Report

QLK2-CT-2002-01546: **Fish Egg Trade**

Work package 2 report:
The application of risk assessment to the study of vertical transmission of fish pathogens

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Work package 2 report: The application of risk assessment to the study of vertical transmission of fish pathogens

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English summary:
Two risk assessment models for the vertical transmission of a fish pathogen have been constructed: a farm-level model to assess the likelihood of the introduction and establishment of a fish pathogen via the movement of fish eggs, and a fish-level model to assess the effect of broodstock screening. Scenario trees were created to illustrate the sequence of steps necessary for the hazards to occur, and the data requirements for each model were identified. Both models were tested using input parameter for bacterial kidney disease (BKD) derived from published studies and expert opinion. Given an undetected *Renibacterium salmoninarum* prevalence of 2% and no broodstock screening, 9% of the consignments were infected (17% if no disinfection was practised). The fish-level model showed that if the prevalence of infection was 30% and only test negative brood fish were used, the mean probability of vertical BKD transmission, resulting in an outbreak was 0.85% per female fish tested, compared with 4.6% without testing. Using BKD as a case scenario, the report identifies the main data requirements and approaches that can be used when applying risk assessment to vertically transmissible fish diseases. Priority areas for future research are proposed.

Norsk sammendrag:
To modeller for risikovurdering hva angår vertikal overføring av fiskesykdomsorganismer er utviklet; én modell på besetningsnivå for å estimere sannsynligheten for å introdusere fiskepatogener med befruktet rogn, og én modell på fiskenivå for å vurdere effekten av stamfisktesting. Scenarier er utarbeidet, og hvilke data som trengs til hver av modellene er identifisert. Beregninger er gjennomført for bakteriell nyresyke (BKD) med parametre fra publisert litteratur samt ekspertvurderinger. Ved en prevalens av *R. salmoninarum* på 2%, uten stamfisktesting, ble risikoen for å selge infisert rogn beregnet til 9%, og til 17% uten desinfeksjon. Modell nr 2 viste at ved en prevalens på 30% smittede stamfisk er sannsynligheten for vertikal overføring 4,6% uten testing, men kun 0,85% dersom man kun bruker test-negative hunnfisk. Med BKD som modell identifiserer rapporten de viktigste tilnærminger og krav til bakgrunnsdata når man skal gjennomføre risikovurderinger for vertikalt overførbare fiskesykdommer. Prioriterte emner for framtidig forskning på dette området foreslås.

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Introduction

What is risk assessment

Risk is generally defined as the probability of the occurrence of an adverse event (hazard) and its impact (consequences), and, therefore, has two components. Risk assessment is a decision making tool that attempts to identify hazards, determine the likelihood of the hazard occurring, assess the consequences of the hazard and of measures that can be taken to reduce both the likelihood of occurrence and the consequences.

Whilst risk assessment methods were originally developed for the nuclear and space industries, they have more recently been applied in the field of animal health. The main application of risk assessment for aquatic animals has been the evaluation of disease risks associated with international trade in animals and their products, known as import risk analysis (IRA) (Rodgers, 2001). IRA has been promoted by the requirements of the Agreement on the Application of the Sanitary and Phytosanitary Measures (SPS Agreement) of the World Trade Organization (WTO) for documented transparent methodologies to assess the risk of disease introduction. Aquatic animal health applications include the investigation of the spread of *Gyrodactylus salaris* from farmed (Paisley et al., 1999) or stocked (Høgåsen & Brun, 2003) Atlantic salmon *(Salmo salar)* to wild fish populations, and the risk for spread of the parasite between river catchments (Peeler et al., 2004). To date, there are no records in the published literature of its application to the assessment of the vertical transmission of fish pathogens. In this paper we will show how risk assessment methods may be used to study the likelihood of vertical transmission of fish pathogens. Bacterial kidney disease (BKD) is thereby used as a model infection to illustrate the data requirements.

