YUMBAH NYAMAT ABALONE FARM ASSESSMENT OF BIOSECURITY AND DISEASE RISKS FOR THE WILD FISHERY

DIGSFISH SERVICES

DigsFish Services Report: DF 18-05
30 December 2018

YUMBAH NYAMAT ABALONE FARM ASSESSMENT OF BIOSECURITY AND DISEASE RISKS FOR THE WILD FISHERY

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Executive Summary

DigsFish Services Pty Ltd was asked by the Victorian Abalone Industry Committee (VAIC) to provide advice regarding potential biosecurity risks to the wild abalone fishery associated with a proposed land based abalone farm (the Yumbah Nyamat Abalone Farm, or YNAF) near Portland, Victoria, so that the VAIC could make informed decisions regarding their submissions to the works approval public consultation process. The VAIC represents the interests of the wild catch abalone fishery in Victoria, which primarily harvests blacklip abalone (*Haliotis rubra*) but also smaller quantities of greenlip abalone (*Haliotis laevigata*) under strict fisheries management quotas. The major area of concern of the VAIC was the adequacy of treatment of effluent water from the proposed farm and whether the proposed biosecurity management strategies at the YNAF would be sufficient to protect wild stocks of abalone on nearby reefs from "backspill" exposure to disease agents of significance to abalone.

Previous disease risk assessments for the abalone fishing and farming industries in Australia have found that the endemic disease agents of most significance to the health of wild and cultured abalone in this country are abalone viral ganglioneuritis disease (AVG), caused by infection with abalone herpesvirus (AbHV), and perkinsosis caused by infection with the protozoan parasite *Perkinsus olseni*. My examination of the proposal found that the biosecurity disease risk assessment was inadequate and biosecurity arrangements and effluent water treatments proposed for the YNAF would not provide adequate protection against disease incursions into YNAF or backspill contamination from YNAF into adjacent abalone fisheries by either pathogen. Perkinsosis is problematic in abalone populations in South Australia, NSW and northern New Zealand whenever water temperatures exceed approximately 19-20°C, so will likely become a problem in Victorian abalone aquaculture sometime in the future under the current global warming scenario. AbHV may also become more problematic in warmer water temperatures, however increased risks for both pathogens were not acknowledged in the relevant sections relating to mitigating risks due to climate change. Risk assessments done by the Tasmanian Government, Federal Government and others have found that discharge of untreated water from abalone farms and holding facilities into the marine environment is a high risk activity with potential for high (regionally significant) to extreme consequences, including spread of AbHV into the environment adjacent to abalone farms which could result in outbreaks of AVG disease in wild abalone populations. However none of the documents provided proposed any treatment of intake or effluent water at YNAF, despite these being identified as critical biosecurity risk categories in the new National Biosecurity Plan Guidelines for Abalone Farming (Spark et al. 2018).

The potential for backspill of AbHV into adjacent abalone stocks via effluent water from land based abalone holding facilities experiencing AVG disease outbreaks has occurred on at least 2 occasions. The first time was after the AVG disease outbreaks in abalone farms near Port Fairy, Victoria in December 2005, which subsequently initiated an outbreak of clinical AGV in wild stocks of abalone in the adjacent ocean in early 2006. A second incident occurred near Bicheno, Tasmania in December 2010, where untreated effluent water from an AVG outbreak in an abalone holding facility with no treatment of its effluent water was responsible for initiating an outbreak of AVG in a nearby abalone farm which had no treatment of its intake water.

In light of this knowledge of how AVG can be spread into adjacent abalone populations via effluent water from abalone holding facilities, I was particularly concerned with the following statement on page 90 of

Yumbah Aquaculture's Works Approval Application: "*The outcomes of a Supreme Court class action law suit against the abalone farms confirmed that the infected abalone farms were not the cause of impact to wild stock abalone and the closure of the commercial abalone wild stock fishery*." This is incorrect, grossly misleading and contradictory to advice from world experts who investigated the matter at the time, contradictory to official biosecurity documents such as the Federal Government Aquavetplan Disease Strategy Manual for AVG, as well as contradictory to subsequent scientific investigations, virtually all of which have acknowledged that the most likely initial source of the AVG outbreak in wild abalone stocks in Victoria in 2006-2007 was from untreated AbHV contaminated effluent from nearby infected abalone farms.

In the case of the Tasmanian abalone farm in December 2010, investigations by local authorities confirmed the most likely source of the AVG outbreak was via intake of untreated water that was contaminated by effluent from a nearby abalone processing facility that had experienced an AVG outbreak. Since that incident, DPIPWE in Tasmania issued legislation relating to water discharge from abalone live holding facilities that requires processors who hold live abalone to treat their effluent water to achieve a minimum 3 log10 reduction in heterotrophic bacterial levels and maintain discharge bacterial concentrations of ≤ 999 bacterial colony forming units per mL. The treatment methods for effluent water that are normally utilised by processors to achieve this outcome include UV irradiation and ozonation.

The regulatory requirements in Tasmania for treatment of effluent from abalone holding facility are consistent with the most recent advice on best practice risk management measures for effluent water outlined in the new National Biosecurity Plan Guidelines for the Australian land-based abalone fishery (Spark et al. 2018). The regulations in Tasmania are therefore instructive to what is required in Victoria to help prevent future backspill outbreaks of diseases such as AVG and perkinsosis in wild abalone adjacent to these large abalone farms. In the case of AVG the consequences of backspill infections of wild abalone are considered high to extreme, given the very high mortality rate experienced by abalone exposed to high doses of AbHV and the fact that wild abalone in Victoria remain extremely susceptible to the infection. Potential methods for mitigating the risk of backspill infections of wild abalone to acceptable levels include:

- Effective quarantine and screening of broodstock for known abalone pathogens
- Treatment /holding of intake water to prevent pathogen entry onto YNAF
- Routine active disease surveillance at YNAF to detect subclinical diseases
- Depopulation of cultured abalone from effluent pipes and settlement ponds
- Depopulation of wild abalone stocks near the YNAF water intake and effluent pipes
- Decontamination of effluent water using UV irradiation (60 mJ/cm^{2}dose) or ozonation (<1 mg/L/min

Even with such risk mitigation methods in place, situations which could still result in release of disease agents into the adjacent receiving environment would include:

- Significant clinical disease outbreaks within YNAF
- Breakdown of water treatment or water recirculation equipment within YNAF
- Failure of the YNAF to meet discharge requirements due to lack of statutory scrutiny

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Abbreviations and Acronyms

ABHV	Abalone herpesvirus (descriptive term to describe all AbHV genotypes (strains) including the 5 strains (Vic1, Tas1, Tas2, Tas3 and Tas4) found so far in Australia)
ABHV-1	Abalone herpesvirus 1 (index strain, first member of the genus *Haliotivirus*)
AAHP	Abalone Health Accreditation Plan
AVG	Abalone viral ganglioneuritis
CS	Coastal Seafarms, a land based abalone farm near Portland
DPIPWE	Department of Primary Industries Water and the Environment (Tasmania)
YNAF	Yumbah Nyamat Abalone Farm, proposed for development near Portland
OIE	World Organisation for Animal Health (formerly Office International des Epizooties).
OsHV-1 µVar	A genetic microvariant of Ostreid Herpesvirus-1 defined on the basis of partial sequence data exhibiting a systematic deletion of 13 bp in a microsatellite zone of the ORF 4 of the genome.
ORF	open reading frame
SOM	Southern Ocean Mariculture, a land based abalone farm near Port Fairy
UV	Ultraviolet light, a form of electromagnetic radiation with a wavelength <400nm, which has germicidal properties by inflicting damage to DNA of microorganisms most effectively at c. 254 nm wavelength
VAIC	Victorian Abalone Industry Committee

Version control

First draft:	24 December 2018
Final report:	30 December 2018

1.0 Introduction

This document was developed after DigsFish Services Pty Ltd was asked by the Victorian Abalone Industry Committee (VAIC) to provide advice regarding potential biosecurity risks to the wild abalone fishery associated with a proposed land based abalone farm (the Yumbah Nyamat Abalone Farm, YNAF) near Portland, Victoria, so that the VAIC could make informed decisions regarding their submissions to the works approval public consultation process. The VAIC represents the interests of the wild catch abalone fishery in Victoria, which harvests blacklip abalone (*Haliotis rubra*) and greenlip abalone (*Haliotis laevigata*) under strict fisheries management quotas. The major area of concern of the VAIC was in relation to the adequacy of proposed treatment (if any) of effluent water from the YNAF and whether the proposed biosecurity management strategies at the YNAF would be sufficient to protect wild stocks of abalone on nearby reefs from exposure to disease agents of significance to abalone. Dr Ben Diggles from DigsFish Services[1] inspected the Yumbah Aquaculture Planning Permit Application (Yumbah Aquaculture 2018a), Yumbah Aquaculture Works Approval Application (Yumbah Aquaculture 2018b) and the Yumbah Nyamat Draft Biosecurity Plan (Yumbah Aquaculture 2018c) and developed this report to provide VAIC with an independent assessment of the veracity of the contents of those documents and the adequacy of the proposed biosecurity arrangements for YNAF in relation to the risk of release of disease agents of abalone into the adjacent wild fishery.

2.0 Diseases of concern for abalone fishing and farming in Australia

Abalone farming in the southern hemisphere has increased in scale significantly over the last 15 to 20 years. The expansion of the industry has been accompanied by increased understanding of disease agents of abalone. Bacterial, fungal, protozoan, metazoan and, most recently, viral disease agents have been recorded from wild and cultured abalone in many parts of the world (Lester and Davis 1981, Goggin and Lester 1995, Friedman et al. 2000, Moore et al. 2001, 2007, Diggles et al. 2002, Hine et al. 2002, Chang et al. 2005, Diggles and Oliver 2005, Hooper et al. 2007, Hills 2007, Corbeil et al. 2010, 2014, 2016, Mayfield et al. 2011). While bacterial and fungal disease agents are (with a few exceptions) ubiquitous opportunistic pathogens, the usual source of other obligate disease agents (virus, protozoa, metazoa) when they appear in abalone farms is generally through cross contamination of cultured abalone with wild broodstock held on site, or through exposure of cultured abalone to untreated water containing infective stages that originate from wild molluscs or other carrier species living adjacent to the facility (Diggles et al. 2002). Of course, there are large knowledge gaps in relation to disease agents that infect aquatic animals in Australia, including abalone (Handlinger et al. 2006). Because of this, there remains a significant risk of emergence of as yet unknown disease agents, even in the absence of their identification (Gaughan 2002). There is also an unquantifiable risk that biosecurity leaks could allow exotic diseases to be introduced into Australia at the border (e.g. via ballast water), and/or that new endemic diseases could emerge in abalone aquaculture in Australia at some time in the future. Because of this, it was considered appropriate to examine whether biosecurity arrangements at YNAF would reduce or eliminate the risk of backspill infection of wild abalone with various economically important diseases of abalone (OIE 2018a, Spark et al. 2018). The various pests and diseases of significance to wild and cultured abalone are summarised in Table 1.

