Guidance and Procedures for Genetic Requirements for Gulf Aquaculture Permits

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Purpose

To provide information on the requirements for broodstock sourcing, as well as information on genetic improvement techniques, for cultured juveniles stocked into offshore aquaculture facilities in the Gulf of Mexico (Gulf). The Fishery Management Plan for Regulating Offshore Marine Aquaculture in the Gulf of Mexico (FMP) and implementing regulations at 50 CFR § 622, Subpart F contain requirements pertaining to broodstock and cultured juveniles aimed at ensuring that escaped cultured animals present minimal genetic risk to the local wild stock from which they originated.

Background

NOAA Fisheries has the authority to issue Gulf Aquaculture Permits (GAPs) under the FMP. Final regulations for this FMP can be found at http://sero.nmfs.noaa.gov/sustainable_fisheries/gulf_fisheries/aquaculture/documents/pdfs/gulf_aquaculture_fmp_fr.pdf. The FMP, which was developed by the Gulf of Mexico Fishery Management Council (Council) under the authority of the Magnuson-Stevens Fishery Conservation and Management Act, requires a GAP for aquaculture operations in federal waters of the Gulf that intend to grow species managed by the Council (with the exception of shrimp and corals, which are not allowed).

A list of species allowed for culture in the Gulf can be found in at http://gulfcouncil.org/Beta/GMFMCWeb/downloads/species%20managed.pdf. ***Note*** that shrimp and coral species ***cannot*** be cultured under the FMP and regulations.

Requirements for Gulf Aquaculture Permit Holders

A. Broodstock Sourcing

Under the regulations, applicants must certify that all broodstock or progeny of such broodstock will be or were originally harvested from U.S. waters of the Gulf, will be or were harvested from the same population or sub-population that occurs where the facility is located,

and that no genetically engineered or transgenic animals will be used or possessed at the aquaculture facility.

The terms population and subpopulation are defined in the NOAA Fisheries Glossary[1] (Glossary) as follows:

> ***Population*** is defined as a number of individuals of a particular species that live within a defined area. It is equivalent to the term stock. Stock is defined in both the Glossary and the Magnuson-Stevens Fishery Conservation and Management Act (amended 2007; §3, 104-297(42)). Therein, a ***stock*** is 1) a part of a fish population usually with a particular migration pattern, specific spawning grounds, and subject to a distinct fishery or 2) a species, subspecies, geographical grouping, or other category of fish capable of management as a unit.
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> ***Subpopulation*** is defined as geographically or otherwise distinct groups in the population between which there is little exchange.

Other relevant fishery terms not defined here that may provide further context, if desired, include species, management (or conservation) unit (often equivalent to stock), and evolutionarily significant unit (see also distinct population segment).

Additional broodstock requirements and restrictions include:

- Permittees must submit certification to NOAA Fisheries that all original broodstock have been harvested from U.S. waters of the Gulf.
- Each individual brood animal must be marked or tagged (e.g., via a Passive Integrated Transponder (PIT), coded wire, dart, or internal anchor tag) at the hatchery to allow for identification of those individuals used in spawning.
- Permittees must submit fin clips for each individual brood animal to NOAA Fisheries. If permittees do not own or operate the hatchery, they must obtain a signed certification from the owner(s) of the hatchery indicating that this requirement has been met and furnish a copy of this certification to NOAA Fisheries. Procedures for procuring and submitting fin clips can be found in Appendix B.
- Permittees must submit certification to NOAA Fisheries that no genetically engineered or transgenic animals are used or possessed at the aquaculture facility.[2] A ***genetically***

[1] National Oceanic and Atmospheric Association (NOAA) (2006) NOAA Fisheries Glossary, Revised Edition. United States Department of Commerce, NOAA, Technical Memorandum NMFS-F/SPO-69.

[2] *Aquaculture facility* means an installation or structure, including any aquaculture system(s) (including moorings), hatcheries, equipment, and associated infrastructure used to hold, propagate, and rear allowable aquaculture species in the Gulf EEZ under authority of a GAP.

engineered animal is defined as an animal modified by recombinant DNA (rDNA) techniques, including the entire lineage of animals that contain the modification. The term genetically engineered animal can refer to both animals with heritable rDNA constructs and animals with non-heritable rDNA constructs (e.g. modifications intended for gene therapy). A ***transgenic*** animal is defined as an animal whose genome contains a nucleotide sequence that has been intentionally modified in vitro, and the progeny of such an animal. ***Note*** that an animal that has been altered such that its ploidy has been changed (e.g., a triploid animal) is not considered to be genetically engineered, provided that that animal does not contain genes that have been introduced or otherwise altered by modern biotechnology.

