



Oyster Biosecurity and Genetic Integrity Protocols

1. Oyster Transport Authorization Requirements

Texas Parks and Wildlife Department (TPWD) must be notified no later than seven (7) days before oyster seed or oysters are imported, stocked, or moved from one site to another, both in-state and out-of-state. An Oyster Transport Request Form (PWD 1439F-V3400) along with any accompanying documents such as Oyster Health Certificate (Section 4), broodstock verification, or ploidy verification (Section 5) must be submitted for all transport of oysters or seed between farms, hatcheries, nurseries, and research facilities. TPWD will review the documents and then issue an Oyster Transport Authorization Letter if all transfer requirements are met. All transfer of seed and oysters must conform to the broodstock region guidance below (Section 5).

- The TPWD Oyster Transport Request Form (PWD 1439F-V3400) asks for the following information:
 - Name / address of applicant (recipient; nursery or grow-out permit number)
 - Name / address of source facility (e.g., out-of-state hatchery)
 - Name/ address of destination (stocking site or facility)
 - Life stage (e.g., larvae, seed) and size (mm)
 - Quantity of each
 - Geographic origin of all broodstock
 - If broodstock was harvested by the permittee under a broodstock permit, the bay system, geographic coordinates, date, and number of broodstock organisms harvested should be reported, as well as the date the broodstock was sent to the hatchery for spawning
 - If broodstock was purchased and sent to a hatchery, the bay system, harvest area, harvest date, and number of broodstock should be reported, as well as the date the broodstock was sent to the hatchery for spawning

2. In-State Hatchery Transfer Requirements

In-state hatcheries must comply with broodstock region guidance (Section 5) and disease testing requirements (Section 4). Out-of-region and out of bay system importation may be subject to disease testing prior to importation. Genetic variants (broodstock, larvae, and seed from differing broodstock regions) shall not comingle. No oysters from the Atlantic or Pacific coast will be permitted in Texas oyster hatcheries. Broodstock (and resulting spawned larvae and seed) from other states shall not comingle with Texas variants. If a hatchery contains stock from a genetic region differing from the location of the hatchery (e.g., a hatchery in the southern region containing broodstock from the northern region), wastewater must be disposed of in a way to ensure no genetic escapement into the bay. Broodstock and seed importation must follow the importation guidelines established in Section 1 & 3. An Oyster Transport Request Form (PWD 1439F-V3400) must be provided to TPWD prior to any importation.

3. Out-of-State Seed or Oyster Importation/Transfer Requirements

Each time seed is imported from an out-of-state hatchery/nursery, a signed copy of the Oyster Health Certificate (Section 4), broodstock verification, chain-of-custody documentation, and an Oyster Transport Request Form (PWD 1439F-V3400) must be provided to TPWD prior to seed or oyster importation. Written authorization from the Department must be received prior to the importation and stocking of diploid, triploid, or tetraploid larvae, seed or broodstock into Texas waters.

Out-of-state hatcheries/nurseries can only be used if their products are certified as disease-free

(Section 4) and genetic integrity of the oysters is preserved (Section 5). Out-of-state hatcheries/nurseries must be screened and approved for use by TPWD.

- Out-of-state hatcheries/nurseries must be in the Gulf of Mexico region.
 - Because of the known threat of introduction of MSX from oyster stocks grown in the waters of the Atlantic Ocean or drainages into the Atlantic Ocean, the sale of oyster seed, larvae or broodstock from Atlantic Coast waters is prohibited for use in Texas coastal waters.
 - Because of the known threat of introduction of Oyster Herpes Virus (OsHV-1) from oyster stocks grown in the waters of the Pacific Ocean or drainages into the Pacific Ocean, the sale of oyster seed, larvae or broodstock from Pacific Coast waters is prohibited for use in Texas coastal waters.
- Out-of-state hatcheries/nurseries will not be approved for use if they accept oysters from the Atlantic or Pacific coast – only hatcheries that work exclusively with Gulf oysters will be permitted.
- Hatcheries/nurseries will not be approved for use if their culture system allows for the intermingling of Texas-bound product with water (effluent) from organisms from the Atlantic or Pacific Coast.
- Hatcheries/nurseries will not be approved for use if their culture practices do not maintain the genetic identity of Texas oysters. If seed is transferred from a hatchery to a nursery, records of maintained chain-of-custody and genetic identity must be provided.