[1] For more information on Dr Diggles' 25+ years experience in relation to matters pertaining to diseases of aquatic animals, see http://www.digsfish.com/publications.html

Table 1. Significant pests and disease agents that have cause economic harm in wild and cultured abalone.

Significant diseases of cultured abalone	**Species affected**	**Geographic range**	**Listed by OIE***	**Exotic to Australia**
Viruses				
1. Abalone viral ganglioneuritis (AVG)	*Haliotis laevigata, Haliotis rubra, H. laevigata* x *rubra Haliotis hannai, H. diversicolor*	Victoria, Tasmania, China, Taiwan	✓	
2. Amyotrophia –like viruses of abalone	*Haliotis discus discus, H. discus hannai*	Japan		✓
Bacteria				
3. Infection with *Xenohaliotis californiensis*	*Haliotis cracherodii, H. sorenseni, H. rufescens, H. corrugata, H. fulgens, H. diversicolor supertexta* and others.	USA, Mexico, Europe, China, Thailand, Europe	✓	✓
Fungi				
4. Deuteromycote fungal infection of abalone shell	*Haliotis iris, H. australis, H. virginea*	New Zealand		✓
Protozoa				
5. Infection with *Perkinsus olseni*	*Haliotis rubra, H. laevigata, H. scalaris, H. cyclobates*	Worldwide	✓	
6. Infection with haplosporidians	*Haliotis iris, H. tuberculata*	New Zealand, Europe		✓
Metazoa				
7. Infection with Mudworms	*Haliotis rubra, H. laevigata, H. scalaris, H. cyclobates, H. iris, H. australis, H. virginea* , and probably all species of abalone are susceptible	Worldwide		
8. Infection with Sabellid polychaetes	*Haliotis rufescens, Haliotis fulgens, Haliotis corrugata, Haliotis midae*, and probably all species of abalone are susceptible	USA, Mexico, Europe, South Africa, South America		✓

*OIE (2018a)

References: 1: Hooper et al. 2007, Handlinger 2007, Hills 2007, Corbeil et al. 2010, 2014, 2016, Baulch et al. 2013, 2: Nakatsugawa et al. 1988, 1999, Harada et al. 1993, Otsu and Sasaki 1997, 3: Gardner et al. 1995, Friedman et al. 2000, 2002, 2003, Moore et al. 2000, 2001, Balserio et al. 2006, Wetchateng et al. 2010, OIE 2018c, 4: Friedman et al. 1997, Grindley et al. 1998, Nollens et al. 2002, 2003, 2004, Diggles and Oliver 2005, 5: Lester 1986, O'Donoghue et al. 1991; Goggin and Lester 1995, Lester and Hayward 2005, Liggins and Upston 2010, Stone 2013, 6: Diggles et al. 2002, Hine et al. 2002, Reece and Stokes 2003, Diggles and Oliver 2005, Balserio et al. 2006, 7: (Kojima and Imajima 1982, Lleonart et al. 2003a, 2003b, Handlinger et al. 2004, Diggles and Oliver 2005, 8: Kuris 1997, Leighton 1998, Kuris and Culver 1999, Finley et al. 2003, Moore et al. 2007.

Previous biosecurity documents and disease risk assessments for the abalone fishing and farming industries in Australia (Tasmania DPIW 2007, Diggles 2011a, 2011b, 2012, Baulch et al. 2013, Spark et al. 2018) have determined that the endemic disease agents of greatest significance to the health of wild and cultured abalone in this country are abalone viral ganglioneuritis disease (AVG), which is caused by infection with Abalone Herpesvirus 1 (AbHV), and perkinsosis caused by infection with the protozoan parasite *Perkinsus olseni*. Perkinsosis is problematic in abalone populations in South Australia, NSW and New Zealand whenever water temperatures exceed approximately 19-20°C, so this disease will likely become a problem in Victorian abalone aquaculture sometime in the near future under the current global warming scenario. Similarly, risk assessments done by the Tasmanian Government found that untreated discharge of water from abalone farms and holding facilities into the marine environment was a high risk activity with potential for high (regionally significant) consequences, including spread of AbHV viruses into the environment adjacent to abalone farms which potentially could result in outbreaks of AVG disease in wild abalone populations (Tasmania DPIW 2007, Baulch et al. 2013, OIE 2018b). More detailed information on these two diseases is presented in Sections 2.1 and 2.2. The definitions for the various likelihood and consequence descriptions used in these sections are contained in Appendix 1.

2.1 Abalone Viral Ganglioneuritis (AVG)

Aetiologic agent: AVG is caused by infection with abalone herpesvirus (AbHV-1), the first member of the genus *Haliotivirus* and second member of the Family *Malacoherpesviridae* (see Savin et al. 2010). At least 6 different AbHV genotypes have been identified to date, including 5 strains (Vic1, Tas1, Tas2, Tas3 and Tas4) found so far in Australia, mostly in Tasmanian waters (Corbeil et al. 2014, 2016).

OIE List: Yes **Reportable in Victoria:** Yes

Australias status: AVG infections have been reported from Victoria and Tasmania, and the disease is reportable in all States except the ACT. It is likely that at least some of the herpesvirus isolates responsible for the outbreaks of abalone viral mortality reported in China and Taiwan originated from Australia and were translocated in live exported abalone (Chen et al. 2012, Cowley et al. 2012, Corbeil et al. 2014, 2016, OIE 2018b).

Epidemiology

In December 2005 disease outbreaks resulting in acute mass mortalities of up to 90% or more in greenlip abalone (*Haliotis laevigata*), blacklip abalone (*H. rubra*) and hybrid abalone (*Haliotis laevigata* × *H. rubra*) due to a novel herpes-like virus occurred in three abalone aquaculture facilities, two land based farms in western Victoria (Coastal Seafarms (CS), near Portland, and Southern Ocean Mariculture (SOM), near Port Fairy) and one sea based farm (Abonex) in Westernport Bay near Flinders in central Victoria (Victoria DPI 2006, Hooper et al. 2007, Hills 2007, Corbeil et al. 2010). The virus was associated with inflammation and necrosis of neural ganglia (mainly those in the cerebral and buccal regions), but also in nerve bundles and pleuropedal ganglia within the foot muscle (Hooper et al.

2007). The ganglioneuritis was associated with sudden high levels of mortalities up to 90% within 14 days of onset (Hooper et al. 2007). All sizes of abalone were affected and exhibited external signs including swollen mouths and prolapse of the radula, and loss of righting reflex (Hooper et al. 2007). Electron microscope and genetic studies confirmed the disease agent causing abalone ganglioneuritis (AVG) was a neurotrophic herpes-like virus (Tan et al. 2008, Corbeil et al. 2010, Savin et al. 2010) closely related to herpes-like viruses responsible for mortalities in abalone in Taiwan (Chang et al. 2005, Corbeil et al. 2010, Savin et al. 2010). Indeed, it appears very likely that the herpesvirus responsible for abalone viral mortality in China and Taiwan originated from Australia and was translocated with the large volumes of live *H. laevigata* and *H. rubra* that are exported into those countries from Australia each year (Chen et al. 2012, OIE 2018b). This is suggested by the fact that the 99% genetic similarity between Australian and Taiwanese isolates of AbHV is within the range of the genetic variation that is evident between the 5 strains (Vic1, Tas1, Tas2, Tas3 and Tas4) found so far in Australia (Chen et al. 2012, Cowley et al. 2012, Corbeil et al. 2014, 2016).

The sea based farm (Abonex) in Westernport Bay central Victoria which had received stock from Coastal Seafarms (CS) in December 2005 noted increased mortality rates due to AVG in early January 2006, and voluntarily destocked and decontaminated the facility (Hills 2007, Hooper et al. 2007). However a 4th farm in Westernport Bay, located 640 meters away from Abonex, became infected in late April 2006 and was depopulated and decontaminated by early May 2006 (Victoria DPI 2006, Hills 2007). Both CS and SOM pumped raw seawater into their facilities, through their tanks then back out into the ocean via settling ponds without treatment. SOM continued to discharge water into the adjacent environment as they did not immediately destock until after a second, much larger scale disease outbreak occurred in late March 2006, after which diseased abalone with AVG were detected in wild abalone populations on reefs adjacent to SOM at Port Fairy in early May 2006 (Victoria DPI 2006). Since then AVG spread in waves radiating outward from this point easterly and westerly along the Victorian coast, initially at rates of up to 5-10 km/month (Hills 2007, Mayfield et al. 2010), significantly impacting wild abalone populations and substantially reducing commercial catches and recruitment in the wild fishery (Mayfield et al. 2011, Conrad 2015).

