Professor Richard Whittington
Chair Farm Animal Health

25 January 2013

John Stubbs
Broken Bay Oysters

Emergency survey of Pacific oysters in the Hawkesbury River for POMS

1. Purpose of survey

To detect POMS (OsHV-1 uvar infection) in Pacific oysters in active leases in the Hawkesbury River during an outbreak of the disease in January 2013. The findings will be used to make recommendations to BBO about the risk of movement of oysters between bays within the Hawkesbury River to enable management for growout and harvest.

2. Assumptions

- In the absence of signs of the disease (epizootic mortality in juvenile and adult oysters) it is assumed that leases may be free of the infection at the time of sampling.
- qPCR is highly sensitive and specific to detect the infection
- If the infection is present it will affect more than 10% of oysters in a lease and more than 10% of leases in a bay
- Infection may be clustered in some parts of a bay
- Some bays may be affected and not others; bays are independent
- Routine movements of live oysters from the known infected areas (Mullet Creek and Mooney Mooney) within the last 2 weeks may have unknowingly transferred the infection to other areas already
- Wild oysters may be infected already but sampling farmed oysters will be representative at bay level

Faculty of Veterinary Science
Farm Animal & Veterinary Public Health
Shute Building C01
425 Werombi Rd
CAMDEN
NSW **2570** Australia

T +61 2 9351 1619
F +61 2 9351 1618
E Richard.whittington@sydney.edu.au
sydney.edu.au

ABN 15 211 513 464
CRICOS 00026A

- The value of the results for this purpose decreases with time after collection of the samples due to the potential for rapid spread of the disease in the river system. As samples can be processed by the EMAI lab commencing Wednesday 30 Jan, sampling should not be undertaken sooner than Tuesday 29 January.

3. Design considerations

Sample size estimation

Sample sizes were estimated using the *2-Stage sampling approach* for demonstration of disease freedom with the leases to be selected in the first stage and oysters within leases in the second stage. All bays were considered to be independent populations as the aim was to demonstrate freedom of infection for each bay.

The assumptions made for calculating sample sizes are presented in Table 1 and the sample sizes calculated for five bays which were assumed to be uninfected on the day of calculation (9am 25 January 2013) using raw data available at 11pm 24 January 2013 in Table 2. These sample sizes would provide 95% probability of detecting disease in a bay at 10% oyster and 10% lease-level design prevalences.

Information about oyster population per lease was not considered in sample size calculation because of active harvesting from some leases. As the number of active leases was also subject to change due to harvest activities, other examples of lease numbers were explored using the software. In general a sample size of 32 oysters per lease for bays with 2 to 21 active leases would achieve the required target system sensitivity of 0.95. This is increased to 35 to enable pooling in lots of 5 (i.e. 7 qPCR tests per lease).

All calculations were made using calculators available online: http://epitools.ausvet.com.au/content.php?page=2StageFreedom (least cost where herd size unknown)

Table 1. Assumptions made for calculation of sample size

Description	Values assumed
Oyster-level design prevalence	10%
Lease-level design prevalence	10%
Test sensitivity	0.9
Test specificity	1
Relative cost of sampling a lease: testing an oyster	500:1
Maximum number of oysters to be sampled per lease	100
Target system sensitivity	0.95

Table 2. Examples of sample sizes, number of leases and number of oysters per lease.

Bay	Number of active leases	Number of leases to sample	Min. number of oysters to sample per lease	Number to sample to enable tests in pools of 5	Estimated lease sensitivity	Achieved bay sensitivity
Coba Bay	11	11	22	25	0.87	0.96
Kimmerikong	5	5	32	35	0.95	0.95
Marramarra	21	16	32	35	0.95	0.95
Porto Bay	19	16	30	30	0.95	0.95
Patonga	6	6	32	35	0.95	0.95

4. Recommended strategy and method

Validity to proceed
An assessment will be made of disease distribution based on mortality in the river on the day that the sampling is to take place. If there is evidence of mortality in the upper river (Coba, Kimmerikong or Marramarra) this part of the survey will not be undertaken. The same applies to the two remaining sites (downriver), but they are independent and would be excluded one by one.

Final sample size
Where there are more than 16 active leases per bay, sample from 16 randomly selected leases. Where there are 16 or fewer active leases per bay, sample all leases.

Sample 35 oysters per lease to enable creation of 7 pools of 5 oysters each for qPCR testing.

Method of sampling
Within a bay:
Select leases using random numbers if required (where there are more than 16 active leases), otherwise sample all leases.

Within a lease:
Use systematic random sampling, take 1 oyster per location. The approach may differ between leases depending on shape and the cultivation system. An audit made by Broken Bay Oysters on 25 January 2013 will be used to determine the number of cultivation units to sample. For example, for Lease OL91/016 where there are 480 trays on 5 racks, every 480/32 = 15th tray will

be sampled. The first tray to be sampled on the first rack will be determined by tossing a coin and then every 15th tray will be opened and an oyster removed with the operator blinded. It is noted that this is an extremely difficult and time consuming task but it is required for the validity of the survey.

Sample handling and packaging:
Oysters will be bagged and placed in an esky. The eskies will be taken to the EMAI laboratory by road.

This document was prepared by Dr Navneet Dhand and Richard Whittington with acknowledgment to Dr Ana Rubio Hornsby Shire Council and Broken Bay Oysters.

Richard Whittington