4. Texas Oyster Disease Testing Requirements

Oyster seed, larvae, or broodstock that are to be used for mariculture purposes coming from out-of-state must be tested for pathogens using two types of testing at a Department approved laboratory (Table 1). Oysters seed, larvae, or broodstock transferring between bay systems in Texas may be subject to disease testing dependent on best available disease information on the area they are transferring from. Each batch (same seed lot, same producer, and held in the same environment) must be tested within 60 days before transfer and an Oyster Health Certificate presented to TPWD. Only batches of oyster seed, larvae or broodstock found to have less than 10% prevalence of Dermo and zero prevalence of other parasites or disease shall be approved for introduction for mariculture purposes. Please note that wild oysters transferred and held in public water under an Introduction Permit need to be disease tested at TPWD discretion when moved between bays, even if not used in mariculture; more details on the specifics will be outlined in their specific Introduction Permit.

Pathogens to be screened for:

- MSX (*Haplosporidium nelsoni*)
- Dermo (*Perkinsus marinus*)
- Bonamiosis (*Bonamia exitiosa*)
- Oyster Herpes Virus (OsHV-1)

Methods to be used:

1. Histological
 - i. A representative sample (n = 60) of the seed, larvae or broodstock must be histologically processed, producing microscope slides showing all major tissue types.
 - ii. Slides must be read by an invertebrate pathologist.
 - iii. All findings of pathogens (MSX, Dermo, Bonamiosis and others) or commensals found within the tissue shall be reported.
2. Rays Fluid Thiglycollate Media (RTFM), PCR or qPCR
 - i. A representative sample (n = 60) of seed, larvae or broodstock must be tested for the presence of *Perkinsus marinus* (Dermo)

Oyster Health Certificate

- An Oyster Health Certificate shall be submitted to TPWD for review and approval before oysters are transferred. The Oyster Health Certificate should include:
 - Dated and detailed pathology report, including the results for each pathology test, and a description of the methods used
 - Name of the hatchery/nursery where the seed is located
 - Certification case number
 - Certification date

5. Genetic Integrity Protocols

Genetic Regions

There are two distinct genetic populations of Eastern oysters in Texas that overlap in the Corpus Christi and Aransas Bay estuarine systems (Anderson et al. 2014) (Figure 1).

- Northern Region – Texas-Louisiana state line to San Antonio Bay
- Southern Region – Upper Laguna Madre to the Texas-Mexico border
- Mixing Zone – Aransas Bay system and Corpus Christi Bay system

This genetic distinction between populations must be incorporated into the management and use of seed oysters in Texas' Cultivated Oyster Mariculture Program. Care must be taken to protect the genetic integrity of Texas oyster populations by ensuring oyster seed are placed into waters compatible with broodstock origin (Figure 2). Documentation of maintained genetic identity and chain-of-custody must be provided to the Department for all oyster seed, larvae, or broodstock used for mariculture purposes or that is intended to be stocked into Texas waters in any way. This documentation also pertains to transfers that occur before importation and/or stocking to Texas waters, such as transfers from a hatchery to a nursery.

Texas Diploid, Triploid/Tetraploid Oyster Seed Requirements

Oyster seed used in Texas oyster mariculture operations must come from Texas broodstock that comes from a compatible population region (Figure 1 & 2). Documentation of broodstock origin must be obtained from the hatchery and provided to the department for transport approval. Ploidy testing results and methods used must also be provided for triploid/tetraploid seed.