In mid 2008, adult wild caught abalone sampled from a commercial processing facility in Tasmania that was recording low levels of mortality were positive for AbHV-1 by PCR at a prevalence of 39% (Crane et al. 2009, Corbeil et al. 2010). Further research found that the disease agent occurs naturally at very low prevalences (3 out of 1625 abalone = 0.18% prevalence) in latent, subclinical infections of wild populations of abalone in Tasmania (Corbeil et al. 2010, Ellard et al. 2011), however the sensitivity of the earlier AbHV-1 surveys was confounded by the fact that the PCR test used did not detect certain strain variants of the virus (MJ Crane, personal communication, Corbeil et al. 2014, 2016). Also, latency characterised by low viral production and/or an abortive viral cycle is well recognised in aquatic herpesvirus infections (LeDeuff et al. 1996, Eide et al. 2011) and in such cases viral particles may not be sufficiently numerous to detect even using PCR (Batista et al. 2007). It is now known that several strains of AbHV (Tas1, Tas2, Tas3 and Tas4) are endemic in wild abalone populations in Tasmanian waters at low prevalences, and another strain of the virus (Vic1) was detected in the coastal waters of western Victoria at high prevalences during the AVG disease outbreak in wild abalone (Crane et al. 2009, Ellard 2011, Corbeil et al. 2010, 2014, 2016). Hence the prevalence of subclinical AbHV

infection in wild populations of abalone in Tasmania is likely to be somewhat higher than the earlier surveys suggest, with current scientific consensus suggesting that at least 4 AbHV variants are endemic to Tasmanian waters, with recent work suggesting prevalence levels approximating 7% (Department of Agriculture 2014). AbHV is only known to infect abalone at this time, and it has not been reported in wild abalone from any other regions of Australia, although it has been detected in onshore holding tanks in Western Australia following movements of abalone from Tasmanian processors (Ellard et al. 2011). While it appears that several Asian abalone species are susceptible to disease caused by AbHV, it appears that the paua (*Haliotis iris*) from New Zealand is refractory to infection (Corbeil et al. 2017).

AbHV was detected during AVG disease outbreaks in various abalone processing plants in Tasmania in 2008 and 2009, but in these cases the outbreaks occurred within closed-loop systems and were therefore contained within the infected premises (Ellard 2011). However, in October/November 2010 outbreaks of AVG occurred in several processing facilities, including one in Bicheno, where AbHV contaminated effluent water was released into the environment within 90-100 meters of the intake of a land based abalone farm, resulting in an AVG disease outbreak in that farm in January 2011 (Ellard 2011, Ellard et al. 2011). The land based farm was issued with an order to cease discharge by the Tasmanian DPIWE and was emptied of all stock and disinfected (Ellard 2011, Ellard et al. 2011, Diggles 2011a).

Surveillance is showing that AbHV is relatively common in abalone processing facilities (Ellard et al. 2011, Department of Agriculture 2014), increasing the risk of AVG outbreaks within these facilities. AVG outbreaks in captive abalone quickly amplify the disease agent to high levels in culture water (Corbeil et al. 2012, 2014), and thus they pose a high risk of backspill infection of wild abalone wherever untreated effluent water is discharged into the environment (Tasmania DPIW 2007, Ellard 2011). Because of this the Tasmanian Government now requires processors who hold abalone to treat their outgoing water so as to achieve a minimum 3 log10 reduction in heterotrophic bacterial levels, and maintain discharge bacterial concentrations of $\leq$ 999 bacterial colony forming units per mL (Baulch and Ellard 2011, Green 2011, Baulch et al. 2013). The increased frequency of outbreaks of AVG in abalone processors in Tasmania suggests that holding of wild caught abalone at high densities in processing plants is a high risk activity likely to stress abalone that are latently infected with AbHV. The stress increases viral replication and shedding, resulting in outbreaks of AVG disease within the holding facilities which, if effluent water is discharged into the environment untreated, greatly increases the risk of backspill AbHV infection within adjacent wild and cultured abalone populations (Ellard 2011, Ellard et al. 2011, Diggles 2011a, Baulch et al. 2013).

AbHV is transmitted horizontally via the water or by mucus trails and infection is by direct exposure of abalone to viral particles (Crane et al. 2009, Corbeil et al. 2012). Elevated water temperatures appear to be the major environmental risk factor associated with disease outbreaks due to herpesviruses in other molluscs (Elston 1997, Renault and Novoa 2004, Garcia et al. 2011, Arzul et al. 2017), as rates of viral replication increase with increasing water temperature. Other stressors such as reduced water quality, reduced oxygen levels and increased production of metabolites such as ammonia are also more likely to occur when water temperatures are high. This suggests that in light of the continuing detection of sub-clinical AbHV infections in wild caught abalone held in live holding tanks at abalone processors at water temperatures >15°C (Ellard et al. 2011), the period of greatest risk of AVG outbreaks in abalone

farms and holding facilities and in the wild is likely to be during late spring and over the summer months (Corbeil et al. 2016).

During an outbreak of AVG in captive abalone, levels of herpesvirus in the water can get very high because viral levels in infected molluscs can quickly increase to exceed 1 x 10^7 viral DNA copies per mg of tissue (see Corbeil et al. 2012a, 2012b). This means that a single moribund juvenile abalone of c. 10 grams (with, say a conservative 5 grams of virus infected soft tissue) could harbour 5 x 1000 x 1 x 10^7 = >5 x 10^{10} viral DNA copies, which (assuming the viral particles remain viable long enough to be transferred to the water after the death of the host) is theoretically enough virus to kill around 5,000 other abalone assuming a minimum lethal dose of 1 x 10^7 viral DNA copies per abalone (a likely lethal dose based on the results from Corbeil et al. (2012b) for abalone exposed to AbHV and Schikorski et al. (2011a, 2011b) for Pacific oysters exposed to another member of the Family Malacoherpesviridae, namely OsHV-1 µVar (see Savin et al. 2010). This massive viral replication in diseased abalone explains why AVG spreads so rapidly in populations of confined abalone. These data demonstrate that AbHV is highly virulent, that confined abalone can become infected once exposed to relatively low concentrations of the virus (Corbeil et al. 2012a, 2012b), and that all virus variants (Vic1, Tas1, Tas2, Tas3 and Tas4) cause disease and mortality in all native abalone stocks tested (greenlip, blacklip and brownlip) (Corbeil et al. 2016) without any signs of resistance developing to date (Crane et al. 2013).

Infection and establishment of AbHV in new hosts occurs only if sufficient viable viral particles are introduced into an area where susceptible abalone are present (i.e. they receive an infective dose). Crane et al. (2009) used viral homogenates from clinically diseased abalone in a dilution series to find the LD50 by injection into the pedal muscle, and found the LD50 to be $10^{-6.39}$ of the stock solution, suggesting that AbHV is highly virulent for abalone. When AbHV was added to the water, the LD50 increased to around 10^{-2} of the stock solution and after a 3 to 8 day prepatent period, death occurred over a period of 7 to 16 days (Crane et al. 2009). AbHV-1 was also transmissable horizontally by co-habitation with infected abalone with 100% mortality observed within 8 days (McColl et al. 2007, Crane et al. 2009). The minimum infective dose for successful horizontal transmission of AbHV-1 is not known, although AbHV-1 is highly virulent for abalone and a 1/100 dilution of viral homogenates from diseased abalone was sufficient to cause infection via the water route (Crane et al. 2009).

During the original outbreak of AVG in Victoria, AbHV-1 was transmitted horizontally at least 640 meters between farms in Westernport Bay (Victoria DPI 2006, Hills 2007). Experience from viral disease agents of finfish (Infectious Pancreatic Necrosis Virus and Infectious Salmon Anaemia) suggests that risk of "backspill" disease transmission to wild fishes increases significantly within 5 km of an aquaculture establishment with clinically diseased fish (Jarp and Karlsen 1997, Scheel et al. 2007, Wallace et al. 2008). Clearly in the absence of any treatment of effluent water from an abalone farm with outbreaks of AVG, the risk of disease transmission to adjacent wild stocks via untreated effluent water is very high for a significant distance away from the effluent pipe outlet. Backspill of AbHV into the environment via effluent water from abalone farms causing AVG disease outbreaks in adjacent abalone stocks has occurred on at least 2 occasions. The first occurrence was after the AVG disease outbreaks in abalone farms near Port Fairy, Victoria in December 2005, which subsequently initiated an outbreak of clinical AGV in wild stocks of abalone in the adjacent ocean in early 2006. The second

incident occurred near Bicheno, Tasmania in December 2010, where untreated effluent water from an AVG outbreak in an abalone holding facility was responsible for initiating an outbreak of AVG in a nearby abalone farm.

Consequence assessment

Outbreaks of AVG disease due to AbHV in abalone have a very serious impact on the productivity and profitability of not only aquaculture businesses, but also wild fisheries (Mayfield et al. 2011, Conrad 2015). Since AbHV is a notifiable disease agent, and there are previous instances where the agent has spread from affected abalone farms and holding facilities into wild populations of abalone, in order to minimise risks to the environment and wild fisheries from outbreaks of AVG in land based abalone farms, these days government responses to AVG disease outbreaks will most likely include orders to immediately cease discharge of effluent water into the receiving environment (Department of Agriculture 2014). While the ability to recirculate water at YNAF may allow for an effective emergency harvest (e.g. allow sufficient time for an emergency harvest to be completed prior to death of all abalone on site due to anoxia), the process of shutdown, destruction, dry out and testing of sentinel animals prior to restart after an AVG incursion will always have a significant impact on the businesses bottom line. At the same time, because a certain level of mortality is always accepted as normal on abalone farms, without treatment of effluent water, there is a high risk of backspill contamination of wild abalone populations adjacent to the effluent pipe outlets prior to farmers noticing the greater than normal mortality rates which would trigger enactment of biosecurity processes such as water recirculation. Thus under such circumstances there still remains a very high risk that increased levels of AbHV would pass into the receiving environment through untreated effluent water, which could precipitate a repeat AVG epizootic in wild abalone as occurred around Port Fairy in 2006. Taking these factors into consideration, the risk of introduction of AbHV into YNAF under the proposed biosecurity conditions are probably low or very low (potentially via contaminated broodstock or intake water), however the consequences of establishment and spread of AbHV in abalone at YNAF for both YNAF and the adjacent wild fishery are considered to be high or extreme, hence additional risk mitigation is required to reduce these risks to an acceptable level.

2.2 Infection with *Perkinsus olseni*

Aetiologic agent: *Perkinsus olseni* and other parasitic protozoa within the Family Perkinsidae.

OIE List: Yes **Reportable in Victoria:** Yes

Australias status: *Perkinsus olseni* infections have so far been reported from all states except Tasmania, and the disease is reportable in all States except the ACT.

