

# Recommendations for periods of basic biosecurity conditions and targeted surveillance for the disease-specific chapters of the WOAAH Aquatic Animal Health Code

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## Executive summary and recommendations

- Chapter 1.4. 'Aquatic animal disease surveillance' of the *Aquatic Code* sets out the principles for declaration of disease freedom via four different pathways: 1. Absence of susceptible species, 2. Historical freedom, 3. Targeted surveillance and 4. Returning to freedom.
- The disease-specific chapters of the *Aquatic Code* provide recommendations for periods of basic biosecurity conditions (BBC) for all four pathways and targeted surveillance (TS) for pathways 3 and 4. Following the adoption of the revised Chapter 1.4. in May 2022, the periods of BBC and TS remained under study pending analysis.
- This report details how recommended periods for BBC and TS have been developed by applying the relevant criteria included in Chapter 1.4. 'Aquatic Animal Disease Surveillance' of the *Aquatic Code*.
- If a pathogen is present, it may be detected via the early detection system or passive surveillance throughout the periods of the BBC and TS.
- Pathogen-specific information relevant to the likelihood of pathogen detection by either the early detection system/passive surveillance or by TS (i.e. seasonality of transmission, persistence in the environment, the rapidity of onset of clinical signs or mortality, and rate of spread) was extracted from the disease-specific chapters of the *Aquatic Manual* and are summarised in the attachments.
- For each pathway, the relevant information was used to rank pathogens and the rankings used to recommend periods for BBC for each pathway and for TS for pathways 3 and 4. For countries and zones, pathways 1 to 4 apply. For compartments, only pathways 3 and 4 apply.

## BBC periods

- For pathway 1, the default minimum period for BBC is 6 months (defined in Chapter 1.4.). Only information on the persistence of the pathogen in the external environment was used for ranking. It is recommended that the period of BBC for pathogens ranked 1 or 2 is 6 months. For pathogens ranked 3, a period of one year is recommended. This pathway is not considered suitable for nine pathogens because as a result of their broad host range demonstrating absence of susceptible species is not considered possible.



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- For pathway 2, the default minimum period for BBC prior to declaring freedom is 10 years (defined in Chapter 1.4.). Only information on the likelihood that infection results in observable clinical signs and a noticeable increase in mortality was used to rank pathogens. For pathogens ranked 1 and 2, the period for BBC prior to declaring freedom is recommended to be ten years. For pathogens ranked 3, a 15 year period for BBC prior to declaring freedom is recommended. For all declarations of freedom utilising pathway 2, the requirements of passive surveillance in article 1.4.8 must be met (e.g. conditions must be conducive for clinical expression of infection). This pathway is not considered suitable for one pathogen.
- For pathway 3, the default minimum period of BBC preceding TS for countries and zones is one year (defined in Chapter 1.4.). The duration of BBC preceding TS should be long enough for the design prevalence used in TS design to be reached, assuming the pathogen became established immediately prior to commencement of BBC. Hence, the rate of spread between populations is critical.
- Pathogens whose transmission only occurs during limited periods (determined primarily by water temperature) require a longer period of BBC to ensure high confidence that the design prevalence has been reached before TS begins.
- During the period of BBC, the pathogen, if present, may be detected through passive surveillance, which is more likely for pathogens that cause observable signs or mortality. As passive surveillance is a secondary form of evidence for pathway 3 (refer to Article 1.4.3. of the *Aquatic Code*), this factor was also used to make recommendations for the period of BBC for pathway 3 (see Table 3).

### **TS periods**

- The default minimum period for TS for countries and zones is two years. For pathogens whose transmission rate is significantly determined by environmental conditions the prevalence may fall below the design prevalence at periods when environmental or biological conditions are not conducive to transmission.
- For pathogens whose transmission is significantly influenced by environmental factors and where infection does not consistently result in observable clinical signs or mortality, it is recommended that the period of TS is extended to three years (see Table 3).
- For compartments seeking freedom in accordance with pathway 3, a period of one year for BBC and TS is considered sufficient for all pathogens, as the conditions required to maintain a compartment will generate a high confidence that the pathogen will be detected irrespective of its characteristics.
- Chapter 1.4. of the *Aquatic Code* requires that countries, zone or compartments attempting to return to freedom via pathway 4 following an outbreak, review measures to prevent the introduction of the pathogenic agent and implement changes for as long as necessary to evaluate success. As the circumstances of each disease outbreak leading to a breakdown in disease freedom are unique, setting the period of BBC (preceding TS to regain freedom) on a pathogen basis is not considered appropriate.
- In principle the minimum period of TS under pathway 4, should be consistent with the requirements for pathway 3. However, guidance in *Aquatic Code* Chapter 1.4., allows for flexibility in applying periods of TS to regain a disease free status if justified by the circumstances of the outbreak.

**Table 1.** Recommendations for periods of BBC using Pathway 1. 'Absence of susceptible' species.

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
6 months	EHNV <i>G. salaris</i> HPR-deleted ISAV <sup>1</sup> IHNV ISAV (including HPR0 and HPR-deleted) KHV SVCV TiLV	AHPND <i>H. penaei</i> IHHNV IMNV MrNV YHV1	AbHV <i>B. exitiosa</i> <i>B. ostreae</i> <i>P. marinus</i> <i>M. refringens</i> <i>X. californiensis</i>	<i>B. salamondrivorans</i>
12 months	SAV			
Pathway not suitable	EUS <i>M. pagrus 1</i> VHSV	crayfish plague DIV1 WSSV	<i>P. olseni</i>	<i>B. dendrobatidis</i> <i>Ranavirus</i>

**Table 2.** Recommendations for periods of BBC using Pathway 2. 'Historical freedom'.

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
10 years	EHNV HPR-deleted ISAV IHNV <i>M. pagrus 1</i> SAV SVCV TiLV VHSV	AHPND crayfish plague DIV1 <i>H. penaei</i> IHHNV IMNV MrNV WSSV YHV1	AbHV <i>B. exitiosa</i> <i>B. ostreae</i> <i>P. marinus</i> <i>M. refringens</i> <i>P. olseni</i> <i>X. californiensis</i>	<i>B. dendrobatidis</i> <i>B. salamondrivorans</i> <i>Ranavirus</i>
15 years	KHV EUS			
Pathway not suitable	<i>G. salaris</i> ISAV (including HPR0 and HPR-deleted)			

<sup>1</sup> For infection with infectious salmon anaemia virus, the standards of the *Aquatic Code* apply to two categories of disease status: freedom from ISAV (including HPR0 and HPR-deleted) and freedom from HPR-deleted ISAV only as described in Chapter 10.4.