Risk assessment models

Risk assessment has been widely used in a number of areas which has given rise to a variety of different terms and definitions. IRA has generally followed the guidelines of the Office International des Épizooties (OIE) on risk assessment (Rodgers, 2003), which are based on the Covello and Merkhofer (1993) approach. Hazard identification is the first step and considered separately from the risk assessment. Risk assessment is further subdivided into stages: i) release assessment (description of pathways necessary for introduction), ii) exposure assessment (description of pathways necessary for the exposure of aquatic species in the importing country to the introduced exotic pathogen and its establishment), iii) consequence assessment (identification of the consequences of disease introduction and establishment), and iv) risk estimation (integration of the release, exposure and consequence assessments). In this study the OIE guidelines are broadly followed, however, a consequence assessment is not being performed.

The risk question

It is recommended that the scope of a risk assessment is described by the "risk question" (Murray et al., 2004). The risk question should define the hazard, the scope of the risk assessment and the denominator (unit of interest). For example the risk question for an import risk analysis for fish eggs would need to define the host species, the type of commodity, i.e unfertilised (green) or embryonated (eyed) eggs, and the exporting country(s).

Scenario trees

A scenario tree is a graphical representation of the pathways by which the hazard may occur. The development of a scenario tree helps to clarify the understanding of the issues. They ensure that the sequence of events is depicted in an order that is accurate both in time and space. Scenario trees also help explain how the risk assessment was performed.

A farm-level model for vertical disease transmission and spread

The hazard

The first assessment examines the following risk question "What is the likelihood that the introduction of a consignment of Atlantic salmon eggs into a hatchery results in the introduction and establishment of vertically transmitted disease, A, within X days of hatching". This scenario is based on eggs being supplied from a farm which has no history of disease and therefore, individual broodstock testing is not performed. Trade is continuing since the disease has not been detected in population monitoring or surveillance. This scenario is typical of a situation where infection occurs as a "surprise event".

The steps in the pathway that results in a pathogen, present in a farmed Atlantic salmon broodstock, ultimately causing a clinical disease outbreak in a hatchery population of progeny parr or pre-smolts are illustrated in a scenario tree (Figure 1). The scenario tree tracks the potential infection through the farm production stages and is split into three sections that address the following specific questions (which combine to create the risk question above):

1. What is the likelihood that an egg batch (individual crossing) produced from infected broodstock is itself infected when incubated?
2. What is the likelihood that an egg consignment (made up of multiple batches) is infected when sold/moved to another facility?
3. What is the likelihood that an infection in an egg consignment results in a subsequent clinical disease outbreak in a hatchery fish population?

Definitions

When undertaking a risk assessment, it is important that terms are clearly defined. In this study an egg batch is defined as "eggs produced from 1 hen crossed with 1 or more cocks". This study is essentially identical to a commodity import risk analysis, and we have defined the unit of the commodity as an "egg consignment" which is defined as the "discrete number of eggs sold in a single transaction".

Assumptions

1. A batch (eggs from an individual female) may be fertilised by one or more males (the total number of males used to fertilise egg batches is farm specific and depends on the fertilisation strategy employed).
2. A consignment is made up of a discrete number of batches.
3. A batch might be infected if either the female or any of the male brood fish used to fertilize the eggs are infected.
4. If a female is infected, there is a probability that the pathogen is internalised into the ovarian tissue resulting in an intra-ovum infection. This is distinct from a surface infection where pathogens contaminate the ovarian fluid when eggs are released into the coelomic cavity at ovulation. Hence, at the batch level, there are two categories of infection: internal (represented by orange boxes in Fig. 1) and external (yellow boxes).
5. Sperm cells cannot be internally infected.
6. Only externally infected batches may be successfully disinfected.
7. There is no disinfection of milt or unfertilised eggs.
8. Batches are incubated separately.