Epidemiology

Members of the genus *Perkinsus* within the Family Perkinsidae are closely related to dinoflagellates (Reece et al. 1997). These obligate protistan parasites are known to infect a wide range of marine molluscs in many regions of the world (Goggin and Lester 1987, Villalba et al. 2004). The life cycle of

Perkinsus spp. involves vegetative proliferation within the host by trophozoites that undergo successive bipartitioning (Goggin et al. 1989). When host tissues infected by *Perkinsus* spp. are incubated in fluid thioglycollate medium (FTM), the trophozoites enlarge and develop a thick cell wall, becoming easy to visualise after staining with lugols iodine (Ray 1966). When these enlarged hypnospore stages are transferred into seawater, they form zoosporangia and production of hundreds to thousands of zoospores occurs within the original cell wall (Villalba et al. 2004). The biflagellated zoospores 3-5 µm in size leave the zoosporangium through discharge tubes and enter the water to reinfect new hosts via the gills, palps and digestive tract (Villalba et al. 2004). Infection of susceptible molluscs can occur horizontally through the water by cohabitation via contact with zoospores, but trophozoites and hypnospores have also been shown experimentally to cause infection (Goggin et al. 1989), and the disease can be transmitted via vectors such as ectoparasitic snails (White et al. 1987).

Perkinsus olseni was originally described from blacklip abalone *Haliotis rubra* near Port Lincoln in Spencer Gulf, SA (Lester and Davis 1981), but was subsequently associated with severe mortalities in greenlip abalone *Haliotis laevigata* around 140 km away in the western side of Gulf St Vincent (Lester 1986, O'Donoghue et al. 1991; Goggin and Lester 1995) in SA. More recently the same parasite has been associated with significant mortality events in abalone along the central and southern coast of NSW (Liggins and Upston 2010), and in paua (*Haliotis iris*) cultured on land based pump ashore abalone farms in New Zealand (Stone 2013). The presence of *P. olseni* in infected abalone of all sizes was characterized by the presence of macroscopic necrotic nodules (0.5-8.0 mm in diameter) in the adductor muscles and mantle (O'Donoghue et al. 1991), and the disease process is facilitated by high water temperatures >20°C (Lester 1986, Lester and Hayward 2005). *Perkinsus*-like parasites have also been reported from 30 out of 84 species of molluscs examined from the Great Barrier Reef (Goggin and Lester 1987), from pearl oysters *Pinctada maxima* from Torres Strait (Norton et al. 1993) as well as several species of molluscs from WA (Hine and Thorne 2000). To date the *Perkinsus* –like parasites from Australian molluscs have all been identified as *P. olseni* (see Murrell et al. 2002, Lester and Hayward 2005). *Perkinsus olseni* has also been recorded in many other regions worldwide, including in cockles (*Austrovenus stutchburyi*) and paua in the North Island of New Zealand, where its distribution is probably limited by temperature (Hine and Diggles 2002). *Perkinsus olseni* has also been associated with mass mortalities of the Manila clam *Tapes philippinarum* in South Korea and has been detected in clams from Japan, China , Vietnam, Europe and Uraguay (Villalba et al. 2004, Park et al. 2005).

The release pathway for introduction of protozoans like *P. olseni* into the YNAF facility is horizontal and direct via infected abalone broodstock held on site, or horizontally through the intake water via protozoans originating from wild abalone or other molluscs living inside or adjacent to the sites water intake. The existing water treatments used at YNAF would not prevent entry of protozoan infective stages pumped in the intake water onto the site. *Perkinsus olseni* has been recorded in molluscs in Victoria, including flat oysters (*Ostrea angasi*) in poor condition[2], but it usually requires minimum water temperatures of around 20°C in order to cause disease in abalone. The water temperatures at YNAF exceed 20°C for several months during summer, thus a window of potential infection already exists when conditions would be suitable for transmission of *P. olseni* into the facility. However, the

[2] https://www.animalhealthaustralia.com.au/wp-content/uploads/2015/09/AHiA2015_Chapter5.pdf

global warming trend is likely to extend this window and increase the probability of establishment of *P. olseni* in Victorian abalone farms such as YNAF at some stage in the future.

Zoospores of *P. olseni* can survive in seawater at temperatures of 20-25°C for up to 28 days (Chu and Greene 1989), but under natural circumstances, susceptible molluscs need to be in close proximity to diseased molluscs for horizontal transmission to occur, possibly due to the fact that a relatively high numbers of infective stages (zoospores, and/or trophozoites and/or hypnospores) are required to initiate infection (around 1 x 10^5 infective stages/oyster for *P. marinus*, see Andrews 1996). However, dead molluscs can liberate very large numbers of infective stages (Andrews 1996), which can then be concentrated within the digestive tract of filter feeding bivalves as well as many other species of grazing or filter feeding molluscs which could act as vectors. *Perkinsus* is known to be excluded from aquaculture facilities by using filtration and UV irradiation (Ford et al. 2001) and it is susceptible to disinfectants (Hine 1996, Goggin et al. 1990).

Consequence assessment

Outbreaks of disease due to *P. olseni* in abalone at YNAF would likely have significant short term impacts on the productivity and profitability of the business. The disease is not treatable and can cause mass mortality as well as reduction in the marketability of surviving abalone. Since *P. olseni* is a notifiable disease agent within Australia and internationally, there would likely be significant impacts on trade. As effluent water is untreated, there would also be a risk of spread of the disease agent to wild abalone adjacent to the facility, and *P. olseni* is known to cause disease and mortality in wild abalone under certain circumstances, particularly with warmer water temperatures in summer (Lester 1986, O'Donoghue et al. 1991, Liggins and Upston 2010). Given the current global warming trend, the potential risks to the receiving environment and wild fisheries from *P. olseni* are increasing. The situation in relation to the need to develop protocols for water and effluent management during a *P. olseni* outbreak to allow an effective emergency harvest without endangering wild abalone populations would be virtually identical to those discussed above for AVG. Taking these factors into consideration, the risk of introduction of *P. olseni* into YNAF under the proposed biosecurity conditions are moderate (potentially via contaminated broodstock or intake water), and the consequences of establishment and spread of *P. olseni* in abalone at YNAF for both YNAF and the adjacent wild fishery are considered to be moderate to high, hence additional risk mitigation is required to reduce risks to an acceptable level.

3.0 Source of the 2006 AVG outbreak in wild abalone in Victoria

A statement as to the role of abalone farms in the index case outbreak of AVG in wild abalone at Port Fairy in 2006 was contained in Section 13.1.2 Abalone disease on page 90 of Yumbah Aquaculture Ltd (2018b). The statement said;

"*The outcomes of a Supreme Court class action law suit against the abalone farms confirmed that the infected abalone farms were not the cause of impact to wild stock abalone and the closure of the commercial abalone wild stock fishery*."

I was particularly concerned with this statement, as it is incorrect, grossly misleading and contradictory to advice from aquatic disease experts who investigated the matter at the time (e.g. Hardy-Smith 2006, Mouton 2006, Handlinger 2007, Prince 2007), contradictory to official biosecurity documents such as the Federal Government Aquavetplan Disease Strategy Manual for AVG (Department of Agriculture 2014), as well as contradictory to subsequent risk assessments and scientific investigations, virtually all of which have acknowledged that the most likely source of the AVG outbreak in wild abalone stocks in Victoria in 2006-2007 was from AbHV carried in untreated effluent water from the nearby infected abalone farms (Tasmania DPIW 2007, Crane et al. 2009, Corbeil et al. 2010, 2014, 2016, Baulch et al. 2013, OIE 2018b).

Instead, in simplest terms the Supreme Court decision (Regent Holdings vs State of Victoria) centred around a question of whether the Victorian government did all that was considered reasonable (by a reasonable (non-expert) person) to prevent spread of the disease at the time, given the knowledge then available. Even so, the fact that virtually all aquatic animal diseases (particularly viruses) are transmitted horizontally from host to host via the water is a matter of general knowledge for aquatic animal health experts and laypersons alike. Instead, the real issue in this case centred around the fact that there was no direct evidence as to when the virus first escaped from SOM's farm. The evidence disclosed that while unusually high mortalities of abalone at CS were first detected on 19th December 2005, and unusually high mortalities of abalone at SOM were first detected on 28 December 2005 (Hardy Smith 2006), officers of the Victorian DPI first learnt of the disease outbreak at SOM on 10 January 2006. In other words, potentially infected effluent water had been flowing to the sea from CS and SOM for at least 3 weeks before the State authorities were alerted to the presence of the disease outbreak, and as such it was possible that the virus had already escaped into the wild prior to the government being alerted to the problem.

After 10 January 2006, without treatment of effluent or intake water at SOM, it is certainly possible that diseased wild abalone on reefs near the SOM water intake may have contributed to increased AbHV in intake water which could explain the explosive nature of the second (and much larger) AVG outbreak at SOM which started in late March 2006. As such, the supreme court could not establish that "reasonable care" by the State tortfeasors would have prevented the virus escaping[3]. However, in my professional opinion, the most likely scenario is that the virus cycled unseen for 2 months in escapee abalone within the effluent drains or intake/ effluent pipework at SOM prior to the disease re-emerging in growout tanks (Prince 2007). In any case, certainly by late May 2006 large numbers of diseased wild abalone were observed on the reefs near the effluent pipe from SOM, and the absence of treatment of intake or effluent water meant there was never any hope of effective biosecurity control during the incident.

In any case, clearly such a scenario is very different to a statement to the effect that "*infected abalone farms were not the cause of impact to wild stock abalone and the closure of the commercial abalone wild stock fishery*". In view of the expert opinion on this matter (see Section 3.1) it is strongly recommended that this grossly misleading statement is adjusted by Yumbah Aquaculture Ltd (2018b) to better reflect the reality of what happened.

[3] *Regent Holdings v State of Victoria* [2013] VSC 601 at [59], [60]. http://www8.austlii.edu.au/cgi-bin/viewdoc/au/cases/vic/VSC/2013/601.html

3.1 Expert opinion

Hardy-Smith (2006) conducted a review of the initial outbreak chronology and concluded "*that the index case in this disease outbreak is most likely to have occurred at CS"* (where diseased abalone were first recorded on 19th December 2005), and that the most likely source of AbHV-1 at the index farm (CS) was "*live wild (broodstock) abalone that were brought onto CS at the start of the outbreak*". Subsequent movements of abalone from CS to SOM and farms elsewhere (Abonex) explained the subsequent emergence of AVG at SOM and Abonex (Hardy Smith 2006, Prince 2007).

