Options for oyster surveillance for POMS on a bay area basis.

Situation: Oysters Tasmania has expressed interest in reviewing POMS (Pacific Oyster Mortality Syndrome) classification status, as is referenced within Oysters Tasmania discussion *Project 6 "Targeted review of specific areas with Intermediate POMS classification".*

Objective: Develop a project for the **s**urveillance of Pacific Oysters to aid in determining pathogen status of Ostreid Herpesvirus 1 microvariant (OsHV-1µ) in defined bodies of water in Tasmania (bays).

Summary:

- There is a lot of uncertainty in relation to being able to accurately predict disease status and/or guarantee that a bay area is free of POMS even with the best executed sampling design.
- The current calculations are based on a number of assumptions, known test parameters and an assumed / expected OsHV-1µ prevalence of 2%.
- Sampling is recommended at 4 occasions over 2 summers.
- Laboratory fees for PCR testing of 150 adult oysters is currently => $9,951 AUD per sampling.
- PCR testing of 30 pooled samples each containing 5 spats to 15mm is a cheaper option but is not recommended to assess bay oyster population status.
- This will result in approximately 5% chance of incorrectly identifying an infected sampled population as pathogen free or test negative (error).
- Ultimately the Tasmanian oyster industry has to determine the level of confidence required.

Background: It should be noted that there are knowledge gaps regarding Ostreid herpesvirus and there are many limitations to this proposed surveillance. This information may be used to help review POMS zone classification, but the information yielded from this surveillance may or may not provide sufficient evidence to alter current POMS zone classifications. POMS and Ostreid Herpesvirus 1 microvariant (OsHV-1µ) are used interchangeably in this paper.

There are currently no international standards for the surveillance and demonstration of "proof of freedom" from OsHV-1µ or the disease POMS. Further, specific reference and recommendations for OsHV-1µ surveillance or reporting have been removed from the World Organization for Animal Health (WOAH) Aquatic Manual and Code (OIE, 2023). The role of Ostreid herpesvirus variants, non-pathogenic variants, and subclinical oysters, as well as the pathogen's various roles in morbidity and mortality (mass illness or death) events, are not fully known (Trancart, 2022). WOAH cite "infection without clinical signs is very common

in aquatic animals and presents a significant risk of spreading pathogens through trade" (OIE, 2011).

The following sampling design is *not* sufficient for a zone or area within Tasmania to demonstrate freedom from disease, or freedom from the presence of Ostreid herpesvirus. Instead, the following surveillance methods will help to detect the presence of the virus OsHV-1µ in a population to a certain level of confidence, under several assumptions which will be described in detail below.

POMS is widely considered a complex and multifactorial disease, where proximity of the pathogen OsHV-1µ and the oyster host alone will not always result in detectable illness or death (Petton et al., 2021). Further, *significant knowledge gaps* remain surrounding diagnostic test performance for OsHV-1µ among asymptomatic (clinically normal Ostreid herpesvirus affected oysters) and POMS-resistant oysters (AHL, 2023; Martenot et al., 2010). Different authors have recommended marine separations of 2-5 NM between populations of differing status but this will depend on the characteristics of the bay waters and the Tasmanian situation has not been studied for POMS. The role of sentinel populations (non-resistant stock) or passive disease surveillance has not been considered,

Sampling: Sampling recommendations are based on *known* parameters regarding detection of OsHV-1µ, as well as principles and methods referenced in the Manual of Diagnostics Test for Aquatic Animals (OIE, 2019). The following recommendations are provided based on best knowledge available at the time of writing and *do not* provide sufficient evidence to reliably demonstrate complete freedom from the virus OsHV-1µ. Typically, sampling numbers required for an investigation, study, or surveillance, are determined using a pathogen's status in a population (prevalence) and information surrounding the accuracy of diagnostic test used to detect the pathogen.

A.) Prevalence: current prevalence (the total number of OsHV-1µ cases existing in a population, divided by the total population), of OsHV-1µ throughout individual Tasmanian waters of intermediate POMS status is *currently unknown*.

