Application to amend the *List of Specimens taken to be Suitable for Live Import* (Live Import List)

(Live animal imports of exotic species/specimens into secure facilities for research purposes only (Part 2 of the Live Import List))

1. Background

This proposal is to support the importation of the nominated species to undertake short term research projects in high containment, quarantine approved premises to assess the susceptibility of various oyster species to infection with oyster herpesvirus type 1 (OsHV-1). This virus was first detected in Australia in 2011 and has devastated Pacific Oyster production in NSW and Tasmania. The information that follows is provided to support an application to amend the Live Import List.

2. Species of interest and origin

The animals to which this application relates are various species of oysters. These are:

Pacific oysters (Crassostrea gigas);

Kumamoto Oysters (Crassostrea sikamea);

Atlantic Oysters (Crassostrea virginica);

European Flat Oysters (Ostrea edulis) and

Olympia Oysters (Ostrea lurida /Ostrea conchaphila)

Each species is farmed commercially in the northern hemisphere and the imported stock would be obtained from hatcheries in the United States.

3. Transport and containment

All oysters being imported are subject to biosecurity controls to meet the import conditions imposed by the Department of Agriculture and Water Resources. Consequently it is essential that animals are transported to the containment facility (see section 8 below) in leak-proof containers secured with tamper proof seals. Only once they are inside the containment facility can the containers be opened and animals removed. Once in the containment facility, the oysters are held in containers of appropriate size (usually with a total volume of <10L, often as small as 50-100mL). A combination of these primary containers and the fully sealed, secure, limited access holding rooms provide a mechanism to ensure containment of these animals. Further details of these containment facilities and disposal of animals and associated waste are described in full in section 8 below.

4. Related risk assessments

The applicants are not aware of any other environmental risk assessments undertaken on the species both in Australia and overseas, including any Import Risk Analyses undertaken by the Commonwealth Department of Agriculture and Water Resources

5. Potential impacts of escape

Pacific Oysters were introduced into Australian waters more than 70 years ago. Diploid oysters have spread extensively from Tasmania in the south to the mid north coast of NSW where higher temperatures limit their viability. Triploid Pacific Oysters are farmed commercially in NSW, Tasmania and South Australia. As the other species nominated have similar biological characteristics and habitat requirements, if they were to escape it is expected that they could potentially spread in a similar manner to Pacific Oysters.

6. Risk reduction measures

Most of the oysters being imported will be too young to reach sexual maturity, with most being from 2-6 months of age. They will always be held in biosecure facilities (see below) and will never be in proximity to waterways where they could survive. Further, few will survive for more than a few weeks after arrival in Australia as the experimental infections are usually lethal. At the end of a round of experiments any remaining oysters are disposed of by autoclaving.

7. Purpose of import

These oyster species are specifically being imported to determine their susceptibility to Oyster Herpesvirus (OsHV-1) infection. This virus was introduced into Australia in 2011, initially into NSW and in late 2015 spread to Tasmania. This forms part of a collaboration between scientists in Australia and the USA to identify both species and genotypes of oysters that are genetically resistant to OsHV-1 virus. Although Pacific Oysters are available in Australia, genetically defined lines from the USA are included in this research as a comparison with stock held in Australia. Such a comparison cannot be undertaken in the USA where the virus is exotic. Further, scientists in Australia have developed an experimental infection model to expose oysters to the virus in a manner that simulates natural infection and removes the need for field based studies with the associated biosecurity and ecological concerns.

The proposed research will be undertaken by staff of the Virology Laboratory at Elizabeth Macarthur Agriculture Institute (EMAI), Menangle NSW. This facility is owned and operated by the NSW government through the Department of Primary Industries. The staff have received international recognition for their achievements in research into this and many other virus diseases of both terrestrial and aquatic animal species.

8. Containment facilities

A range of both laboratory and animal handling facilities are available at EMAI with containment ranging from PC1 through to QC3/PC3+ (for both laboratory and animal containment activities). These facilities meet both the relevant Australian standards and are DAWR accredited containment facilities for imported biological materials. The containers

holding imported oysters will be held in QC2/PC2 animal containment facilities in accordance with DAWR biosecurity requirements. These facilities consist of a single building that is located within a secure outer compound to which entry is gained via a locked change room and shower facilities. Access to these facilities is further restricted to relevant staff either by swipe card or security key system. Within the building complex is a series of adjacent but separate fully enclosed rooms, without any windows and each room has a separate anteroom with a single entry door. The rooms have floors and walls are constructed of impervious coated concrete, have a filtered air supply and operate under negative pressure. There is a concrete paved verandah around the perimeter of the rooms. All liquid waste is captured through a single central drain that is fitted with an appropriate grill and trap to capture solids before liquid drains into the waste drains and is transferred to a decontamination facility. Prior to either chemical or high temperature sterilisation, all liquid waste is passed continuously through a series of mechanical grinders and only particulate material with a diameter of <0.1uM is allowed to pass on to the sterilisation tanks.