Scenario tree

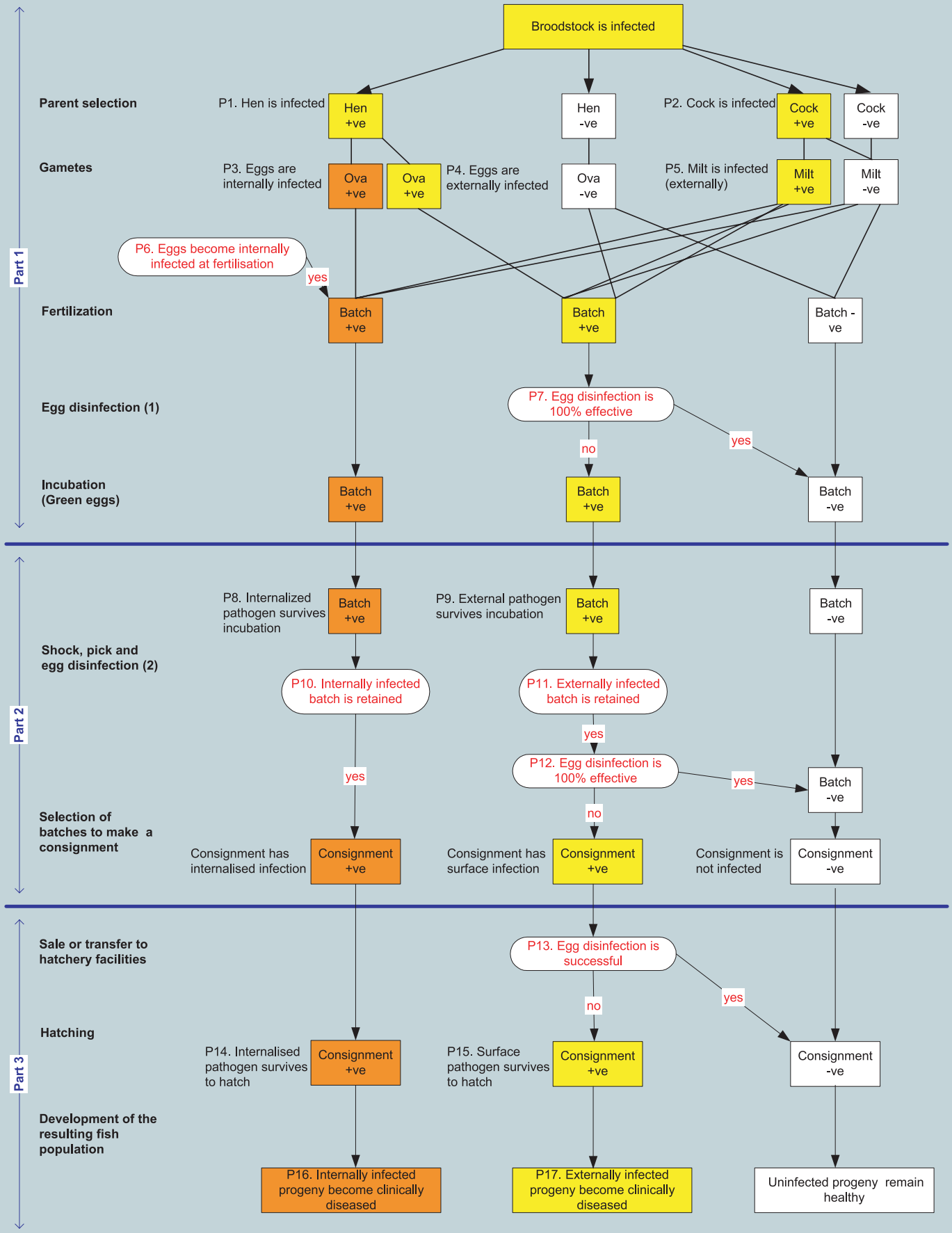
In the risk assessment the unit of observation changes from the broodstock, to the batch of eggs, to the consignment. A quantitative assessment must take into account the number of broodstock used in a mating (e.g. milt from more than one male may be used to fertilise eggs from a single female) and the number of batches used in a consignment. Thus these data are given in Table 1.

