

Brood Stock Distribution -- 2009

From our start in 1998, the hallmark of our program has been the release of brood stock to hatcheries for propagation of seed and eyed larvae. We have always felt that this is the culmination of our breeding efforts. Because progress is continuous in a

breeding program, we expect to release lines yearly that are better than the previous generations. The numbers of brood stock released in the past five years have been climbing steadily: 100, 200, 500, 1000, then 7000 for the 2008 season. Consequently we have

been learning what to do and what not to do. We have adopted the following principles and policies for the distribution of diploids and tetraploids that seem to be most serviceable for all.

Brood stock - lines

In the Fall of each year, we will query hatcheries about their expected needs for brood stock. The contact at ABC for this is Nate Geyerhahn. At that time, we prefer to learn of the total estimated requirements for brood stock for the upcoming year.

Sometimes it will be impossible to predict the entire season, we understand. At the very least, a hatchery should know what is needed for the early season so it can attend to early conditioning.

Brood stock management
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General Principles

- **Timing** – We prefer to release all the brood stock in late Fall of the year prior to spawning season. For example, for the 2009, spawning season, we released brood stock to hatcheries in late—November or December of 2008. It's possible that a hatchery will not know their entire brood stock needs that early. In that case, there likely will be additional brood stock available later in the following spawning season.
- **Strategic timing** – We recommend that hatcheries receive the brood stock needed for early season spawns in the Fall delivery. This will assure that conditioning (see below) and salinity adjustments are within their control. For mid-spring spawns, it is possible to get brood stock, but they will still have to be conditioned. During the spawning season, oysters will be ripe, of course, up until they spawn out. We can not hold brood stock back from natural spawning. In the late season, all of our brood stock will be spawned out and therefore useless to hatcheries. If a hatchery needs brood stock late in the season, the hatchery must make their own provisions to attain them and hold them for late season use.
- **Availability** – To reiterate the points in Table 3, because we have changed the very nature of our breeding program, we expect to have ample brood stock of any specific line available by 2011. In the

meantime, we will distribute a line upon request if we have it available, otherwise we will provide the next best thing according to our experience.

- **Fecundity** – For now – until our new brood stock come on line – it is best to estimate the number of females needed based on an average fecundity of 5,000,000 eggs per female. This is a conservatively small number. Unfortunately, we can not predict sex ratio, which can change from year to year and site to site.
- **Conditioning** – We do not have the resources to get oysters sexually mature for commercial hatcheries, by

conditioning. For early spawns, where conditioning is needed, we release brood stock early.

- **Cost** – Starting with the 2009 release of brood stock (for the 2010 season), there will be a charge for the number of animals distributed. This cost is being determined, but we estimate that \$2-3 per female is probably about right. Obviously, we do not know how many females there are when the brood stock are first released. Therefore, we will ask the hatcheries to record the number of females used over the course of the season.

(See “Intellectual Property and Licensing”).

- **Retaining brood stock** – Any unused brood stock at the end of the spawning season may be retained by the hatchery (in their own grow out) for the ensuing spawning season. These inventories should be communicated to Nate Geyerhahn so that future needs are adjusted accordingly. Alternatively, brood stock should be returned to ABC. Brood stock may not be propagated for quality control reasons.

Brood stock - tetraploids

Distribution

As a rule, we do not release tetraploid oysters (exception, see “Timing” below). Rather, we have been successful in distributing live sperm from tetraploid males prior to a planned spawn. This works well even if the hatchery is remote from us, because the sperm is viable for up to 3-4 days.

For tetraploid sperm, we have on hand populations of

tetraploids from various year classes (and in the future from various lines). A hatchery notifies us that it is planning a triploid spawn(s). Our contact person for this is Karen Hudson. ABC personnel open putative tetraploids and find the males. We then certify that the male is indeed tetraploid and that the sperm is 100% di-haploid. The tetraploid male is then strip spawned and the sperm placed in 1.5mL Eppendorf tubes. The

tubes containing the sperm from tetraploids are then packed in a cooler with ice for storage, delivery, or shipment. When possible, we try to include sperm from at least two males.