**Table 3.** Recommendations for periods of BBC and TS for claims of freedom for countries and zones using Pathway 3. 'Targeted surveillance'.

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
<b>BBC</b>				
1 year	EHNV HPR-deleted ISAV IHNV <i>M. pagrus 1</i> SAV SVCV VHSV TiLV	AHPND crayfish plague DIV1 <i>H. penaei</i> IHHNV IMNV MrNV WSSV YHV1	AbHV	<i>B. dendrobatidis</i> <i>B. salamandrivorans</i>
2 years	EUS <i>G. salaris</i> ISAV (including HPR0 and HPR-deleted) KHV	/	<i>B. exitiosa</i> <i>B. ostreae</i> <i>P. marinus</i> <i>M. refringens</i> <i>P. olseni</i> <i>X. californiensis</i>	<i>Ranavirus</i>
<b>TS</b>				
2 years	EHNV HPR-deleted ISAV IHNV <i>M. pagrus 1</i> SAV SVCV TiLV VHSV	AHPND crayfish plague DIV1 <i>H. penaei</i> IHHNV IMNV MrNV WSSV YHV1	AbHV	<i>B. dendrobatidis</i> <i>B. salamandrivorans</i>
3 years	EUS <i>G. salaris</i> ISAV (including HPR0 and HPR-deleted) KHV	/	<i>B. exitiosa</i> <i>B. ostreae</i> <i>P. marinus</i> <i>M. refringens</i> <i>P. olseni</i> <i>X. californiensis</i>	<i>Ranavirus</i>

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## Abbreviations

BBC	basic biosecurity conditions
TS	targeted surveillance

### Abbreviations for 'listed diseases' of fish

EHNV	Infection with epizootic haematopoietic necrosis virus
EUS	Infection with <i>Aphanomyces invadans</i> (epizootic ulcerative syndrome)
<i>G. salaris</i>	Infection with <i>Gyrodactylus salaris</i>
IHNV	Infection with infectious haematopoietic necrosis virus
ISAV	Infection with HPR-deleted and HPR0 infectious salmon anaemia virus
HPR-deleted ISAV	Infection with HPR-deleted infectious salmon anaemia virus
KHV	Infection with koi herpesvirus
<i>M. pagrus 1</i>	Infection with <i>Megalocytivirus pagrus 1</i>
SAV	Infection with salmon alphavirus
SVCV	Infection with spring viraemia of carp virus
TiLV	Infection with tilapia lake virus
VHSV	Infection with viral haemorrhagic septicaemia virus

### Abbreviations for 'listed diseases' of molluscs

AbHV	Infection with abalone herpesvirus
<i>B. ostreae</i>	Infection with <i>Bonamia ostreae</i>
<i>B. exitiosa</i>	Infection with <i>Bonamia exitiosa</i>
<i>M. refringens</i>	Infection with <i>Marteilia refringens</i>
<i>P. marinus</i>	Infection with <i>Perkinsus marinus</i>
<i>P. olseni</i>	Infection with <i>Perkinsus olseni</i>
<i>X. californiensis</i>	Infection with <i>Xenohaliotis californiensis</i>

### Abbreviations for 'listed diseases' of crustaceans

AHPND	Acute hepatopancreatic necrosis disease
crayfish plague	Infection with <i>Aphanomyces astaci</i> (crayfish plague)
DIV1	Infection with decapod iridescent virus 1
<i>H. penaei</i>	Infection with <i>Hepatobacter penaei</i> (necrotising hepatopancreatitis)
IHHNV	Infection with infectious hypodermal and haematopoietic necrosis virus
IMNV	Infection with infectious myonecrosis virus
MrNV	Infection with <i>Macrobrachium rosenbergii</i> nodavirus (white tail disease)
TSV	Infection with Taura syndrome virus
WSSV	Infection with white spot syndrome virus
YHV1	Infection with yellow head virus genotype 1

### Abbreviations for 'listed diseases' of amphibians

<i>B. dendrobatidis</i>	Infection with <i>Batrachochytrium dendrobatidis</i>
<i>B. salamandrivorans</i>	Infection with <i>Batrachochytrium salamandrivorans</i>
<i>Ranavirus</i>	Infection with <i>Ranavirus</i> species

## Introduction

The World Organisation for Animal Health (WOAH) provides standards for Members to allow them to demonstrate freedom from specified pathogens at the country, zone or compartment level. The disease-specific chapters of the Aquatic Animal Health Code<sup>2</sup> (*Aquatic Code*) set default minimum periods for the duration of basic biosecurity conditions (BBC) before a declaration of freedom can be made by pathways 1, 2 and 3, and the period of targeted surveillance (TS) for pathway 3. Attachment 1 details the minimum periods for each listed pathogen and pathway stipulated in the disease-specific chapters before the adoption of the revised Chapter 1.4. 'Aquatic animal disease surveillance' in 2022. Since 2022, the default minimum periods have been under study.

This paper presents a rationale for determining for each listed aquatic animal disease, the minimum periods of BBC for pathways 1, 2 and 3, and the duration of targeted surveillance for pathway 3, for declarations of freedom for a country, zone or compartment (only pathway 3 applies for compartments). In addition, the guidance for the BBC for a country, zone or compartment to return to freedom under pathway 4 is reviewed.

The duration of the minimum period of BBC required before declaration of freedom using pathway 1 (absence of susceptible species) should be long enough for any pathogen introduced by a fomite (e.g. via trade) before measures were implemented to lose viability.

The duration of BBC before declaring freedom via pathway 2 should allow the early detection system (EDS) and passive surveillance to generate a high level of confidence that if present the pathogen would be detected (EDS and passive surveillance are components of basic biosecurity).