Part 1

(spawning, fertilisation and incubation)

Eggs are stripped from individual females, fertilised, water-hardened, disinfected and incubated separately as discrete batches (see Fig. 1 part 1). Disinfection may take place immediately after fertilisation and water-hardening (P7).

Figure 1. Scenario tree for the vertical transmission of a fish pathogen: a farm-level model



Data requirements

Table 1. Data needed for a farm-level risk assessment of vertical pathogen transmission

	PROBABILITIES	NOTES
P	**PART 1**	
1	prevalence in females	depends on duration of infection in farm, environmental and other factors
2	prevalence in males	
3	probability that infected female produces internally infected eggs (during ooegenesis or in coelomic fluid)	pathogen, age, duration of infection, abundance of pathogen
4	Probability that infected female produces externally contaminated eggs	pathogen, age, duration of infection, abundance of pathogen
5	probability that infected male produces externally contaminated milt	pathogen, age, duration of infection, abundance of pathogen
6	probability that eggs become internally infected at fertilisation (requires external contamination from milt or ovarian / coelomic fluid)	pathogen size, pathogen load, time to water-hardening
7	probability that disinfection is 100% effective (pathogen completely removed from eggs)	pathogen characteristics, pathogen load, disinfection procedure (i.e. disinfectant used, concentration, time)
	PART 2	
8	internalised pathogen survives incubation	embryonic infection, pathogen survival characteristics, environmental factors
9	external pathogen survives incubation	
10	batch with internally infected eggs is retained	high level of egg mortality may lead to discard of the batch (future probabilities need to be adjusted to account for different level of discard of infected and uninfected batches)
11	batch with externally infected eggs is retained	
12	egg disinfection is successful	residual pathogen load, disinfection procedure (i.e. disinfectant used, concentration, time)
	PART 3	
13	egg disinfection is successful at arrival	residual pathogen load, disinfection procedure (i.e. disinfectant used, concentration, time)
14	internal infection survives to hatching	pathogen survival characteristics, environmental factors
15	external infection survives to hatching	
16	internal infection results in disease and shedding and a disease outbreak	pathogen, biosecurity, production system
17	external infection results in disease and shedding and a disease outbreak	pathogen, biosecurity, production system
	OTHER DATA	
	number of males per mating	male fecundity, fertilisation policy
	number of batches per consignment	fecundity, consignment size.

Part 2

(to sale or transfer of eyed eggs for on-growing)

Shortly before hatching, when the eggs are eyed, they are shocked, dead eggs are removed (picking) and the remaining eggs are desinfected (P12). Individual batches are then combined to make consignments that are sold or transferred to new incubating facilities (Fig. 1 part 2).

Part 3

(hatching and on-growing)

If a consignment is sold or transferred to a new farm site, it is common practice (and recommended) that the eggs are disinfected by the receiving hatchery on arrival (P13). Eggs are hatched and resulting fry are reared to smoltification in freshwater facilities (Fig. 1 part 3).

If infection causes death in embryos, these eggs are highly likely to be identified and discarded (procedures to remove dead eggs are routinely used). Secondly, since these eggs do not hatch, the pathogen is not released and infection of the sac-fry cannot take place. Therefore, it is unlikely that eggs which die as a result of intra-ovum infection are an important source of pathogen.

If the disease results in a covert subclinical infection in the sac-fry and pathogen shedding does not occur, the hazard will not occur. A time period for the development of the disease in the offspring should be defined.