- F_1 individuals (i.e., first generation offspring of original wild-caught broodstock) can be used for broodstock purposes without further justification. Permittees who wish to use F_{2+} individuals (i.e., second or higher generation offspring bred in captivity) for broodstock purposes must first submit a genetics management plan to NOAA Fisheries for review and approval. This plan must include a risk assessment. Supporting information may include results from modeling (e.g., OMEGA[3]), pedigree analysis (e.g., using P-LOCI[4] to track parentage), population genetic analyses, certification of sterility in the stocked animals (e.g., via triploidy), or other applicable data.
- When using the offspring of original wild caught broodstock as broodstock, permittees must still abide by all requirements outlined above and in the regulations.

NOAA Fisheries anticipates that the following four species will be initially targeted for offshore aquaculture in the Gulf: almaco jack (*Seriola rivoliana*), cobia (*Rachycentron canadum*), red drum (*Sciaenops ocellatus*), and red snapper (*Lutjanus campechanus*). Appendix A includes guidelines for sourcing broodstock for these species in relation to the geographic location of the aquaculture facility. These guidelines are based on the best available science at this time and may be modified in the future.

[3] The Offshore Mariculture Escapes Genetics Assessment (OMEGA) model is freely available at http://www.nmfs.noaa.gov/aquaculture/science/omega_model_homepage.html.

[4] Matson S.E., M.D. Camara, W. Eichert, M.A. Banks. 2008. P-LOCI: a computer program for choosing the most efficient set of loci for parentage assignment. Molecular Ecology Resources 8:765-768.

B. Genetic Improvement Techniques

Genetic improvement is a process through which the incidence or expression of desirable traits (e.g., improved growth, higher product quality, resistance to stress or diseases) are increased in a cultured population.

Genetic improvement programs that include the use of genetic engineering or transgenics are **prohibited** (see definitions of ***genetically engineered*** and ***transgenic*** above). Allowable genetic improvement techniques may include one or more of the following: selective breeding, chromosome manipulation, hybridization, and sex control. These terms are defined and described below.

1) ***Selective breeding*** is a process by which animals are intentionally bred to produce progeny with desirable traits. Selective breeding is an often long-term process, with potentially permanent heritable genetic gains, as each generation of broodstock is selected based on desired characteristics and individuals are interbred in a controlled manner. Selective breeding programs commonly focus on traits such as growth rate, survival, stress tolerance, disease resistance, and meat quality and yield.

2) ***Chromosome manipulation*** is a modification of the number, identity, or origin of chromosomes within somatic or sex (typically egg) cells. Examples of this technique include induction of polyploidy and maintaining inbred lines.

    a. Polyploidy

    Triploidy is the most commonly produced polyploid state in aquaculture. Triploid animals contain three sets of chromosomes in their somatic cells. Triploid animals are often sterile, which can be an effective management tool for protecting wild populations by preventing reproduction with farmed conspecifics. Moreover, with triploid-induced sterility, physiological resources are used for bodily maintenance and growth rather than producing eggs and sperm, which can result in improved growth, survival, and meat quality.

    b. Inbred lines

    The making of inbred lines involves the creation of genetically identical or nearly identical populations. This technique can be used to produce large numbers of offspring with specific desirable characteristics in one generation by making multiple copies of high performance or selectively bred individuals. Maintenance of inbred lines may be coupled with hybridization (see below) to produce superior characteristics in an F_1 generation (i.e., hybrid vigor).

3) ***Hybridization*** occurs when genetically distinct individuals are crossed to produce heterozygous offspring, which contain two different alleles at a given gene or genes. Hybridization between different breeds, strains, or varieties of the same species (intraspecific) is allowed. Hybridization between species (interspecific) is prohibited. Hybridization may result in heterosis, or hybrid vigor, in which heterozygous offspring display enhanced performance (usually growth). Because heterosis requires hybridization, its effect is often restricted to the F_1 generation and not heritable. Therefore, ensuring a consistent supply of heterotic F_1 individuals requires the maintenance of multiple strains at the aquaculture operation.