The design of the TS to demonstrate freedom (via pathway 3) will be largely based on the selected design prevalence (i.e. the minimum prevalence that will be detected with 95% confidence). Guidance on setting the design prevalence is provided in Chapter 1.4. of the *Aquatic Code*. At a zone and country level, the BBC needs to be in place long enough to generate a high level of certainty that the design prevalence would have been reached prior to the start of TS (assuming the pathogen is present before BBC were implemented). The duration of BBC (preceding TS) may need to be longer than the default minimum period (one year) if the pathogen: i) has a long lifecycle; ii) spreads only slowly within and between populations (e.g. requires a high infectious dose); iii) transmission only takes place during limited periods of the year (i.e. when water temperatures are permissive for replication); or iv) remains viable for only short periods (<14 days) outside the host (survival outside the host correlates with likelihood of transmission).

For pathways 3 and 4, information from passive surveillance can be used as secondary evidence in demonstration of disease freedom. Therefore, in addition to the pathogen transmission (i.e. the rate at which the design prevalence is reached), the likelihood of detection during the period of BBC may also be used to determine the period of BBC. Infections which result in rapid onset of clinical disease or mortality following introduction to a naïve population, are more likely to be detected during the period of BBC compared with pathogens which cause low levels of clinical disease or mortality.

The default minimum period of TS specified in chapter 1.4. is two years for a country or zone and one year for compartments. The rationale for setting the minimum period of TS used in this paper, assumes that the design prevalence has been reached before TS starts. However, for many pathogens transmission, and therefore prevalence, is influenced by environmental factors. Unseasonably low water temperatures in the first year of sampling may result in the prevalence falling below the design prevalence. In addition, the likelihood that a sampled infected aquatic animal will test positive may be

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<sup>2</sup> <https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/>



reduced if levels of infection are lower (e.g. due to a reduced exposure level). A longer sampling period increases the time before freedom is declared, which allows for further pathogen spread (i.e. a higher prevalence and geographic distribution), and thus making detection more likely. Secondly, if sites are sampled on multiple occasions then the lifecycle of the pathogen becomes relevant, as in the second year of sampling the likelihood that the prevalence has increased above the design prevalence increases. Seasonality is the key factor driving variation in prevalence from year to year (i.e. the likelihood detecting the pathogen is strongly influenced by water temperature). As passive surveillance can be combined with active surveillance to demonstrate freedom, the likelihood that infection results in clinical signs or mortality detectable through passive surveillance is also considered in determining the minimum period of TS.

The assessments from this document are applied to the default periods in the disease-specific chapters of the *Aquatic Code*. The guidance in Chapter 1.4. and the disease-specific chapters of the *Aquatic Code* should be used when considering the minimum levels of BBC and TS.

## Terms of reference

1. Develop an approach to determine for each listed pathogen the minimum period of basic biosecurity conditions for demonstration of freedom at country or zone level via pathway 1 (absence of susceptible species) and pathway 2 (historical freedom) and preceding targeted surveillance for pathway 3 (targeted surveillance<sup>3</sup>).
2. Apply the method to WOAHA listed aquatic animal diseases and recommend periods of BBC for pathway 1 and 2, and to precede targeted surveillance to demonstrate freedom at country and zone level (via pathway 3) for the disease-specific chapters of Aquatic Animal Health Code.
3. Review guidance for the minimum period of BBC for compartments seeking disease freedom under pathway 3 (TS)
4. Review the guidance for the BBC for countries, zones or compartments to regain freedom under pathway 4.

## Method

Information on pathogen specific characteristics that influence i) the speed at which the design prevalence will be reached and ii) likelihood of early detection through passive surveillance, was extracted from the *Aquatic Manual* disease-specific chapters (summarised in Attachments 2-5). The characteristics are:

1. lifecycle;
2. rate of spread within and between populations (e.g. infectious dose);
3. period of the year during which transmission takes place (i.e. when water temperatures are permissive for replication);
4. persistence outside the host (in the environment);
5. likelihood of early detection (i.e. rapid onset of clinical disease/ mortality following introduction).

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<sup>3</sup> Described in Article 1.4.3. of the *Aquatic Code*

For pathway 1 (absence of susceptible species), only information on persistence outside the host in the environment was considered relevant to determining the BBC. This factor was used to rank (from 1-3) pathogens at host group level (i.e. fish, molluscs, crustaceans, amphibians). Recommendations for the duration of BBC for each pathogen are made.

For pathway 2 (historical freedom), only information on the likelihood of detection was considered relevant to determining the BBC. This factor was used to rank (from 1-3) pathogens at host group level (i.e. fish, molluscs, crustaceans, amphibians). Recommendations for the duration of BBC for each pathogen groups were made.

For the BBC of pathway 3, pathogens are ranked (from 1-3) at host group level based on all the characteristics assessed (see Table 4 for details). The rankings indicate the relative rate at which design prevalence will be reached and/or a higher likelihood of detection by passive surveillance.

**Table 4.** Rankings used to assess the period of basic biosecurity conditions for pathway 3. ‘Targeted surveillance’.

Rank 1.
<ul style="list-style-type: none"> <li>• little or no seasonal variation in transmission</li> <li>• evidence of rapid onset of clinical signs/mortality following pathogen introduction</li> <li>• evidence of rapid spread between populations</li> <li>• persistence outside of host in the environment for &gt; 14 days</li> </ul>
Rank 2.
<ul style="list-style-type: none"> <li>• seasonal variation in transmission, at least some evidence of low to negligible level of transmission during some period of the year</li> <li>• evidence of rapid onset of clinical signs/mortality following pathogen introduction</li> <li>• evidence of at least moderate rate of spread between populations</li> <li>• persistence outside of host in the environment for &gt; 7 days</li> </ul>
Rank 3.
<ul style="list-style-type: none"> <li>• strong seasonal variation in transmission, good evidence of low to negligible level of transmission during some period of the year</li> <li>• slow onset of clinical signs/mortality following pathogen introduction AND / OR</li> <li>• slow spread between populations</li> </ul>

For the duration of TS (pathway 3), the factors listed in **Table 5** are compared between pathogens for each host group (i.e. fish, molluscs, crustaceans, amphibians) considering:

1. limited period of the year during which transmission occurs, that may vary between years due to environmental factors (e.g. water temperatures);
2. likelihood of early detection (i.e. rapid onset of clinical disease/ mortality following introduction).