Mouton (2006) in her invited independent review of the outbreak, stated "*In the case of Coastal Seafarms, the presence of a herpes like virus was confirmed during January 2006. Abalone were dying on the farm at this time. Considering what was known about the disease then, an argument can be made that the farm should have depopulated immediately. Abalone farms in general are not designed or managed in a way that permits the control of a highly infectious disease. It is not probable that Coastal Seafarms would have been able to stop the disease from affecting the entire farm and subsequent events demonstrated this."*

Handlinger (2007) agreed with the above statement from Mouton and observed that "*There is a case to take the issue of farm design to minimise disease spread as a serious issue for the whole industry. In considering the investment implications of such an approach farms should consider that no sector has probably been more affected by this outbreak than the directly impacted farms, and that the advantages provided would apply equally to locally propagating endemic diseases. Savings from the latter would off-set the cost*."

Prince (2007) in his summary of events at the time, stated "*a herpes-like virus that escaped from an abalone farm near Port Fairy in May 2006 has spread to infect reefs over some 90 km of coastline in the Western Zone of Victoria's abalone fishery*". He further noted that "*The international experts who participated in the WADA study tour agreed unanimously that abalone mariculture inevitably posed a disease risk to the wild stocks of a species and that the best that could be achieved was an active management of that risk*." He also noted that "*A glaring but only indicative example of the lack of bio-security awareness has been the FRDC Family Lines project, which had as a central aim translocating broodstock from all over Australia so that selective breeding could occur*." Given we now know that AbHV variants are endemic to Tasmanian wild abalone populations and occur at prevalences of around 7% in apparently healthy wild abalone (Department of Agriculture 2014), but regularly revert to a clinical disease state when those wild abalone are held in onshore holding facilities (Ellard 2011, Ellard et al. 2011, Baulch et al. 2013), the fact that wild caught broodstock from Tasmania were implicated in the index AVG disease outbreaks at CS in December 2005 (Hardy Smith 2006) is a critical point of evidence as to the mechanism of initial emergence and spread of the disease.

Prince (2007) also observed that '*Contact between farmed and wild animals, via water flows, escapees, brood-stock translocations or disposal of offal and mortalities. A major bio-security issue on all farms is controlling the movement of abalone through the plumbing system. In all the Pump-On farms visited escapee abalone had established populations throughout the system including in the settlement ponds,*

which during disease challenges became infective reservoirs of the disease rather than entrapping sinks.", and in the case of the sea based abalone farms at Abonex, "*Farms should not be placed near natural beds of abalone and in this regard the practice of In-Sea farming needs especially stringent consideration.*"

Soon after the disease outbreak at Port Fairy, the Tasmanian Government undertook a risk assessment of abalone fishing and farming activities using AVG as a case study (Tasmania DPIW 2007). The document stated "*Investigations into the disease event have suggested that the initial pattern of spread amongst farms was strongly linked to movements of broodstock as part of a selective breeding program. Although affected farms undertook voluntary destocking, clinical disease was later detected in wild populations of abalone on reefs in close proximity to farm outflows*" (Tasmania DPIW 2007). The document then went on to classify the following as high risk activities with high likelihood of discharge of AbHV from a facility if diseased abalone were present, stating that high risk activities included: *Discharge of water from processors into the marine environment, Discharge of water from farms into the marine environment,* and *Discharge of water from live holding facilities into the marine environment* (Tasmania DPIW 2007).

The Tasmanian Government has been proactive on biosecurity relating to AVG. This is because it is now known that AbHV is endemic in apparently healthy, subclinically infected wild abalone in Tasmanian waters. Once appropriate diagnostic tests were developed, AbHV was found to be associated with mortalities in various abalone processing plants in Tasmania in 2008 and 2009 (Ellard 2011). It turns out that sporadic mortality events were occurring in these export holding facilities for some years, which probably explains the presence of abalone viral mortalities in Taiwan and China prior to its detection in Australia. However, after an AVG outbreak in a holding facility at Bicheno resulted in a large AVG outbreak in a nearby abalone farm in January 2011 (Ellard 2011, Ellard et al. 2011, Diggles 2011a), stringent controls were implemented requiring treatment of effluent water to reduce the risk of backspill infection of wild abalone (Baulch and Ellard 2011, Green 2011, Baulch et al. 2013). This legislation requires abalone holding facilities to treat their outgoing water to achieve a minimum 3 log10 reduction in heterotrophic bacterial levels, and maintain discharge bacterial concentrations of $\leq$ 999 bacterial colony forming units per mL (Baulch and Ellard 2011, Green 2011). Baulch et al. (2013) explained the reasons behind the legislation as follows "*live holding water that has previously contained abalone shedding virus has potential implications when being discharged directly into the marine environment, and it is highly likely that adjacent populations of abalone will become infected*".

The position of the Tasmanian Government on treatment of effluent water from abalone processors has been mirrored in the latest National Biosecurity Plan Guidelines for Abalone Farming (Spark et al. 2018), where they identified treatment of intake water and treatment of effluent water as critical risk management measures in current best practice biosecurity arrangements for abalone farms in Australia (Table 8 on page 20 in Spark et al. 2018). Based on this evidence provided by aquatic animal disease experts, as well as State and Federal Governments, it is vitally important that all existing and proposed new abalone farms in Australia learn the lessons from past mistakes, embrace current best practice for treatment of intake and effluent water (Sparks et al. 2018), and acknowledge that sub-standard biosecurity on abalone farms poses a significant risk to populations of wild abalone.

4.0 Proposed risk mitigation measures

The information from Yumbah Aquaculture (2018b, 2018c) suggests that the YNAF will be equipped with 100% recirculating capacity designed to maintain water flow across the tanks in the event of a catastrophic event such as an oil spill in Portland Bay or a disease outbreak. The recirculating system is designed to be compartmentalised and sections of the farm can be isolated on recirculating water from the recirculating reservoirs (i.e. all the eggs will not be in one basket).

In section 3.5 Biosecurity (on page 10,11) of Yumbah Aquaculture Ltd (2018b), they state *"Effective biosecurity is integral to any successful production system as it helps minimise unnecessary costs, can improve production outcomes, and helps maintain trade and market access. Those measures aimed at preventing disease entering farms in the first place can lead to a significant return on investment. Preventing disease not only protects businesses but has wider benefits for the industry and communities that would potentially be devastated by a significant disease outbreak"*.

However, the site plans and biosecurity documents (Yumbah Aquaculture(2018b, 2018c) do not present any biosecurity methods or infrastructure which would prevent diseases agents such as viruses (AbHV) or protozoans (*P. olseni*) to gain access to stock at YNAF. Instead, they state that "*The Yumbah Nyamat abalone farm will be equipped with 100% recirculating capacity designed to maintain water flow across the tanks in the event of catastrophic event such as an oil spill in Portland Bay or disease outbreak."*

In other words, the biosecurity plan does not aim to "*prevent disease entering farms in the first place"*, but only aims to restrict disease outbreaks once they are detected by moving to recirculated water. However, as previously identified in Section 2.1, because a certain level of background mortality is always accepted as normal on abalone farms, without treatment of effluent water, there is a high risk of backspill contamination of wild abalone populations adjacent to the effluent pipe outlets prior to farmers noticing the greater than normal mortality rates which would trigger enactment of biosecurity processes such as water recirculation. Thus, under such circumstances there still remains a very high risk that increased levels of AbHV would pass into the receiving environment through untreated effluent water in the days or weeks before a disease outbreak is detected, which could precipitate a repeat AVG epizootic in wild abalone as occurred around Port Fairy in 2006.

In section 10.2.2 Wastewater management options (on page 65) of Yumbah Aquaculture Ltd (2018b), they state there will be "*separate treatment facilities for wastewater from each module prior to discharge to the marine environment to assist with maximising the biosecurity of the farm*". However, the site plans and biosecurity documents (Yumbah Aquaculture(2018b, 2018c) do not present any biosecurity methods or infrastructure which would treat effluent water in such as way as to prevent backspill infection of wild abalone adjacent to the effluent pipes with diseases agents such as viruses (AbHV) or protozoans (*P. olseni*) which could gain access to YNAF via untreated intake water or thought biosecurity breaches in broodstock quarantine, etc.

In section 13.1.4 Best practice in biosecurity (on page 91) of Yumbah Aquaculture Ltd (2018b), they state that "*All Yumbah Farms are all approved under the Abalone Health Accreditation Plan (AAHAP). This formally recognised accreditation has been developed to provide Australian land-based abalone farms with the tools and templates to create fully auditable biosecurity plans. The Victorian Fisheries Authority recognises that in the abalone industry, "best practice is evolving, and a continual improvement process is required to ensure that the protocols are effectively implemented and adapt to new information and practices*".

What is not acknowledged here or elsewhere in these documents is that best practice for biosecurity management in abalone farming in Australia (as outlined in the latest National Biosecurity Plan Guidelines for Abalone Farming, see Spark et al. 2018) has recently evolved to identify treatment of intake water and treatment of effluent water as critical risk management measures in current best practice biosecurity arrangements for abalone farms in Australia (Table 8 on page 20 in Spark et al. 2018). So clearly the existing AAHP does not effectively mitigate all biosecurity risks, does not meet current best practice as outlined in Spark et al. (2018), and there is significant room for improvement in critical biosecurity areas, such as the need for treatment of discharged water, as required in other jurisdictions in Australia (Baulch et al. 2013)..

In section 13.2 Climate change impacts (on page 92) of Yumbah Aquaculture Ltd (2018b), there are several risks from climate change that are identified together with proposed treatments to reduce those risks. These were summarised in Table 31 on page 94 (Climate change risks and proposed treatment). However the fact that increased water temperatures will result in increased risk from diseases such as Perkinsosis (Lester and Hayward 2005, Liggins and Upston 2010) and AbHV-1 (see Corbeil et al. 2016) were not identified, nor addressed in this table. I suggest the following row should be included in Table 31.