4) ***Sex control*** means manipulating sex determination or sex ratio, typically with skew toward a monosex culture. Controlling sex may allow for more efficient exploitation of desirable sex-specific traits.

APPENDIX A: Species-Specific Requirements for Sourcing Wild Broodstock[5]

These guidelines are based on the best available science at this time and may be modified in the future if additional scientific data becomes available. For species that are allowed to be cultured under the regulations, but are not specified in this Appendix, permittees must provide NOAA Fisheries with information supporting the proposed collection range. NOAA Fisheries will use this information to determine whether or not the proposed collection range is suitable.

Permittees must submit a *Request to Harvest Broodstock* form to NOAA Fisheries at least 30 days prior to each time a permittee or their designee intends to harvest broodstock from the EEZ or state waters. NOAA Fisheries must approve any broodstock harvest activities before harvest can occur. This form can be found at http://sero.nmfs.noaa.gov/operations_management_information_services/constituency_services_branch/permits/permit_apps/.

Almaco jack (*Seriola rivoliana)*

There are no studies of population genetic structure in almaco jack in the Gulf or elsewhere. Other commonly cultured seriolids include Japanese amberjack (*S. quinqueradiata*), greater amberjack (*S. dumerili*), and yellowtail amberjack (*S. lalandi*). Population genetic studies in these species show little to no divergence within water masses, similar to other pelagic finfish, such as tuna and billfish. For example, Gold and Richardson (1998a[6]) found evidence of two stocks of greater amberjack off the southeastern U.S., one in the northern Gulf and a second along the western Atlantic coast. Thus, research to date in closely related species indicates that almaco jack within the Gulf may be a single panmictic population.

Collection Range: Wild almaco jack broodstock may be collected within U.S. state or federal waters of the Gulf.

[5] Broodstock collection requirements listed for almaco jack, cobia, red drum, and red snapper only.
[6] Gold JR, Richardson LR (1998a) Population structure in greater amberjack, *Seriola dumerili*, from the Gulf of Mexico and western Atlantic Ocean. Fish Bull 96:767-778.

Cobia (*Rachycentron canadum)*

Gold et al. (2013)[7] found no evidence of structure among western US Atlantic and northern Gulf populations. Thus, research to date indicates that cobia within the Gulf may be a single panmictic population.

Collection Range: Wild cobia broodstock may be collected within U.S. state or federal waters of the Gulf.

Red drum (*Sciaenops ocellatus)*

Gold et al. (1993[8], 1994[9], 1999[10]) and Seyoum et al. (2000[11]) reported weak genetic divergence between Atlantic and Gulf populations. In the northern Gulf alone, Gold et al. (1999[12]) found isolation by distance (positive correlation between genetic and geographic distance), possibly attributable to sex-specific behaviors, and suggested a geographic neighborhood size relative to genetic migration of 500-600 km. Gold and Turner (2002[13]) reported similar results, with a neighborhood size of 700-900 km. Most recently, tagging studies in the Tampa Bay region indicated fairly high spawning site fidelity (~60%) and natal homing, although there was some mixing with a population 132 km to the south and another ~30-40% of tagged fish presumably spawned out of the range of monitoring.[14] Although this level of migration outside of the monitored region would homogenize allele frequencies across a broader geographic range, the known migratory radius is therefore 132 km. Thus, research to date suggests red drum display a minimum geographic neighborhood size of roughly 260 km.

Collection Range: Wild red drum broodstock may be collected within an 82 mile (~132 km radius) of the site of the permitted aquaculture operation.

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[7] Gold JR, Giresi MM, Renshaw MA,Gwo J-C (2013) Population genetic comparisons among cobia from the northern Gulf of Mexico, U.S. western Atlantic, and southeast Asia. N Am J Aquacult 75:57-63.

[8] Gold JR, Richardson LR, Furman C, King TL (1993) Mitochondrial DNA differentiation and population structure in red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. Mar Biol 116: 175-185.

[9] Gold JR, King TL, Richardson LR, Bohlmeyer DA, Matlock GC (1994) Allozyme differentiation within and between red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. J Fish Biol 44: 567-590.

[10] Gold JR, Richardson LR, Turner TF (1999) Temporal stability and spatial divergence of mitochondrial DNA haplotype frequencies in red drum (*Sciaenops ocellatus*) from coastal regions of the western Atlantic Ocean and Gulf of Mexico. Mar Biol 133:593-602.