For each category of host (i.e. fish, molluscs, crustaceans, amphibians), pathogens are ranked on the basis of the characteristics assessed (see **Table 5** for details).

**Table 5.** Definitions of rankings used to determine the minimum period of targeted surveillance for pathway 3

Rank 1.
<ul style="list-style-type: none"> <li>• little or no seasonal variation in transmission,</li> <li>• evidence of rapid onset of clinical signs/mortality following pathogen introduction</li> </ul>
Rank 2.
<ul style="list-style-type: none"> <li>• seasonal variation in transmission, at least some evidence of low to negligible level of transmission during some period of the year</li> <li>• evidence of rapid onset of clinical signs/mortality following pathogen introduction</li> </ul>
Rank 3.
<ul style="list-style-type: none"> <li>• strong seasonal variation in transmission, good evidence of low to negligible level of transmission during some period of the year</li> <li>• slow onset of clinical signs/ mortality following pathogen introduction</li> </ul>

## Results and Recommendations

### Pathway 1: Assessment of duration of basic biosecurity conditions (absence of susceptible species)

The rankings of pathogens within host group are set out in Table 6.

**Table 6.** Summary rankings of pathogens to determine the minimum period of BBC for pathway 1. 'Absence of susceptible species'. Pathogens marked \* are considered unsuitable for application of this pathway.

Ranking	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
1	<i>G. salaris</i> KHV	AHPND WSSV* YHV1		
2	EHNV HPR-deleted ISAV IHNV ISAV (including HPR0 and HPR-deleted) <i>M. pagrus 1*</i> SVCV TiLV VHSV*	DIV1* <i>H. penaei</i> IHHNV IMNV MrNV TSV	AbHV <i>B. exitiosa</i> <i>B. ostreae</i> <i>P. marinus</i> <i>M. refringens</i> <i>X. californiensis</i>	<i>B. dendrobatidis*</i> <i>B. salamandrivorans</i> <i>Ranavirus*</i>
3	EUS* SAV	crayfish plague*	<i>P. olsenii*</i>	

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Based on the analysis, it is recommended that for cases demonstrating freedom at a country or zone level, pathogens ranked 1 and 2 should retain the default minimum six month period of BBC. For pathogens ranked 3, it is recommended that the BBC is extended to 12 months.

This pathway is not considered suitable for pathogens with a broad host range and for which new susceptible species are expected to be determined with further research or spread of the pathogens to new geographic areas. For these species, demonstrating absence of susceptible species in a country or zone is not considered possible. Diseases have been determined to have a broad host range consistent with the criteria for application of *Aquatic Code* Article 1.5.9.

Pathway 1 is thus unsuitable for eight species - EUS, *M. pagrus* 1, VHSV, crayfish plague, DIV1, WSSV, *P. olsenii*, *B. dendrobatidis* and *Ranavirus*. See Attachment 1.

Pathway 1 is not appropriate to demonstrate freedom at the compartment level as the *Aquatic Code* does not currently include provisions for compartment freedom via pathway 1.

## **Pathway 2: Assessment of duration of basic biosecurity conditions (historic freedom)**

The rankings of pathogens by host group are set out in Table 7. All fish pathogens with the exception of EUS, KHV, *G. salaris* and ISAV (including HPR0 and HPR-deleted) have a high likelihood of detection by early detection systems or passive surveillance, and hence the default minimum period of ten years will generate a high likelihood of detection (for populations that meet the requirements of Article 1.4.8. and assuming an annual surveillance systems sensitivity of at least 30%). For KHV and EUS annual surveillance systems sensitivity may be less than 30% and therefore an extended period of 15 years is recommended.

For infection with infectious salmon anaemia virus, the standards of the *Aquatic Code* apply to two categories of disease status: freedom from ISAV (including HPR0 and HPR-deleted) and freedom from HPR-deleted ISAV only. For HPR-deleted ISAV, infection in populations of Atlantic salmon may lead to clinical signs and an observable level of mortality and pathway 2 is applicable. For HPR0 ISAV, clinical disease is not expected so pathway 2 is not considered appropriate to claim freedom from the category with all forms of ISAV (including HPR0 and HPR-deleted). For *G. salaris* clinical disease may not be seen in all susceptible species when infected with the pathogenic agent and it was considered that pathway 2 was not appropriate to claim freedom from *G. salaris*.

All crustacean pathogens have a high or moderate likelihood of detection and the default minimum period of ten years can be recommended. It should be noted that for all pathogens the passive surveillance requirements of Article 1.4.8. must be met. For example, this pathway may be suitable for declarations of freedom from crayfish plague (*A. astaci*) in populations of susceptible species in which infection results in clinical signs and observable levels of mortality (e.g. native European species). However, it may not be appropriate to declare freedom for species in which *A. astaci* causes subclinical infection (e.g. North American species of crayfish).

Many mollusc species only cause mortality in older animals and thus may not be detected for some years after introduction. If the pathogen is introduced shortly before the period of BBC starts, mortality will become apparent within the default minimum ten year time period. Hence a period of ten years for BBC can be recommended.

Claims of freedom from *B. dendrobatidis*, *B. salamondrivorans* and *Ranavirus* need to provide evidence of the presence of susceptible species in which infection will cause mortality and clinical signs.

**Table 7.** Summary rankings of pathogens to determine the minimum period of BBC for pathway 2. 'Historic freedom'.

Ranking	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
1	SAV	AHPND Crayfish plague DIV1 <i>H. penaei</i> IHHNV IMNV MrNV WSSV YHV1	AbHV	<i>B. dendrobatidis</i> <i>B. salamondrivorans</i> <i>Ranavirus</i>
2	EHNV IHNV HPR-deleted ISAV <i>M. pagrus 1</i> TILV SVCV VHSV		<i>B. exitiosa</i> <i>B. ostreae</i> <i>M. refringens</i> <i>P. marinus</i> <i>P. olseni</i> <i>X. californiensis</i>	
3	EUS KHV			
Not suitable	<i>G. salaris</i> ISAV (including HPR-deleted and HPR0)			

It is recommended that pathogens ranked 1 and 2 retain the default minimum ten year period for BBC. For pathogens ranked 3, the minimum BBC period is extended to 15 years.