Potential receptor of climate change	Potential risk	Potential risk treatment
Abalone diseases	Increased risk of infection with *Perkinsus olseni* , AbHV and other significant disease agents of abalone (e.g. haplosporidians) when water temperatures exceed 19-20°C	Filtration/UV irradiation of intake water to exclude potential infective stages/vectors of AbHV, *P. olseni* and other disease agents of abalone (e.g. Ford et al. 2001) Treatment of effluent water to minimise risk of backspill infection of wild abalone stocks (e.g. Baulch et al. 2013)

In section 13.4.2.2 Best practice in abalone production (on page 99) of Yumbah Aquaculture Ltd (2018b), they state that "*Broodstock which typically have a higher value than commercial farm stock will housed in a separate quarantine area and managed with suitably robust biosecurity protocols.*" However, nowhere in Yumbah Aquaculture Ltd (2018b) or Yumbah Aquaculture Ltd (2018c) are any details of these biosecurity protocols for broodstock disclosed. Further, they state that "*The abalone*

grow out areas will be compartmentalised so that each of the four separate modules are independent from each other. The four modules will be replicas but will be supplied with seawater from a distinct network of compartmentalised pipes. This increases the biosecurity across the farm." Given that none of the intake water is being treated prior to entry into the farm, it is not clear how dividing untreated intake water up into different intake pipes will increase biosecurity.

In this section they also state "*Concrete grow out tanks will be constructed on the finished ground level. This allows for optimal seawater flows through the concrete tanks. Abalone are light sensitive, and they require some shading from direct sun light. The grow out shelters will be constructed with black shade cloth over a cable grid. Black shadecloth creates the optimal light intensity necessary to replicate the abalone's natural environment."* While use of shade cloth may replicate the abalones natural environment, it does not reflect best practice in abalone production if the raceways are not completely enclosed to prevent access by birds or scavengers which could otherwise access cultured abalone and move them quickly across the farm and into the wild environment. If the shadecloth setup does indeed fully enclose the raceways and prevent access by birds or scavengers, this is an important biosecurity feature that should be mentioned in the documents.

In section 13.4.2.3 Best practice for seawater intake (on page 100) of Yumbah Aquaculture Ltd (2018b), they state that "*The purpose Yumbah Nyamat's proposed marine intake system is to draw in seawater from the ocean and deliver it to the abalone farm in a way that minimises adverse ecological impacts and ensures a supply of sufficient quantity and quality to the farm. The seawater intake system has been designed to maximise abalone productivity and avoid or minimise, to the extent practicable, adverse effects on the receiving environment, consistent with applicable legislation*." And "*Screens will be installed at the intake point to prevent entry of marine organisms and debris. Screens will be regularly cleaned to prevent biofouling, which could affect the reliability of the supply*." And "*Design of the intake pipe allows for efficient pigging to clean pipes and control marine fouling, so the reliability and quality of supply is not restricted."*

While screening of the intake and designing it so it can be cleaned on a regular basis is better than nothing, without further treatment of the intake water to prevent ingress of known pathogens such as AbHV and *P. olseni*, (as recommended by Spark et al. (2018) in the latest National Biosecurity Plan Guidelines for Abalone Farming), there is no evidence to suggest that the seawater intake design at YNAF meets current best practice for abalone farming in Australia.

In section *13.4.2.5 Outlet system* (on page 101) of Yumbah Aquaculture Ltd (2018b), they state that "*The purpose of the marine outlet system at Yumbah Nyamat is to dispose of the return from the abalone farm in a way that minimises negative environmental impacts and ensure that the return water quality is compliant with objectives*." However, without treatment of the effluent water to prevent backspill of known pathogens such as AbHV and *P. olseni* into wild abalone populations (as recommended by Spark et al. (2018) in the latest National Biosecurity Plan Guidelines for Abalone Farming), there is no evidence to suggest that the seawater outlet system at YNAF meets current best practice for abalone farming in Australia.

In their Table 32 entitled "*Potential non-routine upset situations and their management*", Yumbah Aquaculture Ltd (2018b) state the following measures to reduce risk in relation to issues that could potentially adversely impact the wild fishery.

Type of upset	Potential environmental impact	Measures to reduce risk
Threat of disease outbreaks (page 105)	Sick, dying or dead abalone Requirement to destock abalone Requirement to humanely destroy abalone Requirement to decontaminate sections or all of the farm Significant volume of contaminated waste requiring disposal	Four grow out modules each in isolation with the ability to shut off sections of the farm Biosecurity protocols enforced for staff and visitors. No wild stock will be brought on site Ongoing monitoring of farmed abalone will be conducted to identify any early onset of disease. Communication and monitoring of issues with Fisheries Vic, and commercial and rec divers. Regular communication with Fisheries Victoria will be maintained. Diseased abalone will be treated or destroyed in order to contain or control disease outbreak 100% emergency recirculation capacity
Impact to seabed in receiving environment (p 106)	Eutrophication resulting in excessive algal growth in vicinity of discharge pipes Eutrophication resulting in excessive algal growth within the mixing zone. Excessive suspended solids discharged results in smothering of reef	Discharge pipes will be angled away from the seabed. A marine monitoring program will be implemented to assess the condition of the seabed
Discharge treatment is ineffective (p 106)	Increased nutrients in discharge water Nutrient concentrations exceed licence requirements Excess build-up of sediment Build-up of sediment causes offensive odour	Discharge water settlement processes have been designed to remove sediment and nutrients prior to discharge. The performance of the system will be continuously assessed and optimised, where required. Accumulated sediment will be removed from treatment system before it becomes anoxic Discharge water is compliant with ANZECC guidelines with without water treatment.

In relation to "*Threat of disease outbreaks*", there is a conspicuous lack of specific information on, for example on the actual biosecurity protocols that will be enforced for staff and visitors, the source of broodstock in the hatchery (if no wild caught abalone are ever to be bought on site), how ongoing health

monitoring of farmed abalone will be conducted (methods, frequency, independent oversight), what triggers will determine when background mortality rates become unacceptable and indicate the potential outbreak of a disease event, how diseased abalone will be treated or destroyed in order to contain or control a disease outbreak, what triggers will instigate implementation of the 100% emergency recirculation capacity, and so on. The draft Biosecurity Plan document (Yumbah Aquaculture Ltd 2018c) is inadequate in that it does not specifically address any of these key operating protocols or trigger points, nor does it reference key documents such as the Aquavetplan for AVG (Department of Agriculture 2014), hence more details are required in order to determine whether the measures proposed to reduce risk presented in their Table 32 are adequate and sufficient to effectively mitigate the risks identified. Similar problems exist in the rows dedicated to "*Impact to seabed in receiving environment*" (p 106), and "*Discharge treatment is ineffective*" (p 106), particularly in relation to discharge treatment when it is considered that the ANZECC guidelines do not address minimum pathogen loads (e.g. AbHV, *P. olseni*) in the effluent water, as do more recent effluent standards such as those enforced by the Tasmanian Government for abalone processors (i.e. they must treat their outgoing water so as to achieve a minimum 3 log10 reduction in heterotrophic bacterial levels, and maintain discharge bacterial concentrations of ≤ 999 bacterial colony forming units per mL, see Baulch and Ellard 2011, Green 2011, Baulch et al. 2013).

In their Table 33, on page 107, in a statement on "Environmental commitment to Marine Protection" in of Yumbah Aquaculture Ltd (2018b), they state an objective to "*Manage activities that can directly or indirectly impact the marine environment to protect the biodiversity and natural environment of Portland Bay*." However, this is clearly not being done if national guidelines for best practice (such as treatment of effluent water to reduce or eliminate risks of spillback of disease agents into Portland Bay) are not being heeded.

In relation to the Yumbah Nyamat Biosecurity Plan (Yumbah Aquaculture Ltd 2018c), this draft document has some significant shortcomings and oversights. For example, their section 5.2 on major disease transmission routes does not identify intake water as a potential disease risk, however it is widely known that untreated intake water is one of the most common pathways of introduction of disease agents onto aquaculture facilities due to horizontal transfer of infectious particles or stages via the intake water (Kasai et al. 2002, Lightner 2005, Wyban 2009, Yoshimizu 2009). Furthermore, the document was silent on specific important biosecurity aspects such as standard operating protocols for disinfection, destruction, disposal, movements of personnel on site, and so on (Amass et al. 2000, Gavine et al. 2009, Kent et al. 2009, Spark et al. 2018). Furthermore, Yumbah Aquaculture Ltd (2018c) also did not propose any treatment of intake or effluent water at YNAF, despite these being identified as critical risk categories in the National Biosecurity Plan Guidelines for Abalone Farming (Spark et al. 2018).

5.0 Additional risk mitigation measures to minimise risk of spillback infection of wild abalone

Given the inadequacy of the documentation provided regarding the planned steps to mitigate biosecurity and disease risks at YNAF, and given the lack of proposed treatment of intake and effluent water (contrary to recommendations given by Spark et al. (2018) in the latest National Biosecurity Plan

Guidelines for Abalone Farming), this proposal provides inadequate protection against both AVG and perkinsosis for not only disease incursions into YNAF, but also backspill contamination from YNAF into adjacent abalone fisheries. Biosecurity in the context of aquaculture is the practice of exclusion of specific pathogens from cultured aquatic stocks in broodstock facilities, hatcheries, and farms, or from entire regions or countries for the purpose of disease prevention (Lightner 2005). Water treatments that result in pathogen free water are a fundamental component of aquaculture biosecurity programmes which aim to exclude important pathogens for improved growth and survival and/or achievement of outcomes such as development of specific pathogen free spat (Brock and Bullis 2001, Kasai et al. 2002, Matson et al. 2006, Yoshimizu 2009, Wyban 2009, Table 2). Because of this, several additional risk mitigation methods are proposed in order to reduce risks to the wild fishery to an acceptable level. These are outlined in the following sections.