[11] Seyoum S, Tringali MD, Bert TM, McElroy D, Stokes R (2000) An analysis of genetic population structure in red drum, *Sciaenops ocellatus*, based on mtDNA control region sequences. Fish Bull 98:127-138.

[12] Gold JR, Richardson LR, Turner TF (1999) Temporal stability and spatial divergence of mitochondrial DNA haplotype frequencies in red drum (*Sciaenops ocellatus*) from coastal regions of the western Atlantic Ocean and Gulf of Mexico. Mar Biol 133:593-602.

[13] Gold JR, Turner TF (2002) Population structure of red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico, as inferred from variation in nuclear-encoded microsatellites. Mar Biol 140:249-265.

[14] S Lowerre-Barbieri, Florida Fish and Wildlife Conservation Commission, personal communication.

Red snapper (*Lutjanus campechanus)*

Several studies have found no evidence of red snapper population genetic structure in the Gulf (e.g., Gold and Richardson 1998b[15], Garber et al. 2004[16], Pruett et al. 2005[17]) despite evidence of relative site fidelity of adults and homing in juveniles from tagging (e.g. Szedlmayer 1997[18], Workman et al. 2002[19]). More recent work employing genetics, tagging, and otolith microchemistry, however, suggests a metapopulation stock structure in which semi-independent, local populations are variably connected by migration, extinction, and recolonization (Pruett et al. 2005[20], Patterson 2007[21], Saillant et al. 2010[22]; see also Smedbol et al. 2002[23]). Patterson (2007), for example, found that while many adults display site fidelity, some may move hundreds of km, and larger fish moved greater distances than smaller and younger fish. These non-equilibrium conditions may homogenize allele frequencies among populations, accounting for the lack of stock structure in earlier research.

Stock assessments for red snapper treat the species as two relatively independent stocks separated by the Mississippi River[24], a conclusion putatively based on otolith elemental signatures (Patterson et al. 1998[25]; Cowan et al. 2002[26]; Patterson et al. 2008[27]). However, this is based on water mass signatures and may not reflect smaller

[15] Gold JR, Richardson LR (1998b) Genetic homogeneity among geographic samples of snappers and groupers: evidence of continuous gene flow? Proc Gulf Caribbean Res Inst 50:709-726.
[16] Garber, AF, Tringali MD, Stuck KC (2004) Population structure and variation in red snapper (*Lutjanus campechanus*) from the Gulf of Mexico and Atlantic Coast of Florida as determined from mitochondrial DNA control region sequence. Mar Biotechnol 6:175-185.
[17] Pruett CL, Saillant E, Gold JR (2005) Historical population demography of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico based on analysis of sequences of mitochondrial DNA. Mar Biol 147:593-602.
[18] Szedlmayer ST (1997) Ultrasonic telemetry of red snapper, *Lutjanus campechanus*, at artificial reef sites in the northeast Gulf of Mexico. Copeia 1997:846-850.
[19] Workman I, Shah A, Foster D, Hataway B (2002) Habitat preferences and site fidelity of juvenile red snapper (*Lujanus campechanus*). ICES J Mar Sci S43-S50.
[20] Pruett CL, Saillant E, Gold JR (2005) Historical population demography of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico based on analysis of sequences of mitochondrial DNA. Mar Biol 147:593-602.
[21] Patterson III WF (2007) A review of movement in Gulf of Mexico red snapper: implications for population structure. Am Fish Soc Symp 60:221-235.
[22] Saillant E, Bradfield SC, Gold JR (2010) Genetic variation and spatial autocorrelation among young-of-the-year red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. ICES J Mar Sci 67:1240-1250.
[23] Smedbol RK, McPherson A, Hansen MM, Kenchington E (2002) Myths and moderation in marine 'metapopulations'? Fish Fisheries 3:20-35.
[24] C Porch, NOAA Fisheries Southeast Fisheries Science Center, personal communication.
[25] Patterson III WF, Cowan Jr JH, Graham EY, Lyons WB (1998) Otolith microchemical fingerprints of age-0 red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico. Gulf of Mexico Science 16:83– 91
[26] Cowan Jr JH, WoodsM, Patterson III W, Nieland D (2002) Otolith microchemistry (and reproductive biology) *In*: Stock structure of red snapper in the northern Gulf of Mexico: is their management as a single stock justified based on spatial and temporal patterns of genetic variation, otolith microchemistry, and growth rates. National Marine Fisheries Service, Marine Fisheries Initiative (MARFIN) Grant NA87FF0425.
[27] Patterson III WF, Cowan Jr JH, Wilson CA, Chen Z (2008) Temporal and spaitial variability in juvenile red snapper otolith elemental signatures in the northern Gulf of Mexico. Trans Am Fish Soc 137:521-532.

