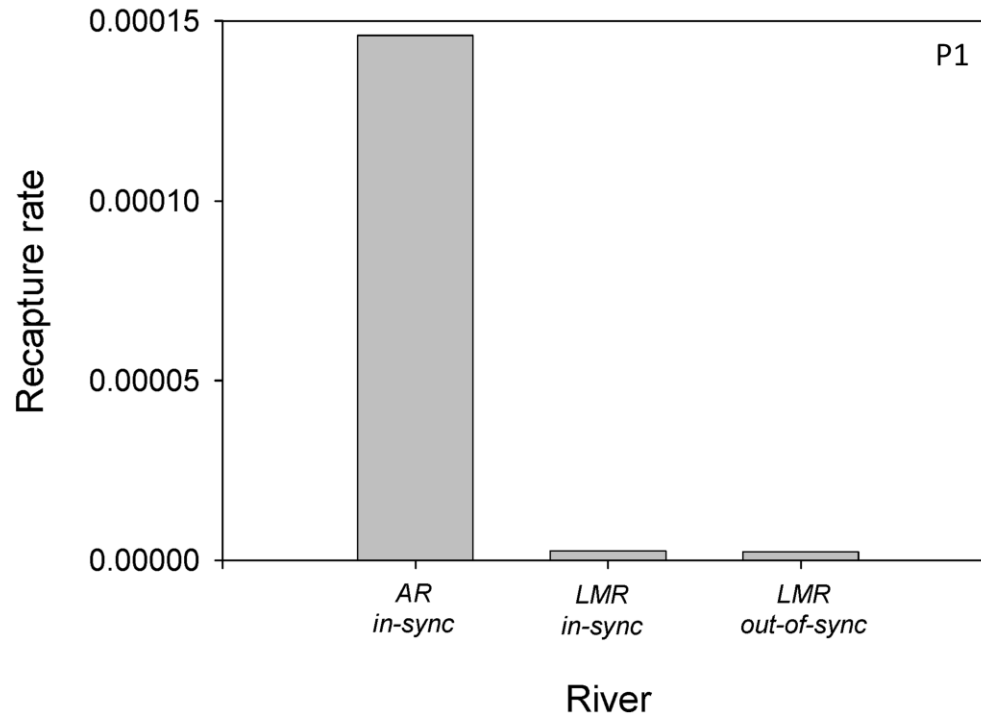
A total of 1,340,098 phase-1 fish were released in the Alafia River (AR). All AR fish were released in sync with natural production. A total of 2,386, 879 phase-1 fish were released in the Little Manatee River (LMR). LMR fish were released either in sync (n = 738,226) or out of sync (n = 1,648,953) with 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.

Release Size
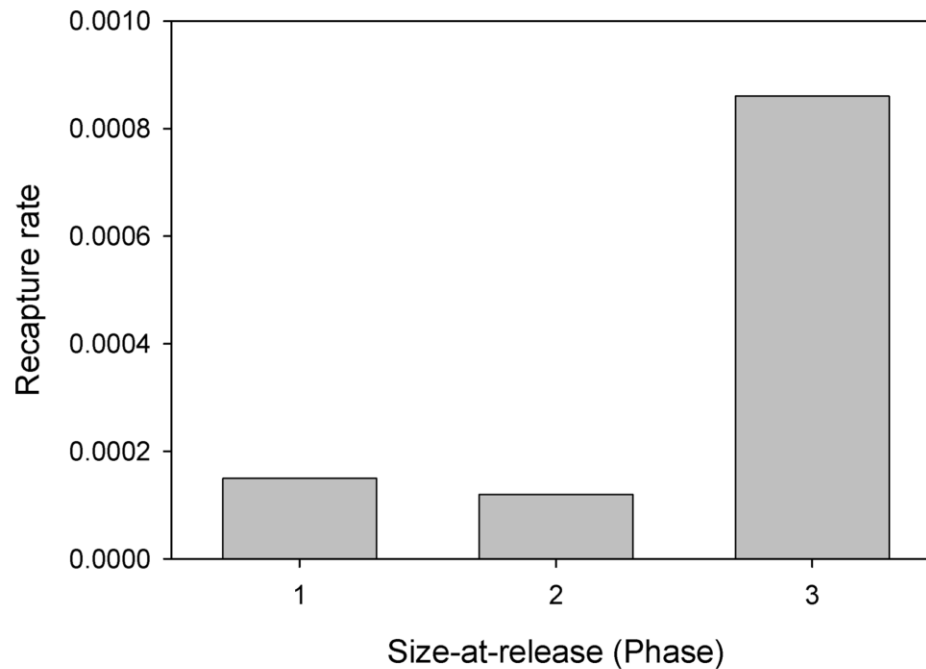
The effect of size-at-release on recapture rate was evaluated for releases in the Alafia River. Phase-1 fish = 25–45 mm standard length (SL) (~1 month old); phase-2 fish = 60–110 mm SL (~5 months old); phase-3 fish = 130–180 mm SL (~8 months old). Recapture rates for phase three fish were 6-7 times higher 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.

Release Habitat by Size
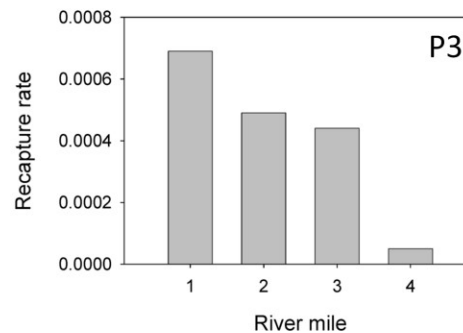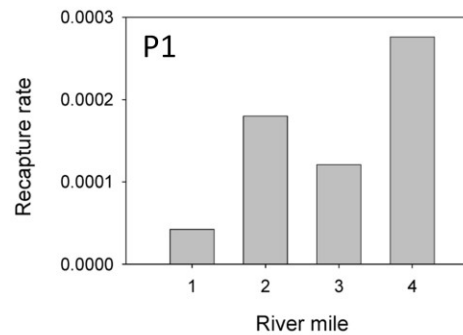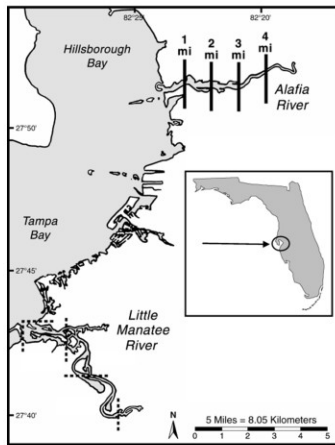
Spatial (microhabitat) trends evident in recapture rate and these varied by release size. Phase-1 fish survival was highest for those fish released in the 4th river mile of the AR. In contrast, phase-3 survival was highest for those released close to the river mouth. Habitat suitability and availability, as they relate to specific release-size classes, are important factors and should be included in research/assessment components of future efforts.

Other Presentation References

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Thanks