Practical Notes on Oyster Embryo Bioassay

Plan to use oysters on day of arrival.
Ideally oysters should be used not later than 24hours out of water, but tests can be performed successfully if oysters have either arrived a day or two late or if the test needs to be delayed provided the following procedures are undertaken:

- **If delivered late** place oysters in cool sea water (10-15°C) with some aeration for an hour to recover. If they are placed in water at a temperature of 20°C or above there is a danger of oysters spawning. If this is unavoidable then we suggest placing each oyster in separate 1 litre containers and keeping an eye on them.
- If a test needs to be delayed it is possible to hold oysters for 2 to 3 days in cool (10-12°C) aerated or running seawater. Otherwise the oysters can be held out of water for up to 24 hours in damp conditions at 6-10°C and then allowed to recover the next day as described above.
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For Procedures where gametes are stripped from gonad:

Selection of oysters. Select the best oysters and gametes before pooling them. Good oysters have a large area of creamy coloured gonad and are particularly good when the surface is veined. Gametes should ooze out easily when incised. Under the microscope good eggs have uniform pear shapes which round up after a while once in seawater and especially after sperm has been. Good sperm becomes active on contact with sea water

Tissue debris in gametes: A 75-100 micron screen removes large tissue debris in the gametes, but if there is a lot of very small debris this should be removed by screening eggs on a 17 to 20 micron screen (small eggs and debris will fall through whilst eggs will collect on the screen)

Sperm Quality and quantity: Sperm can vary greatly in concentration and activity and it is sometimes difficult to know how much to add to fertilize eggs. We suggest that eggs are examined 5 to 10 minutes after inoculation to check that there are about 3 to 10 sperm cells per egg, adding a bit more if there is any doubt. Sometimes sperm is inactive either because the oysters have got very cold in transit or they have been out of water too long. If this is the case a higher concentration of sperm than normal may be needed. .

30 minutes after fertilization Check eggs again. If there are less than 80% showing first signs of cleavage or with polar bodies then either add more sperm or start the test again with fresh gametes. There is no need to wait for 2 hours before doing so.

REFERENCES

THAIN J.E. (1991) Biological effects of contaminants: the oyster (C. gigas) embryo bioassay In: Techniques in Mar.Env. Sci., ICES
Environment Agency The direct toxicity assessment of aqueous environmental samples using the oyster (Crassostrea gigas) embryo-larval development test (2007)
http://www.environment-agency.gov.uk/static/documents/Research/oyster209jan30_1388168.pdf

Oyster embryo test: Troubleshooting

A test failure is when either very low numbers of embryos develop or there is too high a percentage abnormal development.

Possible causes:

1 Reference seawater

- (i)Seawater of unknown/dubious quality is being used.
- (ii) Seawater cannot be stored for too long- max 4 weeks

2. Oysters

- GSF oysters are selected by biopsy and then examining a small sample of gametes from each oyster. We cannot guarantee the quality of the oyster as a whole. This is why we send 5 of each sex so that the operator can be more selective once the oysters are opened. So it is important to :
- i) Select the best oysters: look for fat creamy coloured gonad, usually with "veining" on surface.
- ii) Take care where eggs are taken from gonad- avoid area from where needle has been inserted for biopsy (sometimes discoloured green)
- iii) Select best eggs (round and dense coloured)- allow them to round up in seawater for 15 minutes before adding sperm then decide which to use. Do not pool eggs too soon.
- v) Check eggs for fertilisation. 5 minutes after adding sperm you should see 5 to10 sperm around each egg, and 15-25 minutes later polar bodies should be evident. If quality of sperm seems poor-ie not very motile or slow to fertilise- it may be because oysters are too cold. Allow the sperm to recover in seawater at 25 C. If this does not work adding more sperm (double normal amount) may help. At the stage of 2-4 cell divisions the cells should be compact and reasonably symmetrical). If in doubt be prepared to start again with fresh gametes.

3. Oysters affected in transit

- i) Although oysters are cooled down prior to packing and shipped in polystyrene boxes it is possible that they may have been subjected to extremes of temperature.
- ii) If they have been over 24 hours in transit, place in cool water 10-15 C for an hour to recover. They can be held overnight either out of water in damp conditions at 6-10 C, or preferably in cool (10-15 C) flowing seawater: anything warmer than that may cause them to spawn. The next day they must be allowed to recover by placing in 20 C seawater for about an hour. Caution : they may spawn when put into water or after increasing temperature. Keep males and females, or preferably each oyster, in separate containers.

4. Mishandling of eggs and embryos

- Poor fertilisation – be prepared to add more sperm if not active but avoid more than 20 sperm cells per egg.
- Eggs not kept in suspension
- Eggs at too high a density – keep at less than 5000/ ml
- Temperature should be maintained at 25C