Discussion

Part 1

A batch may be initially infected if either the female or any of the males used to fertilise the eggs are infected. Risk of infection increases with the number cocks used to fertilise each batch. If the female is infected there is a risk that the eggs are internally infected. As the batches are disinfected individually, there is a possibility that those with a surface contamination may be cleared of infection before incubation. Disinfection will not change the infection status of internally infected eggs. Since this risk assessment is being conducted at the level of the consignment, disinfection is defined as failing if any viable pathogen remains associated with the egg batch. The likelihood that an individual batch is infected when incubated is then dependent on the prevalence of infection in the broodstock, the probability that an infected female produces internally infected eggs and the efficiency of the disinfection procedure.

Part 2

A consignment may be infected if any of its constituent batches remain infected after shocking, picking and further disinfection. In this risk assessment it was assumed *a priori*, that consignments consisted of a number of discrete batches. However, if batches of eggs were mixed prior to making up a consignment, the risk that a consignment is infected would increase. As the batches are assumed to be disinfected individually, there is a further opportunity to clear infection from batches that are not internally infected. Therefore, the probability that a consignment contains infected eggs increases with the number of batches it contains.

Because batches are from distinct crossings, there is likely to be a large variation in fertility, and survival to the eyed egg stage. The incubation of discrete batches allows each to be assessed after shocking and the option of rejecting those that are of poor quality. Depending on the pathogen, survival to the eyed stage (and therefore probability of rejection) may be dependent on infection status (see P10 and P11). If batches with externally or internally infected eggs are more likely to be discarded compared with uninfected batches, the probability that a consignment will contain a batch with infected eggs may be reduced. The number of batches required to make up a consignment will be a function of the consignment size and the mean fecundity of the female brood fish (batch size). The risk that a consignment is infected increases with the number of batches in a consignment.

Part 3

Consignments may remain internally or externally infected on arrival at a new rearing facility. It is common practice to disinfect eggs transferred from a different hatchery, so again it is possible to clear infection from those with a surface contamination only. The likelihood of a hatchery population developing a clinical disease will depend on its infection status after hatch. Fish must have an internalised infection for this to occur, so any remaining surface pathogen would first have to invade fish tissue.

Risk estimation

In order to assess the overall risk of vertical transmission of a pathogen associated with one egg consignment at the farm level, the number of routes through the scenario tree in Figure 1 must be established (for the sake of clarity some routes have been combined in Figure 1). The probability of pathogen transmission for each route is the product of the probability associated with each step (P1-P17) encountered. The overall risk is the sum of the probabilities for each pathway.

A fish-level model for vertical disease transmission given broodstock testing

In this second scenario, broodstock are routinely tested individually for significant pathogens that can be vertically transmitted. The individual fish level model described in this section assesses the risk of vertical transmission given broodstock screening. This is a typical scenario when using broodfish from a population of unknown or poorly investigated infection status (i.e. wild broodfish), or from populations in disease endemic regions where there is fear of latent infection. In such cases, disease prevalence may be extremely low. The use of risk assessment to examine the effect of broodstock testing is investigated in this report.

The hazard

The scope of the risk assessment is defined by the following risk question: "What is the likelihood that the use of eggs from a broodfish which have tested negative for pathogen X with test Y, fertilised with milt from a test negative male fish, will result in an outbreak of disease Z in the fry/fingerling and disease spread in the hatchery?"

Assumptions

The scenario tree is based on a number of assumptions:

- Vertical transmission may occur if i) the eggs are internally infected, ii) surface of the eggs are contaminated with the pathogen and disinfection is not practised or is ineffective or iii) infected milt is used and disinfection is not practised or is ineffective.
- Contamination of milt with a vertically transmitted pathogen is effectively surface contamination; true intra-sperm infection does not take place.
- Eggs are disinfected after fertilisation.
- Disinfection has no effect on intra-ovum infections.
- Surface contamination does not increase risk of vertical transmission in eggs with true intra-ovum infections.
- If eggs have surface contamination or true intra-ovum infections use of contaminated milt does not increase the risk of vertical transmission.