Diploid Seed

For diploid seed oysters to be stocked into the state of Texas the following is required:

- For diploid seed to be deployed in the northern region estuaries of Texas, broodstock must originate from the northern region - Texas waters between the Texas-Louisiana state line to San Antonio Bay
- For diploid seed to be deployed in southern region estuaries of Texas, broodstock must originate from the southern region - Texas waters between Upper Laguna Madre to the Texas-Mexico border.
- For diploid seed to be deployed in the mixing zone, broodstock can originate from Texas estuaries in any region.
- Oysters from the mixing zone can only be used as broodstock for seed that will be deployed in the mixing zone - Aransas Bay system or Corpus Christi Bay system. Mixing zone oysters cannot be used as broodstock for any other region bay systems.

Triploid/Tetraploid Seed or Broodstock

For polyploid (3N or 4N) gametes, seed or broodstock oysters to be used for mariculture in the state of Texas the following is required:

- Description of methods used to create the polyploid (e.g., tetraploid X diploid or chemical induction of triploid, etc.)
- Ploidy (3N, 4N)
- Ploidy testing results from a representative sample of seed purchased and description of how ploidy of seed was tested
- For triploid seed to be deployed in the northern region estuaries of Texas, triploid seed must be either
 - (1) a crossbetween an established Gulf of Mexico tetraploid broodstock line and diploids from the northern region Texas estuaries (Texas-Louisiana state line to San Antonio Bay),
OR
 - (2) a cross between northern region Texas estuary broodstock diploids that have been chemically induced to the triploid condition.
- For triploid seed to be deployed in the southern region estuaries of Texas, all broodstock must originate entirely from southern region Texas estuaries (Upper Laguna Madre south to Lower Laguna Madre).
 - Triploids produced by crossing southern Texas estuary diploids with tetraploids from the northern Texas region or any other state cannot be used to stock farms in the southern estuaries of Texas (Upper Laguna Madre to the Texas-Mexico border).
- Triploid seed deployed in the mixing zone can be either
 - (1) a cross between an established Gulf of Mexico tetraploid broodstock line and a diploid from Texas estuaries in any region,
OR
 - (2) a cross between any region Texas estuary broodstock diploids that have been chemically induced to the triploid condition.



Figure 1 Map of oyster genetic regions in Texas.