Pathway 2 should not be used to demonstrate freedom at compartment level.

### **Pathway 3: Assessment of duration of basic biosecurity conditions preceding targeted surveillance to demonstrate freedom**

The current default minimum BBC period of one year is considered the minimum period. The results of the assessments for each pathogen (Attachments 2-5) are summarised in the following sections.

#### **Fish pathogens**

Details summarised below can be found in Attachment 2.

- All the fish pathogens had direct lifecycles and therefore lifecycle information was uninformative and not used for ranking pathogens.
- Information in the *Aquatic Manual* chapters did not allow for levels of 'infectiousness' to be compared between pathogens; this criterion could not be used for ranking.

- Based on seasonality and persistence in the environment, only SAV achieved a ranking of 1.
- All pathogens with exception of KHV and *G. salaris*, had a high likelihood of rapid detection post-introduction by passive surveillance. For EUS, the likelihood of rapid detection is dependent on predisposing factors.
- The ranking and recommendations for ISAV was for each of the two categories of disease freedom available in the *Aquatic Code* (i.e. either ISAV [including HPR0 and HPR-deleted] or HPR-deleted ISAV only). HPR0 ISAV is not known to cause clinical disease and exists at very low prevalences in wild Atlantic salmon populations.

### **Crustacean pathogens**

Details summarised below can be found in Attachment 3.

- All crustacean pathogens have simple direct lifecycles.
- Information on survival outside the host and on environmental factors affecting replication/transmission was not available for most pathogens.
- No basis was found to recommend different durations of BBC on pathogen characteristics.
- All pathogens have high rates of spread and high likelihood of detection by passive surveillance so the minimum period of one year can be applied to all crustacean pathogens.
- The ranking for *Aphanomyces astaci* (crayfish plague) applies to infection in populations of susceptible species in which infections leads to signs and mortality. Demonstration of freedom in populations of crayfish species which do not display clinical signs and experience mortality, cannot be used as evidence from passive surveillance to demonstrate disease freedom.

### **Molluscan pathogens**

Details summarised below can be found in Attachment 4.

- Little information is available on environmental persistence of molluscan pathogens.
- All molluscan pathogens showed seasonality in prevalence/mortality indicating transmission was restricted or reduced for a period of the year (usually during winter months).
- Likelihood of early detection is low for all molluscan pathogens (except abalone herpesvirus) as onset of clinical signs/mortality occurs months to years after exposure.
- *Marteilia refringens* is an outlier, having an indirect lifecycle and the best evidence for restricted periods of transmission.

### **Amphibian pathogens**

Details summarised below can be found in Attachment 5

- Little evidence of strong seasonal impact on the rate of transmission of *B. salamondrivorans* or *B. dendrobatidis*.
- Evidence of limited spread between infected populations leads *B. salamondrivorans* to be ranked lower than *B. dendrobatidis*.
- Ranavirus is listed as a genus. Rate of spread and transmission varies considerably between hosts and viral species (multiple), making ranking at genus level difficult thus a conservative approach to ranking was taken.

The rankings are summarised in Table 8.

**Table 8.** Summary rankings of pathogens to determine minimum periods of basic biosecurity conditions for pathway 3. 'Targeted surveillance'.

Ranking	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
1	SAV	All	AbHV	<i>B. dendrobatidis</i>
2	EHNV IHNV HPR-deleted ISAV <i>M. pagrus 1</i> SVCV TILV VHSV			<i>B. salamondrivorans</i>
3	EUS <i>G. salaris</i> ISAV (including HPR0 and HPR-deleted) KHV		<i>B. exitiosa</i> <i>B. ostreae</i> <i>P. marinus</i> <i>P. olsenii</i> <i>M. refringens</i> <i>X. californiensis</i>	<i>Ranavirus</i>

It is recommended that for pathogens ranked 1 and 2, the default minimum BBC period of one year is retained. For pathogens ranked 3, the period is extended to two years.

### Compartments

The default minimum period of BBC is one year for compartments, zones and countries demonstrating freedom using pathway 3 (targeted surveillance). At a compartment level, a case can be made to apply a one year minimum period for all pathogens. Compartments are epidemiologically isolated and factors associated with spread between populations (assessed in this paper) are not relevant. In addition, the high level of management required by Competent Authorities authorising a compartment, should generate a very high likelihood of detection via passive surveillance (e.g. through monitoring of feed consumption and growth rates) even for infections with pathogens that result in few clinical signs or only low mortality. On this basis, the period of BBC (preceding TS) of one year can be adopted for all pathogens.

### Pathway 3. Assessment of duration of targeted surveillance to demonstrate freedom

The results of the assessments can be found in Attachments 2-5, and summarised in the following sections.

#### Fish pathogens

Details summarised below can be found in Attachment 2.

- Based on seasonality and persistence in the environment, SAV is the only pathogen to rank 1.
- All pathogens, with exception of KHV and *G. salaris*, have a high likelihood of rapid detection following introduction into a naïve population by passive surveillance. For EUS, the likelihood of rapid detection is dependent on predisposing factors

#### Crustacean pathogens

Details summarised below can be found in Attachment 3.

- Little evidence for seasonality of transmission of any pathogens.

- All pathogens have a high likelihood of rapid detection following introduction into a naïve population by passive surveillance.

### Molluscan pathogens (Attachment 3)

Details summarised below can be found in Annex 4.

- All pathogens showed seasonality in prevalence/mortality indicating transmission was restricted or reduced for a period of year (usually during winter months).
- Likelihood of early detection is low for all molluscan pathogens (except abalone herpesvirus) as onset of clinical signs /mortality occurs months to years after exposure.
- *Marteillia refringens* is an outlier, having an indirect lifecycle, and the best evidence for seasonally restricted periods of transmission.

### Amphibian pathogens

Details summarised below can be found in Attachment 5.

- Little evidence of strong seasonal impact on the rate of transmission of *B. salamondrivorans* or *B. dendrobatidis*
- Good evidence of rapid onset of mortality and morbidity in many (but not all) host species for *B. salamondrivorans* and *B. dendrobatidis*
- Ranavirus is listed as a genus. Rate of spread and transmission varies considerably between hosts and viral species (multiple), making ranking at genus level difficult thus conservative approach to ranking was taken.