Scenario tree

Figure 2 illustrates the routes by which vertical transmission of a pathogen can result in a disease outbreak. Figure 2 and Table 2, stages P1 to P10 describes the transmission of the pathogen from the broodstock to the eggs, and are equivalent to the release assessment. In this model three routes can result in vertical transmission; i) intra-ovum infection (internal), ii) surface contamination of eggs (external) or iii) or surface contamination of milt (external).

Intra-ovum infection (P3) can occur via: i) internalisation of the pathogen during oogenesis, ii) internalisation during or shortly after fertilisation, and iii) exposure and infection of the embryo. In Figure 2, these three routes are captured in one stage (P3); however, it would be possible to extend the scenario trees to account for these different routes.

The likelihood that the milt is infected is assessed in a separate scenario tree (Figure 3), the results of which are used in Figure 2. Infection of the fry /fingerlings is a necessary but not sufficient factor for a disease outbreak. An outbreak of clinical disease and spread of the pathogen from the infected fry / fingerlings must occur for the disease to become established. The exposure assessment (stages following P10) assess the likelihood of exposure, i.e. the establishment and spread of the pathogen from the eggs.

Figure 2. Scenario tree for the vertical transmission of a fish pathogen

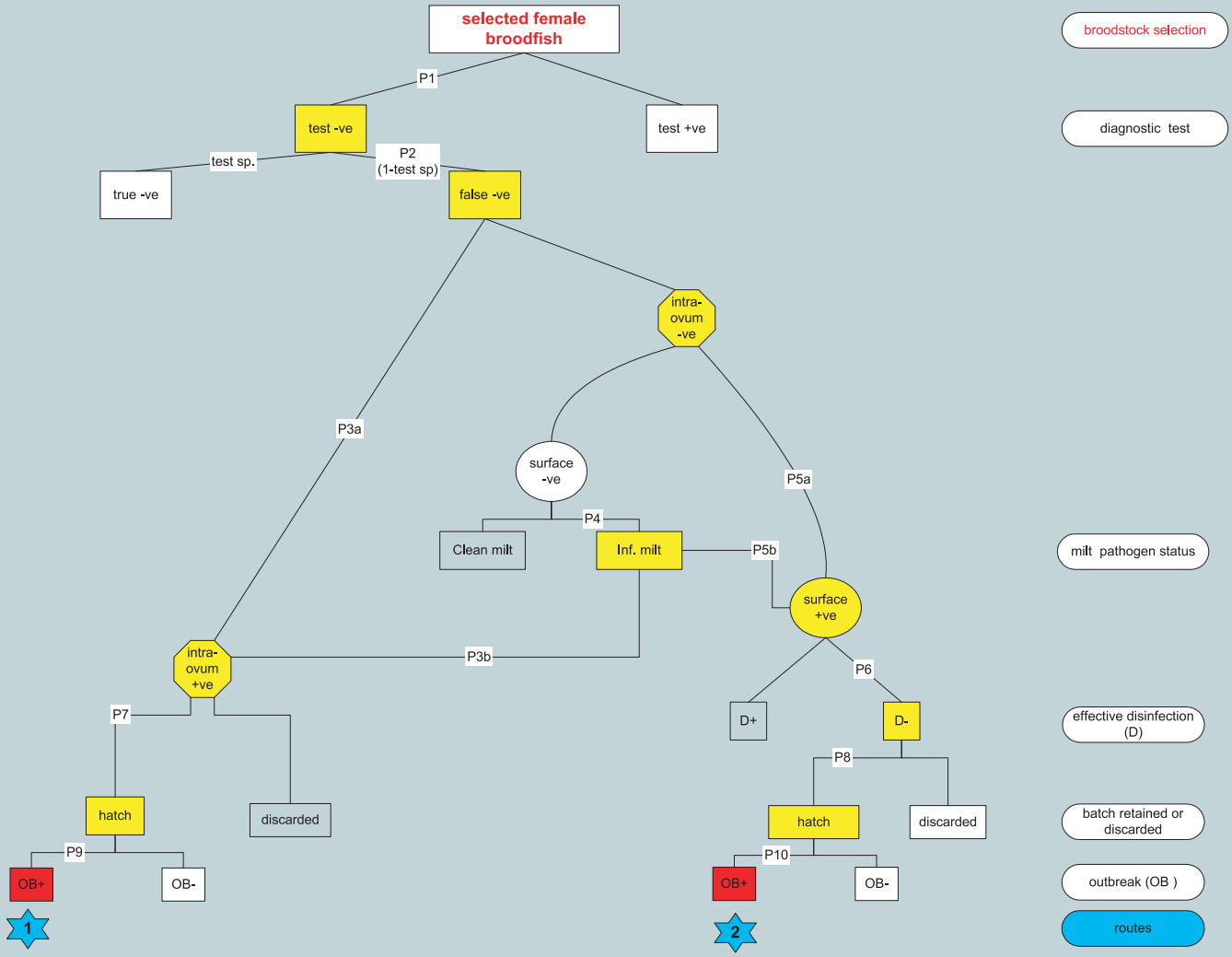
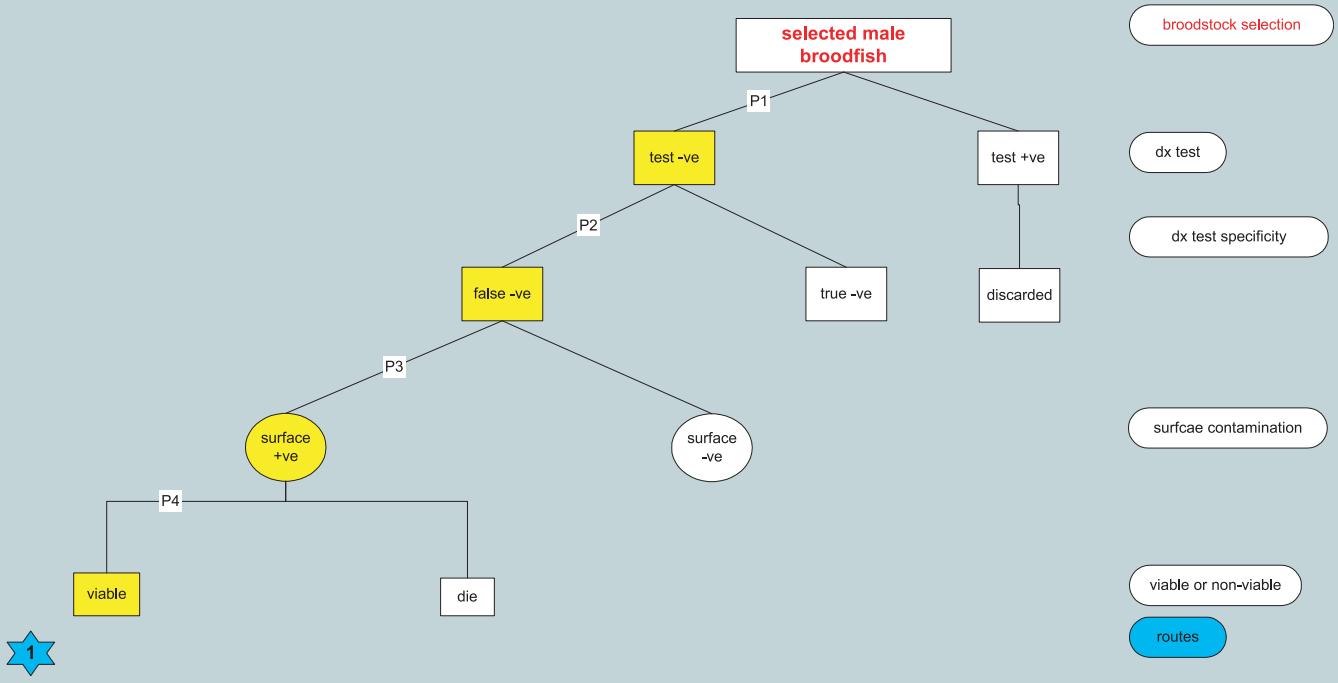