5.1 Effective quarantine and screening of broodstock for known abalone pathogens

Because broodstock abalone represent a very high risk pathway for introduction of diseases onto abalone farms (Hardy Smith 2006), it is recommended that, at a minimum, every broodstock abalone is obtained from populations known to be historically free from AbHV and *P. olseni* for at least 2 years, and then examined and tested for AbHV and *P. olseni* prior to bringing them into the broodstock quarantine facility. Once in the broodstock holding facility, the intake water should be adequately sanitised to reduce the risk of incursions of important disease agents (see Section 5.2), while the effluent water from the broodstock quarantine facility must be adequately sanitised to prevent any risk of release of disease agents such as AbHV and *P. olseni* in the effluent water stream. Potential methods for treating intake and effluent water with UV irradiation and/or Ozone to inactivate these pathogens are covered in more detail in Sections 5.2 and 5.3 and Table 2.

5.2 Exclusion of significant disease agents by treatment/holding of intake water

The intake and distribution of seawater into YNAF were described in their section 5.3.3 (Pumping infrastructure) on page 26 and section 5.3.4 Wastewater treatment (on page 27) of Yumbah Aquaculture Ltd (2018b). On page 26 they stated that "*The Nyamat abalone farm will require approximately 6,000 litres per second of fresh seawater. Pump stations will be constructed in the south western section of the site and will each house about five individual pumps. A maximum of 20 pumps will be used to pump seawater through the site.*" On page 27 they stated that "*Yumbah Nyamat will be divided into 4 modules, each with 500 abalone tanks (i.e. total 2000 tanks). The tanks will be 20 m x 2.8 m and have a nominal water depth of 50 mm (2.8 m3). Seawater will be pumped continuously into each tank at a constant rate of 2.6 L/s to flow slowly down the length of the tank.*

While the volume of water proposed to be pumped onto YNAF is large (360 tonnes/minute) if best practice is to be achieved, this involves protection of growout abalone in raceways from disease agents carried in intake water. Fortunately, modern aquaculture technology allows these volumes of water to be mechanically filtered down to below 100µm using large drum filters (e.g. Faivre 160 or 200 Series, Integrated Aqua, etc.) operating in parallel. Given that the farm will be divided into 4 discrete modules, there are a number of filter arrangements that could be used to properly treat this volume of intake water to remove pathogens or carrier species prior to their entry into raceways. A wide range of protozoan

and metazoan disease agents and disease carriers can be eliminated from water supplies through relatively coarse filtration (50-100µm), while bacterial and viral pathogens cannot be filtered in any practical way for aquaculture purposes due to their small size compared to the large volumes of water used (Table 2). Nevertheless, in land based prawn and salmon farms, large volumes of water are filtered to remove planktonic hosts of White Spot Syndrome Virus (WSSV) and prepare water for UV treatment to inactivate infective stages of whirling disease (*Myxobolus cerebralis*), respectively (Hedrick et al. 2007), using drum filters (nominal particle size removed usually = 40 to 100 µm).

In contrast, most other viruses, including AbHV cannot be directly excluded from aquaculture facilities by using filtration (though carriers or vectors of the virus can be). For another malacoherpesvirus, OsHV-1µVar which infects Pacific oysters, the virus is known to survive for less than 48 hours outside the host, hence storage of intake water for 48 hours prior to its use has been shown to be effective at reducing OsHV-1µVar incursions into shellfish hatcheries. Corbeil et al. (2012b) found that AbHV survived seawater for over 1 day in 15°C, but not 5 days. Even assuming that, like OsHV-1 µVar, AbHV cannot survive for more than 2 days outside the host, storage of 2 days of seawater supply at YNAF would minimise risk of exposure of cultured abalone to viable AbHV, but this would require storage of an impractically large volume of water, so this option would not appear to be feasible.

Research has shown that AbHV is susceptible to disinfectants such as buffodine, Impress and calcium hypochlorite (Corbeil et al. 2012b), but these would be impractical for treatment of intake water in such volumes. However, herpesviruses are known to be susceptible to UV irradiation (Wolff and Schneweis 1973), as shown for other aquatic herpesviruses including OsHV-1 (Schikorski et al. 2011b), koi herpesvirus (Kasai et al. 2005) and channel catfish virus (Robin and Rodrigue 1980, Kasai et al. 2002). Germicidal ultraviolet (UV) light in the UV-C spectral region (190-280 nm) is effective for inactivating a variety of microorganisms by disrupting their cellular membranes and damaging their DNA or RNA (Maisse et al. 1980, Liltved et al. 1995, 2006, 2011, Kasai et al. 2002, Chevrefils et al. 2006). Treatment of water with UV irradiation is an effective means of reducing or eliminating pathogen loads without leaving toxic residues (Brown and Russo 1979, Kasai et al. 2002, Yoshimizu et al. 2005, Liltved et al. 2006, Hedrick et al. 2007). It is particularly useful for inactivating viral and bacterial disease agents that are too small to remove by filtration, and indeed smaller disease agents are generally more vulnerable to UV irradiation than are larger protozoa and metazoa (Kasai et al. 2002, Yoshimizu et al. 2005, Yoshimizu 2009). Nevertheless, water should always be settled and/or prefiltered to remove as many particles as possible before UV disinfection, thereby reducing shading and improving the overall bacterial removal efficiency by reducing the risk of introducing UV-shielded bacteria (Liltved and Cripps 1999). Total microbicidal UV dosage is usually calculated in mJ/cm² based on the relationship of 1 mJ/cm² = 1,000 µW/cm² per second,

i.e: total dose in mJ/cm^2 = (intensity (µW/cm²) x duration of exposure (sec))/1000.

If the objective of a UV disinfection protocol is to reduce the risk of exposure to aquatic herpesviruses such as AbHV, in the absence of minimum dose information for this particular virus at this time, it is pertinent to examine the dose rates required to inactivate other aquatic herpesviruses. These include the 4 mJ/cm^2 reported by Kasai et al. (2005) to be effective for inactivation of Koi Herpesvirus (KHV), and

the 20 mJ/cm^2 required to inactivate channel catfish herpesvirus (Robin and Rodrigue 1980, Kasai et al. 2002). While there is no information regarding the exact UV dose required to inactivate AbHV at this time, the study of Schikorski et al. (2011b) reported inactivation of the closely related OsHV-1 from Pacific oysters by exposure to a very large UV dose (15 min exposure to 1080 µW/cm² = 972 mJ/cm^2). In the absence of a dose response curve for UV irradiation for malacoherpesviruses (OsHV-1 and AbHV), it is difficult to determine whether exposure to 4, 30, or even 60 mJ/cm^2 UV will result in their complete inactivation. However, given that 20 mJ/cm^2 is the highest reported dose required for inactivation of other aquatic herpesviruses in the literature, it is assumed that a minimum dose of 30 mJ/cm^2 would be sufficient to provide an approximate 3 log10 reduction in viral titres (or more), as this is a high UV dose sufficient to inactivate a wide range of bacteria, protozoa and viruses (Chevrefils et al. 2006). Even if levels of virus and/or bacteria were in the vicinity of 10^3-10^4/ml at the YNAF water intake, further treatment of this water with UV at >30 mJ/cm^2 would be likely to reduce the levels of pathogens to c. 10^1/ml, which is a low dose similar to the number of OsHV-1 viral copies seen in healthy Pacific oysters in the latent carrier state (Dundon et al. 2011) and thus unlikely to cause infection.

Larger protozoans are more difficult to kill using UV irradiation (Kasai et al. 2002, Yoshimizu et al. 2005, Yoshimizu 2009). For example, *Perkinsus* spp. is relatively stable because of its thick cell wall, requiring a minimum dose of >28 mJ/cm^2 to inactivate *P. marinus* trophozoites and at least 60 mJ/cm^2 has been shown to be required to kill *P. olseni* hypnospores (Lester and Hayward 2005, OIE 2018d). Other protozoans such as haplosporidian infective stages as well as fungal zoospores can also be inactivated by exposure to around 30 mJ/cm^2 (Kasai et al. 2002, Bovo et al. 2005, Table 2). Rickettsia-like organisms are obligate intracellular disease agents which are relatively fragile and are susceptible to UV irradiation (Kasai et al. 2002, Yoshimizu et al. 2005) as well as antibiotics (Friedman et al. 2003). So a UV dose of >30 mJ/cm^2 is likely to be sufficient to provide protection against the vast majority of known significant disease agents of abalone (excluding *P. olseni* hypnospores, which require 60 mJ/cm^2) (Table 2).

So UV treatment of intake water is potentially effective for inactivating disease agents such as AbHV and *P. olseni*, however safety factors should not be set unnecessarily high because of the potential downsides of excessive UV treatment which include increased running costs, and reduced biofilm formation by microalgae such as diatoms that are needed for nursery rearing and provide a useful supplementary feed source in the growout raceways. Also, changes in the microbial balance of the water can occur due to bacterial activity after UV treatment due to increased availability of dead organic matter in the absence of those species of bacteria that were sensitive to the UV treatment (Gregg et al. 2009).

Marine microalgae (diatoms and other phytoplankton) are UV sensitive (Litchman and Neale 2005, Liltvelt et al. 2011), but UV effects on microalgae are species-specific, with some species more susceptible to UV damage than others (Wulff et al. 2008). In general, microalgae require higher UV dosages for complete inactivation than bacteria and viruses due to their larger size and pigmentation (Rigby and Taylor 2001, Liltvelt et al. 2011), and they also can have DNA repair mechanisms that allow them to recover photosynthetic activity after UV exposure (Wulff et al. 2008). Nevertheless, the higher

UV doses used for deactivation of microorganisms can be lethal to some diatoms , resulting in 90% (1 log10) reduction in viable cells of diatoms at doses around 45 mJ/cm^2 and a 3 log10 reduction (99.9%) at 3 times this dose (135 mJ/cm^2 see Siemens (2011). However, Gregg et al. (2009) and Liltvelt et al. (2011) showed that for some microalgae such as *Tetraselmis* spp. and *Prorocentrum minimum*, UV dose rate can be as high as 100-120 mJ/cm^2 before 1 log10 reductions in algal cell viability were observed.