Rankings for TS are summarised in Table 9.

**Table 9.** Summary rankings of pathogens to determine the minimum period of targeted surveillance for pathway 3. Targeted surveillance

Ranking	Fish	Crustacean	Molluscs	Amphibian
1	SAV	ALL	AbHV	<i>B. dendrobatidis</i>
2	VHSV IHNV SVCV <i>M. pagrus 1</i> HPR-deleted ISAV TiLV EHNV			<i>B. salamondrivorans</i>
3	EUS <i>G. salaris</i> ISAV (including HPR0 and HPR- deleted) KHV		<i>B. exitiosa</i> <i>B. ostreae</i> <i>P. marinus</i> <i>P. olsenii</i> <i>M. refringens</i> <i>X. californiensis</i>	<i>Ranavirus</i>

It is recommended that for pathogens ranked 1 and 2, the minimum period for TS is two years and for pathogens ranked 3 it is three years.



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## Compartments

The current default minimum period for TS is one year for compartments for pathway 3. A case can be made to keep a one year period for TS for all pathogens. The high level of management required by Competent Authorities authorising a compartment, should generate a very high likelihood of detection via passive surveillance if the pathogen was present. On this basis, TS for a minimum period of one year is sufficient for all pathogens.

## Pathway 4: returning to disease freedom

In Chapter 1.4. of the *Aquatic Code* a default minimum period for BBC before TS to regain freedom is not specified. Instead the guidance requires that ‘the pathway of disease introduction should be investigated and basic biosecurity conditions should be reviewed and modified’ and that ‘mitigation measures should be implemented following eradication of the disease, and prior to commencement of any targeted surveillance’. As the circumstances of each disease outbreak leading to a breakdown in disease freedom are unique, setting periods for BBC (preceding TS to regain freedom) on a pathogen basis is not required.

Chapter 1.4. of the *Aquatic Code* suggests that for ‘a country or a zone, the default minimum period of surveillance to regain freedom is consistent with the requirements for pathway 3’, and thus the periods of TS recommended in this paper can be used for pathway 4. However, it should be noted that guidance in Chapter 1.4. allows for earlier self-declarations of freedom ‘if the relevant Competent Authority can demonstrate that the approach would provide an appropriate standard of evidence for the circumstances of the outbreak and the disease’. As outbreaks leading to a breakdown in disease freedom will vary considerably in size and circumstance, flexibility in applying periods of TS to regain a disease free status is justified.

## Discussion

### Pathway 1. ‘Absence of susceptible species’.

Based on the analysis in this paper, it is recommended a minimum period of 6 months for BBC before claiming freedom based on the absence of susceptible species is sufficient for most pathogens. However, for pathogens for which there is evidence of persistence in the environment for months, a minimum period of 12 months is recommended. The viability of pathogens in the environment (outside the host) will be influenced by environmental factors, which following guidance in Chapter 1.4. of the *Aquatic Code*, should be considered in any claim for disease freedom using pathway 1.

### Pathway 2. ‘Historical freedom’.

In editions of the *Aquatic Code* before revision of Chapter 1.4., a minimum period of ten years over which the pathogen had not been observed was required for all but a few diseases (see Attachment 1). Evidence that the pathogen has not been observed is only reliable if BBC (including passive surveillance) have been implemented. A ten year period of BBC will generate a high likelihood of confidence that the pathogen is present for all but two fish diseases (KHV and EUS). The pathway is not suitable for ISAV (Including HPR0 and HPR-deleted) and *G. salaris* because HPR0 ISAV is not expected to cause clinical signs and *G. salaris* as clinical signs are not seen in all susceptible species. The pathway is however suitable for HPR-deleted ISAV. Guidance in Chapter 1.4. is clear that pathway 2 can only be used if infection results in observable clinical signs. As well, in addition to meeting standards for duration of BBC set in the *Aquatic Manual* disease-specific chapters, evidence of the effectiveness of the passive surveillance component of BBC is required in any application for recognition of disease freedom.

### **Pathway 3. 'Targeted surveillance' (period of BBC).**

The BBC period will only formally start once a Competent Authority is confident that the disease is absent (as a result of stamping-out or a long period of no detections). For pathogens with high rates of spread and high likelihood of detection (i.e. ranked 1 and 2), it is reasonable to assume that one year is a sufficient minimum period for the design prevalence to be reached (assuming introduction just preceding implementation of BBC) or detection through passive surveillance.

For pathogens ranked 3, a longer BBC may be required to allow either a second window for spread, or for clinical signs or mortality to occur. For example, infection with a number of molluscan diseases may only become apparent in older animals and thus a longer period is needed for detection during the period of BBC via passive surveillance. For pathogens ranked 3 with limited periods of transmission and low likelihood of detection by passive surveillance, the period of BBC should be extended to two years. All fish disease were ranked 1 or 2, except KHV and *G. salaris* (ranked 3), both of which had limited periods of transmission during some periods of the year and low likelihood of detection by passive surveillance. It is recommended that BBC be extended to 2 years for these pathogens.

Compared with fish diseases, less evidence is available to rank crustacean diseases. On the basis that they are all i) highly infectious and cause rapid onset of morbidity and mortality after introduction to a naïve population, and ii) observational evidence of rapid spread between population, all crustacean diseases met the criteria for a rank of 1. By contrast, for all the molluscan parasites seasonal variation in prevalence indicates water temperature dependent rates of transmission. Only abalone herpesvirus has a high likelihood of detection by passive surveillance within one year of introduction into a naïve population. It proposed that the BBC (preceding TS) is one years for abalone herpesvirus and 2 years for all the other pathogens.

*Ranavirus* genus has a large variation in characteristics between the multiple hosts and pathogens and thus is difficult to fully assess. As such, *Ranavirus* was ranked as a 3 which provided a longer BBC and TS to account for the limited information available and the variation which exists within this genus. Based primarily on observations on a low level of spread between populations, it is suggested that the BBC for *Batrachochytrium salamandrivorans* is at least 2 years. The largely observational evidence for *B. dendrobatidis* indicates higher rate of spread and rapid onset of clinical signs and a one year BBC is appropriate.