ABC is contracted to provide quality control on triploid production in commercial hatcheries. We do this by flow cytometry. Below, we outline the process of certification sampling and procedures. Please contact Karen Hudson if you have any

concerns or questions regarding the certification or sampling procedures. Any questions regarding the certification requirements, allowable percentage triploidy, or charges

should be directed to 4Cs Breeding Technologies, Inc. (4Cs) (See “Intellectual Property and Licensing”).

Tetraploid management
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General Principles

- **Timing** – ABC will make every effort to have conditioned tetraploids by late March, extending to at least Aug 15th – this is the period of time live sperm will be available from ABC.
- **Strategic timing** – If there are plans to spawn earlier than March, we will make arrangements for the transfer of limited numbers of tetraploid oysters for conditioning at the hatchery facility. These tetraploid are “on loan” and records of oysters sacrificed or dead must be kept. All remaining tetraploids must be returned. Remember, that if you are stripping tetraploids at your hatchery, the sperm still should be certified. See certification and sampling below.

If there are plans to use tetraploids later in the season

than mid-August, we need to know about these plans by early Spring, and we will try to negotiate a solution. It is increasingly difficult to keep fecund tetraploids late into the season.

- **Availability** – We have been successful in expanding our tetraploid stocks and do not anticipate any problems with availability of sperm from tetraploids.
- **Fecundity** – Roughly speaking, one tube of sperm should be sufficient to fertilize about 50,000,000 eggs. We recommend using a higher sperm to egg ratio in $4n \times 2n$ crosses because tetraploid sperm are somewhat less active. Probably, it should be double your normal application. Another way to put it is that to obtain 10 sperm per egg, you will need about twice as much

sperm as you would use from diploid oysters. To assure sufficient quantity of sperm, it would be best to specify the approximate numbers of fertilized eggs you are shooting for.

- **Conditioning** – For tetraploids ABC does the conditioning, unless very early spawns are anticipated.
- **Cost** – There is no cost associated with ABC’s distribution of sperm to hatcheries. Other costs, such as licensing fees and certification costs are described in “Intellectual Property and Licensing”.
- **Retaining brood stock** – Unused tetraploid brood stock at the end of the spawning season must be returned to ABC. Tetraploids may not be propagated.

Certification

Certification steps

1. sperm to assure it is 100% di-haploid
2. 2-7 day old larvae to assure they are triploid
3. “pre-sale” (eyed larvae or seed) to assure no contamination in handling

4Cs requires certification of triploid spawns in the early larval stage and in the stage at which they are sold (eyed larvae or spat), and ABC is contracted to do this work. Certification of the sperm before the spawn assures

that there is no contamination from the tetraploid(s). The early larvae sample is for the benefit of the hatchery to ensure that time and effort is not wasted on a spawn contaminated with diploids. There is no set day to

Sampling

Sperm certification – ABC is responsible for providing di-haploid sperm on request. Certification occurs in our labs prior to shipment. Pick-up can also be arranged through Karen Hudson. ABC will be responsible for shipping costs of sperm, with reimbursement from 4Cs.

If tetraploids are being used at hatcheries early in the season, then it will be necessary to send a sample of the sperm from the hatchery to ABC. There are several options for doing this. One, tetraploids can be spawned and a sample of sperm saved in the refrigerator. It can be returned to ABC for certification with 2-day old larvae (but not older since the sperm

may degrade for testing). Two, tetraploids may be opened in the hatchery and strip spawned. Sperm can be held in tubes in the refrigerator while a sample is sent to ABC (overnight). Spawning could be done immediately after the sample is certified. Results will be phoned in to the hatchery on the day the sample is delivered to ABC. Three, tetraploids may be opened in the hatchery and sampled, but not strip spawned. In this case, after sampling, the top shell should be replaced and the whole oyster wrapped in moistened toweling and stored

sample early larvae, but the earlier the better (days 2-7). The “before sale” certification is to assure that no mishandling has occurred during the larvae or seed process. These certifications were put into place to assure that growers are getting high quality triploids.

in the refrigerator. Again, results will be phoned in to the hatchery on the day the sample is delivered to ABC.