While tanks or containers holding animals are being used, water is changed manually at regular intervals. The used water is held in a large drum and disinfected by addition of chlorine prior to release into the floor waste. In the event of any accidental spill, decontamination is effected by using a DAWR approved disinfectant. At the conclusion of any experiment or project, all solid waste (animals and any other material such as feed) is removed in sealed bags and sterilised by autoclaving. For the collection of specimens, animals are removed to a suitable laboratory area and, on completion of dissection, waste is disposed of by autoclaving. On completion of a project the entire room is disinfected and, when necessary, sterilised by fumigation.

In conclusion, the procedures associated with operation of these containment facilities not only provide complete containment of any animals or their germplasm but ensure that no viable material can leave the facility, thereby completely eliminating any potential environmental risk posed by the species.

9. CITES status

These species are not subject to control under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and are not listed on CITES Appendix I, II or III.

10. Ecology

The following information is provided for completeness only as all animals will be restricted to containment facilities, as previously outlined. Further, juveniles (and hence male only populations) are the primary target for import, further limiting the likelihood of any possible release of viable populations.

- ***lifespan of the species:*** Up to 30 years
- ***size and weight range***; up to 450mm shell length
- ***the natural geographic range***;

Pacific oysters (Crassostrea gigas); Northwest Pacific (including Russia, Japan, China and Korea)

Kumamoto Oysters (Crassostrea sikamea); Eastern Asia (including Japan, China, Taiwan and Korea)

Atlantic Oysters (Crassostrea virginica); Northwest Atlantic from the Gulf of St. Lawrence to Panama and Venezuela

European Flat Oysters (Ostrea edulis): western and southern coasts of Europe from Norway to Morocco

Olympia Oysters (Ostrea lurida /Ostrea conchaphila): northern Pacific coast of USA (O. lurida); Pacific coast of Mexico (O. conchaphila)

- ***habitat;*** intertidal (*Crassotrea* spp.) and subtidal (*Crassostrea* spp and *Ostrea* spp) marine environments

- ***diet***: filter feeding molluscs with no natural potential to feed on terrestrial agricultural plants

- ***social behaviour and groupings***: all listed species are sedentary and once settled onto a substrate will not relocate

- ***territorial and aggressive behaviours***: none known

- natural predators: wide variety of fish species, crustacea (eg. crabs), predatory molluscs (eg. oyster drills), starfish, worms (eg. mudworm), and wading birds

- ***characteristics that may cause harm to humans and other species***: sharp shells have the potential to act as a hazard where dense oyster beds exist (this is an existing situation as is the case with many native and feral oyster populations in Australia)

11. Reproductive biology

The following information is provided for completeness only as all animals will be restricted to containment facilities, as previously outlined. Further, juveniles (and hence male only populations) are the primary target for import, further limiting the likelihood of any possible release of viable populations.

- ***age at maturity (first breeding***); Crassostrea spp. have been reported to reach maturity as early as 4 months but usually at 1-2 years; O. edulis at 3 years, and O.lurida/conchophila at 1 year

- ***how frequently breeding occurs***; spawning is governed by water temperature, but multiple spawnings are possible under conducive water temperatures

- ***if the female can store sperm***; gametes are shed into the water column during spawning and viability of sperm is relatively limited; hence sperm is not stored by the female

- ***how many eggs or live-born young are produced at each breeding event***; dependent on size of the oyster; C. gigas (the most fecund of the listed species) 8-15 cm length females produce between 50–200 million eggs in a single spawning

- ***if the species has hybridised with other species*** (both in the wild and in captivity) or has the potential to hybridise with any other species; and if the species can hybridise, are the progeny fertile? Hybridization of *C. sikaema with C. gigas* has occurred among cultured stocks on the West Coast of the US (Hedgecock et al. 1993). The two species can hybridize, but only C. gigas sperm and C. sikamea eggs produce viable offspring; the inverse cross is unsuccessful (Banks et al. 1995). Crosses between C. virginica and C.gigas do not yield viable offspring (Allen et al., 1993). No known fertile hybrids occur between listed Crassostrea spp. and Australian oyster species. No specific information is available regarding the potential for hybridisation for O. edulis, O, lurida and O. conchaphila. However, O. edulis has been reported to be present in natural O. angasi populations in Albany, Western Australia (Morton et l., 2003)

12. Relevant legislation

Provide information on all other Commonwealth, state and territory legislative controls on the species and proposed research

Diploid Pacific oysters (C. gigas) are listed as noxious species in NSW (except for Port Stephens), and Qld,

C.virginica is listed as a noxious species under WA legislation.