scale population heterogeneity. In terms of genetics, Saillant et al. (2010) reported significant spatial autocorrelation among young-of-the-year at ~50-100 km, with a potential isolation by distance effect at < 100 km and patchiness at > 100 km, which indicates largely local recruitment with restricted dispersal, and concluded that management should maintain local spawning populations throughout the Gulf.

Most recently, Gold and Portnoy (2014[28]) found genetic heterogeneity among northern Gulf populations, indicating that the species is not a single panmictic stock. Thus, research to date suggests red snapper display a metapopulation stock structure, although the structuring is weak and geographic stock boundaries have yet to be determined, with the most definitive genetic research suggesting greater potential for genetic similiary within a neighborhood of roughly 200 km.

Collection Range: Wild red snapper may be collected within a 62 mile (~100 km) radius of the site of the permitted aquaculture operation.

[28] Gold JR, Portnoy DS (2014) Population structure and genetic demography of red snapper (*Lutjanus campechanus*) in the U.S. south Atlantic and connectivity with red snapper in the Gulf of Mexico. Southeast Data, Assessment & Review (SEDAR) Report SEDAR41-RD32.

APPENDIX B: Procedures for Collecting Broodstock Fin Clip Samples

Purpose

Permittees are required to submit fin clip samples to NOAA Fisheries for each brood animal used in spawning. This requirement will allow for identification of source broodstock and for comparison of broodstock to offspring stocked into offshore cages. It will also allow for enforcement and monitoring in the event that the use of genetically modified or transgenic organisms is suspected.

Fin clip samples should be collected prior to, or immediately following, spawning events and should be sent to NOAA Fisheries within 30 days of collection. Fish are to be sexed and each brood animal is required to be individually marked or tagged (e.g., PIT, coded wire, dart). For additional information or questions, please contact NOAA Fisheries at 727-824-5301 or nmfs.ser.aquaculture@noaa.gov.

Procedures

Follow these steps to obtain a fin clip sample:

1) Clean all instruments used to extract samples with ethanol. Remove dirt and any visible parasites from tissues as these can affect genetic analyses. Obtain two hole punches or one dime-sized sample of the fin from each brood animal. Clean all instruments with ethanol between samples to minimize sample cross-contamination.

2) Place hole punch samples from each fish into separate clean vials (or, cut dime sized sample into half and place into separate vials). Fill each vial with enough 70-100% non-denatured ethanol[29] to cover the sample and store the sample in a freezer (-20°C to -80°C) until it is shipped to NOAA Fisheries. ***Note****:* Samples are to be sent to NOAA Fisheries within 30 days of collection.

3) Using a permanent marker, clearly label each vial with an ID# specific to the brood animal (e.g., PIT tag number, sequential number). Each ID# should be logged on the *Fin Clip Log* spreadsheet with all required information for that animal. The Fin Clip Log spreadsheet can be found at http://sero.nmfs.noaa.gov/sustainable_fisheries/gulf_fisheries/aquaculture/. Permittees should store samples from each animal in a freezer (-20°C to -80°C).

[29] A license is required to purchase non-denatured ethanol as this is listed as a controlled substance.

4) Send one sample from each fish along with the *Fin Clip Log* spreadsheet to NOAA Fisheries. Include a completed chain of custody form with each shipment. Contact NOAA Fisheries at least 24 business hours prior to shipping to coordinate receipt of samples. Samples should be shipped early in the week to ensure that someone is available to receive the package during normal business hours. Pack samples in excepted quantities and ship according to hazardous materials guidelines[30]. Permittees should store the other half of the sample (or second hole punch) from each fish at their facility in a freezer (-20^{o}C to -80^{o}C) as a back-up.

[30] Federal rules have been established which govern the shipment of ethanol. Please consult with your shipping company regarding any special instructions.