### **Pathways 3. 'Targeted surveillance' (duration of targeted surveillance).**

It is suggested that for pathogens ranked 1 and 2 in this analysis, the minimum period of TS is two consecutive years (the default minimum period stipulated in Chapter 1.4. of the *Aquatic Code*). The design of the surveillance should follow guidance in Chapter 1.4. that requires surveillance to take place in consecutive years. Sampling should take place when conditions for pathogen detection are optimal, which may occur during a period of weeks or months during each year of the surveillance period. Whilst transmission for pathogens ranked 1 and 2 are not strongly seasonal, stochastic inter-annual variation in transmission (and therefore prevalence) justifies the default minimum period of two years for TS.

For pathogens ranked 3, three consecutive years of TS can be justified. This means that sampling is done at the time of year when likelihood of detection is highest in at least three consecutive years, on the basis that environmental conditions in the years one and two may result in a low likelihood of detection by either TS (sampling) or passive surveillance. It is therefore recommended that the minimum period of TS is three years for pathogens ranked 3.

Conditions making detection of the pathogen suboptimal may persist for more than two or three years. Therefore, it is important that Members follow guidance in Chapter 1.4. when making a case for disease freedom and provide evidence that sampling took place when conditions were optimal for pathogen detection.

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## Conclusion

The aim of this assessments is to provide a justification for the durations of the BBC and TS for the disease-specific chapters of the *Aquatic Code*. Therefore, the analysis was focused on pathogen characteristics and has not attempted to provide recommendations based on host and environment. Arguably, it may be problematic to assess the importance of pathogen characteristics without considering the host (for pathogens with multiple hosts) and environment (for pathogens with a wide geographic distribution). To some extent the rankings are based on the pathogen characteristics in the major hosts and on environmental conditions in the main areas where these hosts are found. Nevertheless, it is possible to cite specific examples where pathogen/host/environmental combinations for which the ranking is not appropriate. Therefore, it is important that the provisions of Chapter 1.4. requiring that passive surveillance is effective (as infection will cause observable clinical signs), and sampling is undertaken when conditions are optimal for detection and populations with higher likelihoods of infection are preferentially sampled.

It is important to recognise the lack of data, especially for environmental persistence for many of the pathogens, and especially those of molluscs and crustaceans. Ideally, quantitative assessments from observational epidemiological studies would be available to assess the rate of spread between populations. However, in general these data are not available and are not necessarily thoroughly reviewed in the disease-specific chapters of the *Aquatic Manual*.

Despite these possible criticisms and weaknesses in the available data, the analysis presented provides a sound evidence base to justify recommendations for duration of the BBC and TS that should be used when developing surveillance programmes to claim freedom from WOAHA listed diseases as described in Chapter 1.4. 'Aquatic animal disease surveillance' of the *Aquatic Code*.

## Attachments

**Attachment 1.** Summary of the previously recommended minimum periods of BBC and TS for all listed diseases and all pathways in the 2021 Aquatic Code (i.e. preceding the adoption of Chapter 1.4. in 2022). Periods for country freedom are shown. NA = not applicable (pathway not available).

	Epizootic haematopoietic necrosis disease	A. invadans (EUS)	Infection with <i>Gyrodactylus salaris</i>	ISA virus (HPR0 and HPR-deleted)	ISA virus HPR-deleted	Infection with salmonid alphavirus	Infectious haematopoietic necrosis	Koi herpesvirus disease	<i>Megalocytivirus pagrus 1</i>	Spring viraemia of carp	Viral haemorrhagic septicaemia	Infection with abalone herpesvirus	Infection with <i>Bonamia ostreae</i>	Infection with <i>Bonamia exitiosa</i>	Infection with <i>Marteilia refringens</i>	Infection with <i>Perkinsus marinus</i>	Infection with <i>Perkinsus olseni</i>	Infection with <i>Xenohaliotis californiensis californiensis</i>	Acute hepatopancreatic necrosis disease	Crayfish plague ( <i>Aphanomyces astaci</i> )	Infection with yellow head virus	Infectious hypodermal and haematopoietic necrosis	Infectious myonecrosis	Necrotising hepatopancreatitis	Taura syndrome	White spot disease	White tail disease	Infection with <i>Batrachochytrium dendrobatidis</i>	Infection with ranavirus	Infection with <i>Batrachochytrium salamandrivorans</i>	
1. Absence of susc species	2	NA	2	2	NA	2	2	2	2	2	NA	2	2	2	3	3	NA	3	2	2	2	2	2	2	2	2	2	2	2	2	
2. Historical freedom																															
-Not observed	10	10	10	NA	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	25	10	10	10	10	10	10	10	10	10	10	
-Basic biosec conds	10	10	10	NA	10	10	10	10	10	10	10	2	2	2	3	3	3	3	2	10	2	2	2	2	2	2	2	2	10	10	10
3. Targeted surv																															
-Basic biosec conds	2	2	5	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	2	5	2	2	2	2	2	2	2	2	2	2	
-Targeted surv	2	2	5	2	2	2	2	2	2	2	2	2	2	2	3	3	3	2	2	5	2	2	2	2	2	2	2	2	2	2	
4. Return to freedom	2	2	5	2	2	2	2	2	2	2	2	2	2	2	3	3	3	2	2	5	2	2	2	2	2	2	2	2	2	2	

**Attachment 2.** Fish pathogens: Assessment of duration of BBC preceding TS to demonstrate freedom (pathway 3).

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
VHSV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted (when water temp <14 C)	Moderate- Days to weeks	2
IHNV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted (when water temp <14 C)	Moderate- Days to weeks	2
SVCV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted (when water temp 11-17 C)	Moderate- Days to weeks	2
KHV	Simple-direct	High – very infectious, low minimum infectious dose Slow spread between populations when water temp <16 C	Low: Subclinical infection at low water temp	Restricted (when water temp <16 C)	Low - days	3
SAV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Unrestricted (seasonal variation observed but outbreaks occur throughout the year)	High – weeks to months	1
EHNV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted (outbreaks occur at water temperatures between and 11-20 C)	Very high – months to years	2
<i>M. pagrus 1</i>	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted to summer months (water temp >25 C)	unknown	2
HPR-deleted ISAV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Unrestricted with mortality peaks in early summer and winter	Low persistence – hours to days	2
ISAV (including HPR0 and HPR-deleted)	Simple-direct	High- very infectious, low minimum infectious dose	Very low: HPR0 ISAV is not expected to cause clinical signs	Unrestricted with mortality peaks in early summer and winter	Low persistence – hours to days	3