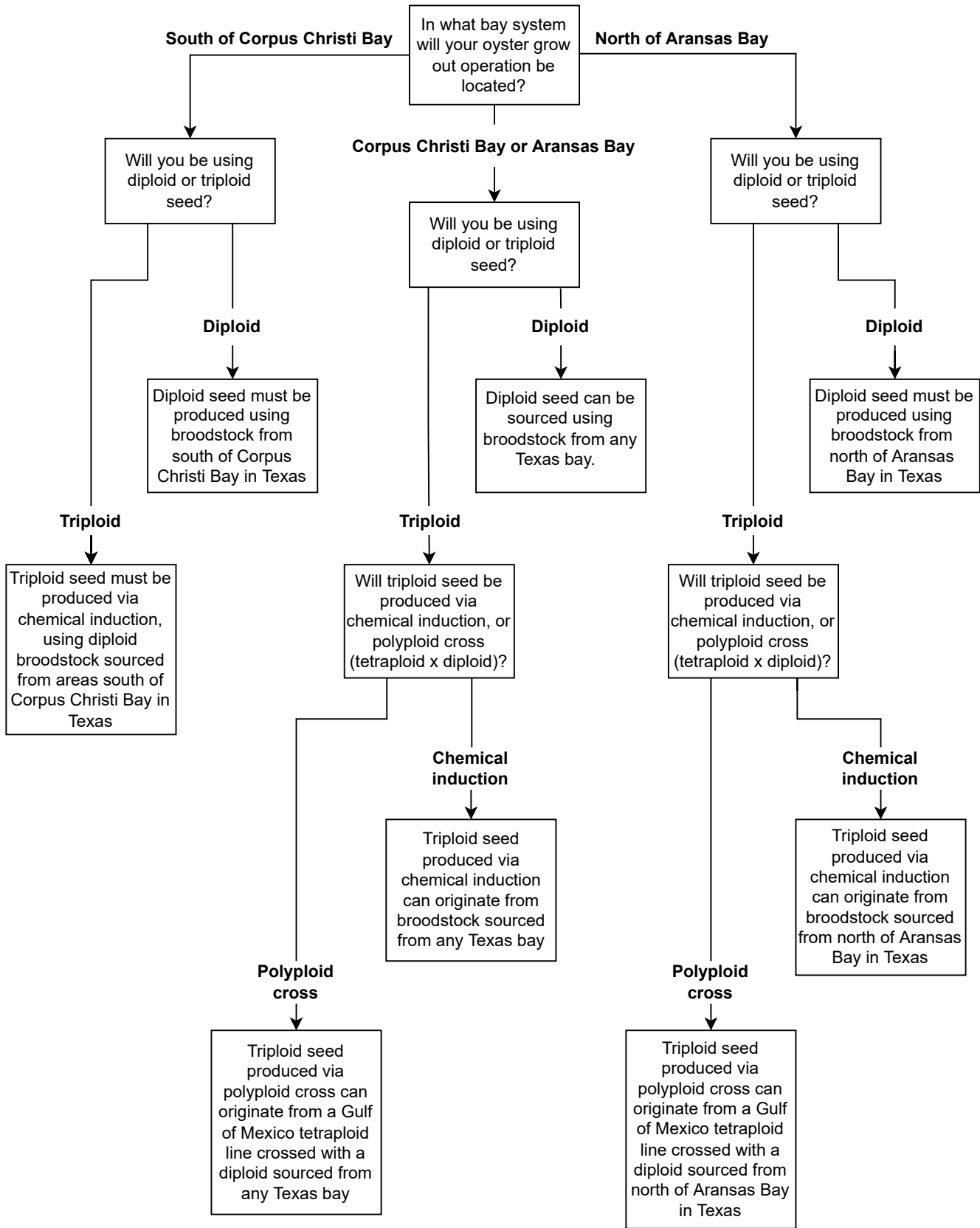


Figure 2. Flowchart of genetic regional requirements for broodstock, ploidy, and seed.

Table 1. Disease testing laboratories. This is not an exhaustive list of all laboratories, if there is a laboratory not on this list you would like to use please check with TPWD as to its acceptability.

Facility	NC State Univ.- Center of Marine Science & Technology	Rutgers NJ Haskin Shellfish Research Lab.	Roger Williams Univ. Aquatic Diagnostics. Laboratory	FAU Harbor Branch Oceanic Institute	Virginia Institute of Marine Science
Address	303 College Circle	6959 Miller Avenue	1 Old Ferry Rd.	1 North	1375 Greate Road
City	Morehead City, NC 28557	Port Norris, NJ 08349	Bristol, RI 02809	Fort Pierce, Florida 34946	Gloucester Point, Virginia 23062
Phone	252-222-6312	856-785-0074 ex-4320	401-254-3299	(772) 242-2525	804-684-7713
Contact	Dr. Tal Ben-Horin	Emily McGurk	Dr. Roxanne Smolowitz	Dr. Susan Laramore	Dr. Ryan Carnegie
Email	tbenhor@ncsu.edu	emily.mcgurk@rutgers.edu	rsmolowitz@rwu.edu	slaramo1@fau.edu	carnegie@vims.edu
Tests Required by TPWD:					
Ploidy information	No	Yes	No	No	Yes
MSX (<i>Haplosporidium nelsoni</i>)	Yes	Yes	Yes	Yes	Yes
Dermo (<i>Perkinsus marinus</i>)	Yes	Yes	Yes	Yes	Yes
Bonamiosis (<i>Bonamia exitiosa</i>)	Yes	Yes	Yes	Yes	Yes
Oyster Herpes Virus (OsHV-1)	Yes	Yes	Yes	Yes, but not usually done	Yes, but not usually done