- A 2% prevalence has been suggested by both WOAH (OIE, 2022) and the Australian Department of Agriculture (Agriculture, 2015) in an effort to detect subclinical OsHV-1µ which could be present in clinically normal Pacific oysters, and based on the assumption that the aquatic population is infinitely large. It has been suggested that aquatic pathogens, such as OsHV-1µ, may not be uniformly distributed across a population which may reduce the chance of detection (OIE, 2023; Paul-Pont et al., 2014).

B.) Diagnostic Test Performance: communication with Animal Health Laboratory advise the diagnostic sensitivity (Se) and specificity (Sp) for TacMan qPCR, used to detect OsHV-1µ in *clinically normal* Pacific oysters, has not been completed at the time of writing (June 2023).

- Diagnostic performance of TacMan qPCR (Martenot, 2010) estimate a DSe (the proportion of samples from *known infected* reference animals that test

positive; tested in duplicate series) of 99% and a DSp (proportion of samples from *known uninfected* reference animals that test negative; tested in duplicate series) of 95% (AHL, 2023; Paul-Pont et al., 2014).

- TacMan qPCR diagnostics Se and Sp among *clinically normal* Pacific oysters has not been completed by laboratory authorities and results will not be available until Summer 2023, at the earliest. It is likely that the Se and Sp will be lower in clinically normal Pacific oysters, and these lower figures would, in turn, require a much larger sample number to detect OsHV-1µ at a prevalence of 2% in a population.

C.) Pooled Samples: pooled samples of 5 juvenile (<15 mm) are an accepted method to screen for OsHV-1µ and meet interstate movement requirements of oyster spat. However, AquaVetPlan and WOAH Aquatic Manual do *not* recommend pooling of sample during surveillance studies. This is because it is difficult to detect viral presence in a clinically normal host. When several samples from clinically normal hosts are pooled together, any presence of a virus becomes further diluted and it becomes even more difficult to detect a virus.

D.) Timing: According to the *Aquatic Manual of Diagnostics for Aquatic Animals, WOAH* if disease status is historically unknown within a zone or area, then the following conditions should be met in order to optimize detection of the pathogen in a scenario with clinically normal oysters and non-uniform (not every oyster host exposed to the virus will carry or contain the virus) distribution of the virus:

i.) Two surveys per year, conducted at last 3 months apart within each summer.
ii.) Surveillance for two consecutive years
iii.) Water temperature exceeds a minimum of 16C and, when possible, sampling should occur when water temperature exceeds 18C for at least two weeks (Petton et al., 2021)
iv.) Pacific Oyster juveniles <15 mm.

Concurrent sampling of feral, asymptomatic adult Pacific oysters in closer proximity to the juveniles is recommended to increase pathogen detection. Adult samples would not be pooled as Se will be further decreased secondary to diluted viral material, particularly in asymptomatic adults (OIE, 2019). Sampling of POM-resistant stock is expected to provide negative test results for OsHV-1µ (Trotter et al., 2021).

E.) Sample Size: sample size needed to detect presence of a pathogen should be determined based on: the diagnostic test Se and Sp performance, expected OsHV-1µ prevalence, the level of confidence desired of the survey results, and the precision desired as feasible (OIE, 2023). The size of an aquatic population is assumed to be infinitely large.

- Note the following unknowns contributing to the proposed surveillance number: The prevalence of OsHV-1µ in Tasmanian waters is unknown. The Se and Sp for the TacMan qPCR diagnostic test used to detect OsHV-1µ

among asymptomatic pooled adults is unknown. The level of confidence desired is minimally 95%.

F.) Sample Size Recommendation per survey (figure 1):
See figure 1, a range of sample sizes are proposed based on the previously described assumptions and current knowledge surrounding detection of OsHV-1µ. These sample sizes would be collected randomly, during each survey. Oysters should be chosen at random, aligning with the principles provided in WOAH *Aquatic Manual Section 1.1*. Briefly, the efforts needed to create random sampling of an aquatic zone are complex and can require a large labour force, inter-sectoral organisation, and planning.