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
TiLV	Simple - direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Outbreaks generally when water temp >22 C	unknown	2
<i>A. invadans</i> (EUS)	Simple-direct	High (single spore sufficient for pathogen to establish)	Low to High: Rapid onset clinical signs, but require predisposing factors for clinical expression.	Restricted 18-22 C.	Month-years (encysted form)	3
<i>G. salaris</i>	Simple-direct	High (single parasite sufficient for infestation to establish) Evidence of slow spread between wild populations	Low: Months to years to detect populations declines in wild <i>Salmo salar</i> ; Clinical signs not apparent in rainbow trout	Rate of replication and spread low below 6.5 C (and on rainbow trout)	Hours to days on dead host; temperature dependent	3

LH = likelihood

**Attachment 3.** Crustacean pathogens: Assessment of duration of BBC preceding TS to demonstrate freedom (pathway 3).

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
AHPND	Simple-direct	100% prevalence achieved indicating high rate of spread	High: Rapid onset mortality	Unrestricted	9-18 d	1
<i>A. astaci</i>	Simple-direct	Very rapid spread in susceptible species crayfish, reaching 100% prevalence	High: Rapid onset mortality (in susc. spp.)	Unrestricted – Infection over wide temp range	Several weeks, spores 2 months	1
DIV1	Simple-direct	Rapid spread and high prevalence recorded in shrimp and crayfish	High: Rapid onset mortality	Infection recorded over a wide temperature range	No information available	1
<i>H. penaei</i>	Simple-direct	Little some information but evidence of rapid spread in farmed <i>P. vannamei</i>	High: Rapid onset mortality	Unrestricted – High rate of spread at high temp and salinity	No information available	1
IHHNV	Simple-direct	Very rapid spread in <i>P. stylirostris</i> ; low in <i>P. vannamei</i> , <i>P. monodon</i> (may go undetected for months)	High; <i>P. stylirostris</i> Low: <i>P. vannamei</i> , <i>P. monodon</i>	Unrestricted – reduced replication at high temp	No information available	2
IMNV	Simple-direct	Little information	Medium : mortality following stress events in endemic areas	No information available	No information available	1
MrNV	Simple-direct	Rapid spread on introduction to naïve populations	High: Rapid onset mortality in juveniles	No information available	No information available	1
TSV	Simple-direct	Dependent of strain/spp susceptibility	High Rapid onset mortality Rapid onset mortality	No information available - (outbreaks more frequent when salinities are below 30 ppt	No information available	1
WSSV	Simple-direct	High rates of spread and mortality	High Rapid onset mortality	Outbreaks generally at water temp between 18-30 C.	3-4 d in pond water, 3-5 wks in sediment	1
YHV1	Simple-direct	Very rapid – 100% mortality with 3-5 d of clinical signs	High Rapid onset mortality	Little information – probably unrestricted	viable in aerated seawater for 3 d	1

LH = likelihood

**Attachment 4.** Molluscan pathogens : Assessment of duration of BBC preceding TS to demonstrate freedom (pathway 3).

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
abalone herpesvirus	Simple-direct	High – rapid rise in prevalence and onset of mortality in all age classes	High	Evidence of seasonal variation in transmission: Outbreaks at 16-19 C but impact of temp not established.	No information available	1
<i>B. exitiosa</i>	Simple-direct	Slow - spread in <i>O. chilensis</i> , causing mortality of 80% over 2-3 years; lower prevalence /mortality in <i>O. edulis</i>	Low	Evidence of seasonal variation in transmission: Peak infection in <i>O. chilensis</i> in autumn & winter; seasonality not established for infection in <i>O. edulis</i>	No information available	3
<i>B. ostreae</i>	Simple-direct	Slow – infection observed >3 mon after introduction – highest prevalence 2 yr old animals	Low	Evidence of seasonal variation in transmission: Peak infection in late winter/early spring	>7d in seawater	3
<i>M. refringens</i>	Indirect via intermediate host	Slow – prevalence peaks 1 yr post-introduction.	Low	Evidence of seasonal variation in transmission: When water temp > 17 C; higher transmission at high salinity	Up to 21 d	3
<i>P. marinus</i>	Simple-direct	Slow - prevalence highest in animals 1 yr post introduction; mortality observed 1-2 yr post introduction	Low	Evidence of seasonal variation in transmission: Peak transmission when water temp high	No information available	3
<i>P. olsenii</i>	Simple-direct	Slow – mortality 1-2 yrs post introduction; low mortality	Low	Evidence of seasonal variation in transmission: Transmission low/ negligible when temp < 15 C.	Several months (spores)	3
<i>X. californiensis</i>	Simple-direct	Slow – prevalence increases with age (size); infection may persist months without signs (3-7 month pre-patent period) esp. at lower water temp	Medium	Evidence of seasonal variation in transmission: Transmission higher at elevated when water temp >15	Demonstrated but not quantified	3

LH = likelihood



**Attachment 5.** Amphibian pathogens: Assessment of duration of BBC preceding TS to demonstrate freedom (pathway 3).

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
<i>B. dendrobatidis</i>	Simple - direct	Very high: in susceptible species	High: Rapid onset mortality in susceptible populations (host species dependent)	Unrestricted: Transmission probably higher in cooler months	Suspected but not confirmed	1
<i>B. salamondrivorans</i>	Simple - direct	High within susceptible species in the invasive range; spread between populations is limited	High: Rapid onset mortality in susceptible populations (host species dependent)	Unrestricted:	Encysted spores viable for up to 31 d	2
<i>Ranavirus</i> species	Simple - direct	Host species / viral species dependent	Host species / viral species dependent	Not known: Outbreaks area seasonal	Months	3*

LH = likelihood

\* Due to the numerous viral species and broad host range of those viral species for *Ranavirus* species a conservative approach was utilised and *Ranavirus* species was ranked 3.