These data suggest that if the aim is to disinfect intake water to inactivate AbHV, *P. olseni* and other abalone pathogens, while retaining reasonable microalgal growth in biofilms, it would seem appropriate to keep the UV dose to between 30-60 mJ/cm^2 to allow a certain margin for error as insurance against all known abalone pathogens (or increase in flow rates in the future once more information is available on UV sensitivity of AbHV), without completely stopping colonisation of biofilms by diatoms during the treatment process.

5.3 Prevention of backspill infection of wild abalone by treatment of effluent water

Disinfection of effluent water from land based fish farms has long been recognised as an important component of any biosecurity program (Jacobsen et al. 1989, OIE 2018a, Spark et al. 2018). In land based facilities holding abalone in Australia, disinfection of effluent water is considered to be a vitally important step to reduce the risk of backspill infection of wild abalone stocks with most pathogens (Spark et al. 2018), but particularly AbHV (see Baulch and Ellard 2011, Green 2011, Baulch et al. 2013).

Decontamination of effluent water from abalone farms can be done after settlement ponds prior to release of the effluent water using UV irradiation (60 mJ/cm^{2}dose, see Section 5.2 above) or ozonation. As mentioned previously, water should always be settled and/or prefiltered to remove as many particles as possible before UV or ozone disinfection, thereby reducing shading and improving the overall bacterial removal efficiency (Liltved and Cripps 1999). Ozonation can be a very useful method for inactivating aquatic disease agents (Liltved et al. 1995, 2006), and use of ozone is particularly suited to treatment of effluent water (Jacobsen et al. 1989). While the minimum dose required to inactivate AbHV using ozone has not yet been established (representing a significant data gap), ozone has been used successfully to control a wide variety of microbial pathogens in the effluent water from fish farms at levels of 0.08–1.0 mg/L/min residual oxidants (OIE 2009b, Diggles 2017). Determination of the minimum ozone dose for inactivation of AbHV would be a highly useful research process which could inform best practice for disinfection of effluent water from not only abalone processors in Tasmania (Baulch et al. 2013), but for abalone farms across Australia. Until such time as this is done, a conservative approach would suggest that ozone levels of around 1.0 mg/L/min residual oxidants would provide a very high chance of complete inactivation of not only viruses such as AbHV, but also other disease agents (including *P. olseni*) likely to be present in abalone farm effluent water.

5.4 Routine active disease surveillance on farm to detect subclinical disease

The Yumbah Nyamat Biosecurity Plan (Yumbah Aquaculture Ltd 2018c), states that like other Yumbah Aquaculture farms, the YNAF would be subject to mandatory annual audits carried out by government Veterinary Officers and would also carry out ongoing health testing and health surveillance of their stock. However, no specific details of what this entails were disclosed. Spark et al. (2018) note that abalone farms operating under the National Abalone Health Accreditation Program must undertake regular batch testing or disease surveillance in compliance with jurisdictional regulations, however it is not clear in the documentation provided regarding the frequency and methodology of testing conducted under these programs.

As noted previously in Section 2.1, because a certain level of background mortality is always accepted as normal on abalone farms, without treatment of effluent water, there is a high risk of backspill contamination of wild abalone populations adjacent to the effluent pipe outlets prior to farmers noticing the greater than normal mortality rates which may trigger enactment of biosecurity processes such as water recirculation. Furthermore, AVG is exceptional in that it tends to cause acute mortalities of captive abalone, which means that its presence in abalone farms is easier to detect using b\passive surveillance. In contrast, virtually all other diseases of cultured abalone have slower, more chronic disease presentations, which unless active surveillance is undertaken regularly, would allow untreated effluent water to transport increased numbers of infective stages into the adjacent environment for several months before any disease process was identified. Thus, under such circumstances, there may still remain a very high risk that increased levels of infective stages of chronic disease agents such as *P. olseni* may pass into the receiving environment in the months before a disease outbreak is detected. Because of this, in the absence of treatment of effluent water, active disease surveillance needs to be conducted more often (minimum 3-4 times a year) so that chronic disease processes can be detected as soon as possible to minimise risks of those diseases spreading to adjacent wild abalone populations.

5.5 Depopulation of cultured abalone from effluent pipes and settlement ponds

Another very useful risk mitigation method which was not discussed in detail was the need to ensure that there are no reservoirs of abalone in the effluent drains, pipes and settlement ponds of abalone farms which could act as hidden sources of infection for abalone on the farm or outside the farm. Instead, all abalone should be removed from drains, pipes and settlement ponds, leaving only specific populations of sentinel abalone housed in key locations in the effluent stream that are examined for disease surveillance on a regular basis so that an accurate and timely representation of the disease status of the various sections of the farm can be conveniently obtained (see Section 5.4 above).

5.6 Depopulation of wild abalone stocks near intake and effluent pipes

A logical extension to the theory of depopulation of farm effluent drains, pipes and settlement ponds outlined in Section 5.5 is the concept of destocking wild abalone populations in the areas immediately surrounding the YNAF intake and effluent pipes. In the absence of effective treatment/disinfection of effluent water, the next best option to reduce the risk of wild abalone being exposed to an infectious

dose of important pathogens which may establish in the YNAF would appear to be establishment and maintenance of a suitably large abalone-free area around the intake and effluent pipes, which would effectively allow for dilution of effluent water to levels which may reduce the infective doses experienced by nearby wild abalone populations to below the minimum infective dose. In Figures 11 and 12 on page 78 of Yumbah Aquaculture Ltd (2018b), an area encompassing the modeled 5 fold dilution mixing zone is portrayed. In the absence of disinfection of effluent water, an option to regularly survey this area and remove all wild abalone from it should be seriously considered in order to provide a (albeit minimal) buffer zone between the farm and the remainder of the wild abalone population.

Table 2. Summary of potential risk mitigation methods for significant diseases of abalone

Significant disease agents of cultured abalone	**Notifiable disease ?**	**Filtration**	**Ozonation *Ct* (mg/L/min)***	**UV irradiation (mJ/cm^2)**		**Physical / Chemical Treatment**
Endemic Viruses				Published effective range	Minimum recommended	
1. Abalone viral ganglioneuritis (AbHV-1)	Yes	Not practicable	?	972	>30	Heat, 60°C for 60 min, desiccation/sunlight 48 hours, 2 mg/L calcium hypochlorite for 15 min, 1 mg/L iodine 20 min, 500 mg/L benzalkonium chloride for 20 min
Endemic Protozoa						
2. Infection with *Perkinsus olseni*	Yes	1 µm	?	4.7- 60	60	Heat 50°C for 60 min, freshwater for >6 hours, 300 mg/L sodium hypochlorite for 30 min
Endemic Metazoa						
3. Mudworm infection *(Polydora, Boccardia*)	No	100 µm	?	?	?	Desiccation for 2-4 hours at >15°C@ <63% relative humidity, freshwater for 12 hours
Exotic diseases						
4. Amyotrophia-like viral agents	No	Not practicable	?	?	?	?
5. Withering syndrome/*Xenohaliotis californiensis*	Yes	0.2 µm	0.1-0.9**	?	>30	>10 mg/L calcium hypochlorite
6. Infection with haplosporidians	No	c. 25 µm	?	30	>30	Salinity < 15 ppt
7. Deuteromycote fungi in paua	No	1 µm	0.1-0.3**	10-250	>30	?
8. Infection with sabellid polychaetes	No	75 µm	?	?	?	Heat, >28.5°C for >48 hours, Freshwater for 63 sec (larvae), 16 hours (adults),

* 0.8-1 mg/L/min recommended for most aquatic pathogens (OIE 2009b)
** data for related pathogens, see Diggles (2017a)
References: 1 (Robin and Rodrigue 1980, Kasai et al. 2002, Yoshimizu et al. 2005, OIE 2009a, Corbeil et al. 2012), 2 (Goggin et al. 1990, Bushek et al. 1997a, 1997b, Lester and Hayward 2005, OIE 2018d). 3 (Handlinger et al. 2004), 5 (OIE 2018c), 6 (Ford et al. 2001), 7 (Chevrefils et al. 2006, Yoshimizu 2009), 8 (Leighton 1998, Finley et al. 2003, Moore et al. 2007).

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7.0 Appendix 1: Definitions of likelihood and consequence descriptions

Table A1. Nomenclature for the qualitative likelihood estimations used in this RA.

Likelihood	Definition	Annual Probability
High	The event would be very likely to occur	$0.7 < P \leq 1$
Moderate	The event would occur with an even probability	$0.3 < P \leq 0.7$
Low	The event would be unlikely to occur	$0.05 < P \leq 0.3$
Very Low	The event would be very unlikely to occur	$0.001 < P \leq 0.05$
Extremely low	The event would be extremely unlikely to occur	$0.000001 < P \leq 0.001$
Negligible	The event would almost certainly not occur	$0 < P \leq 0.000001$

Table 4. Definition of terms used to describe consequences of establishment of unwanted disease agents.

Consequence	Definition
Extreme	Establishment of a disease agent would cause substantial biological and economic harm at a regional or even national level, and/or cause serious and irreversible harm to the environment.
High	Establishment of a disease agent would have serious biological consequences (high mortality or morbidity) and would not be amenable to control or eradication. Such organisms would significantly harm economic performance at a regional level and/or cause serious environmental harm which is most likely irreversible.
Moderate	Establishment of a disease agent would cause significant biological consequences and may not be amenable to control or eradication. Such diseases could harm economic performance at a regional level on an ongoing basis and/or may cause significant environmental effects, which may or may not be irreversible.
Low	Establishment of a disease agent would have moderate biological consequences and would normally be amenable to control or eradication. Such diseases may harm economic performance at a local level for some period and/or may cause some environmental effects, which would not be serious or irreversible.

Very Low	Establishment of a disease agent would have mild biological consequences and would be amenable to control or eradication. Such diseases may harm economic performance at a local level for a short period and/or may cause some minor environmental effects, which would not be serious or irreversible.
Negligible	Establishment of a disease agent would have no significant biological consequences and would require no management. The disease would not affect economic performance at any level and would not cause any detectable environmental effects.

These descriptions are based on information available in other RAs (Jones and Stephens 2006, Biosecurity Australia 2009, Diggles and Arthur 2010, Diggles 2011b, 2017b), the scientific literature, unpublished data, as well as the professional judgment of the analyst.