ABC will provide sampling materials for these early sampling needs. Remember, this is an exception for early production only, otherwise hatcheries will receive already certified sperm from ABC. Instructions for sampling will be sent with tetraploid oysters.

Early larvae certification –

The hatchery is responsible for sending early larvae samples. About 2000-5000 larvae should be collected during a drop, and volume condensed so the larvae can fit into a small tube. Larvae should be shipped in seawater and kept cold (not frozen) during

Samples for certification
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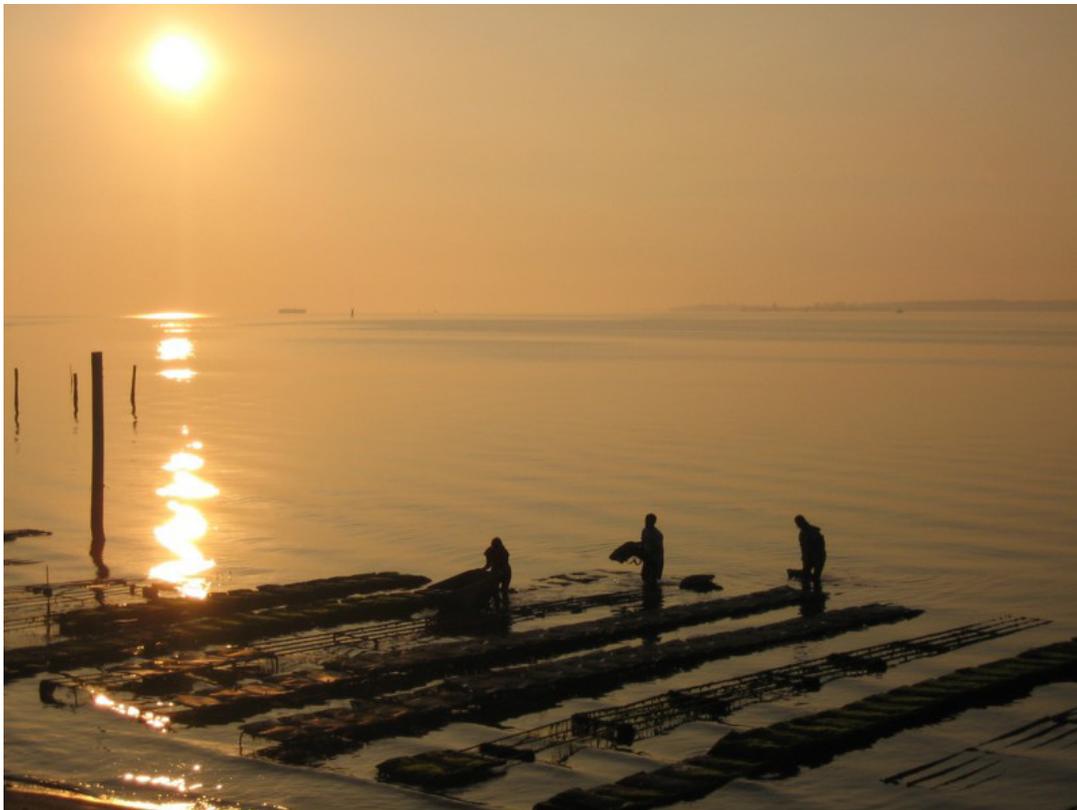
storage and shipping. Labeling needs to include the culture name and/or unique code, or date, to identify that specific cohort in the future. Shipment should be overnight.

“Before sale” certification (larvae) – For eyed larvae sales, the same procedure can be used as for early larvae certification,

except that only about 1000 larvae are needed. Each major batch of larvae, even if it is from the same spawn over a number of days, should have its own certification.

“Before sale” certification (spat) – For spat, ~200 1-2mm spat should be shipped in damp toweling, cold (not frozen) overnight (or delivered for local hatcheries). There will be

a minimum certification of 50 spat, and if there are more than three diploids, another 50 will be run to get to a final sample size of 100.



Breeding farm on York River at sunrise