Figure 1.) Epitools output for sample size

Sample sizes for varying prevalence and precision values

Samples sizes required for sensitivity = **0.99**, specificity = **0.95** and a range of true prevalence and precision values are shown below:

	TP = 0.01	TP = 0.02	TP = 0.05	TP = 0.1	TP = 0.2	TP = 0.5
Precision = 0.01	2430	2786	3809	5359	7885	10852
Precision = 0.02	608	697	953	1340	1972	2713
Precision = 0.05	98	112	153	215	316	435
Precision = 0.1	25	28	39	54	79	109
Precision = 0.2	7	7	10	14	20	28

Source: (Sergeant, 2018)

150 samples with an assumed OsHV-1µ prevalence of 2%, and an assumed test performance of DSe 99% and DSp 95% (both figures require duplicate serial testing of *each* sample) would result in an approximately 5% chance of *incorrectly identifying* an infected sampled population as *pathogen free* or test negative. In other words, there is at least a 5% chance of error, based the many assumptions previously described surrounding OsHV-1µ virus transmission and the accuracy of the diagnostic test. An area or zone may appear to have no presence of OsHV-1µ, even after sampling 150 Pacific oyster, but in fact the virus is present, and translocation of population located within said area or zone may pose a biosecurity threat to other areas or zones.

Sample Number Options:

i.) 150 whole Pacific oysters,
- It would be intended that these are adult feral Pacific oysters growing in the bay which might have a greater cumulative probability of harbouring OsHV-1µ due to the persistent latent nature of the virus infection, or

ii.) 30 pooled samples each containing 5 spats will provide approximately 150 samples
- Pooling samples can reduce time and costs associated with diagnostics. Peter Kirkland Principle Veterinary Virologist NSW DPI EMAI, “pools of 5 animals do not affect diagnostics sensitivity for surveillance of subclinical carriage of OsHV-1µ”. Oysters exceeding 15 mm cannot be pooled (AHL, 2023). Again, it should be emphasized that both AquaVetPlan and WOAH do

not recommend pooling of samples for use in surveillance due to the reduction in test Se performance, which is as a result of dilution of OsHV target viral material (Agriculture, 2015). This is a common approach for the assessment of health status of spat moving interstate.

It is possible to combine the above testing approaches but the resulting increase in confidence has not been calculated.

G.) COST: Assay cost only, as of June 2023 Animal Health Laboratory quotes $66.34 AUD/OsHV1 PCR on a single Fresh Whole Oyster* or a pool of 5 spat.

i.) Example 1: $66.34x150 whole oyster => $9,951 AUD per survey. Four surveys will be performed over two years. Approximately $40K per bay for lab test costs.

ii.) Example 2: $66.34x30 pooled spat of 5 juveniles <15 mm => $1991 AUD per survey (as above). Four surveys will be performed over two years. Approximately $10K per bay for lab test costs.

Note: these estimates are for diagnostic assay estimates only. These figures are estimates and were correct at time of writing, June 2023. These estimates do not include operational costs for collection, transport, supplies, labour, and support for random sampling field design.

*Note: this figure does not include shucking costs as applicable.

Conclusions:

There are limitations surrounding this sampling discussion. Sampling 150 randomly selected Pacific oysters would be expected to provide 95% confidence of detecting the OsHv-1µ antigen in a population where the pathogen is *uniformly distributed* at a prevalence of 2%, with diagnostic test performance as described.

If assumptions are incorrect, for example, if OsHv-1µ were present at a prevalence less than 2%, then the likelihood of detecting the virus would decrease, and the risk of moving disease through stock translocation would increase as a consequence of an incorrect status decision. Other assumptions based on limited information include diagnostic test sensitivity and specificity, uniformity of OsHv-1µ virus distribution in the population, and the ability to conduct true random sampling. Any incorrect assumption will contribute to a reduced ability to detect the virus OsHv-1µ if present.

Ultimately the Tasmanian oyster industry has to determine the level of confidence required. This decision could have important consequences when oyster movements to warmer northern waters which might favour disease expression are intended.

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