Figure 3. Scenario tree for the contamination of milt with a vertically transmitted fish pathogen



Data requirements

The information required to assess each step of the scenario trees (Figures 2 and 3) are described in Table 2.

Risk estimation

When undertaking a risk assessment for the vertical transmission of a fish pathogen, an estimate, either quantitative or qualitative, of the probability for each step in the scenario tree (P1 to 10) is required. The probability for each of the six routes (Figure 2) is the product of the probabilities of the steps along each route. The overall probability is the sum of the probabilities for each pathway.

Table 2. Stages in the scenario tree and data requirements

P	Description	Factors affecting probability
female brood stock (Figure 2)		
1	probability of a selected female fish testing negative	prevalence of the infection from surveillance results, test specificity
2	probability that test negative fish is actually infected	equals (1-test sensitivity) prevalence
3a	probability that infected fish produces infected eggs (true intra-ovum infection)	age, infection status, antibody titre, immune status
3b	probability that use of externally infected milt results in internal infection	level of contamination, pathogen size
4	probability that milt is infected (output from Figure 3)	see below
5a	probability of external infection of eggs	age, duration of infection, antibody titre, immune status; evidence of pathogen in ovarian fluid
5b	probability of external infection of eggs from use of externally infected milt	level of contamination of milt
6	probability that disinfection of eyed eggs does not take place or is ineffective	type of disinfectant and concentration, pathogen survival
7	probability that infected eggs are retained and hatch	experimental data and field observations on the influence of infection on egg viability
8	probability that eggs with surface contamination (from eggs) are retained and hatch	experimental data and field observations on the influence of infection on egg viability
9	probability of pathogen spread from fry / fingerlings from infected eggs	stage at which clinical disease is seen, prevalence, severity, level of pathogen shedding, contact between batches of fry, biosecurity, pathogen characteristics (survivability outside host etc.)
10	probability of pathogen spread from fry from surface contaminated eggs	level of contamination, biosecurity (separation of infected batch from other eggs, fry etc), pathogen characteristics (virulence, survivability outside host etc.)
male brood stock (Figure 3)		
1	probability of a selected male fish testing negative	prevalence of the infection, test specificity
2	probability that test negative fish is actually infected	equals (1-test specificity) prevalence
3	probability of surface contamination	age, duration of infection, antibody titre, immune status, evidence of pathogen in seminal fluid
4	probability that the milt is viable and used	influence of pathogen contamination on viability of sperm testing of milt prior to use

Bacterial kidney disease

BKD is an infectious disease of salmonids caused by infection with *Renibacterium salmoninarum* (Rs), and is one of the most important vertically transmitted diseases of fish. Rs infects all salmonid species belonging to the genera *Salmo, Salvelinus*, and *Onchorhyncus* as well as related freshwater species such as grayling *(Thymallus thymallus)* and Danube salmon *(Hucho hucho)*. Its vertical transmission and control has been extensively investigated and it was therefore chosen to illustrate how the risk assessment models described above can be implemented and to assess the availability of the necessary data.

Bacterial kidney disease can be transmitted horizontally (Balfry et al., 1996), and vertically via true intra-ovum infections (Evelyn et al., 1986a; Evelyn et al., 1986b) and due to surface contamination; Rs is found in the ceolomic/ovarian fluid of infected broodfish (Evelyn et al., 1986b). Vertical transmission may be an important route of transmission leading to persistence of the pathogen in an infected population.

Farm level model

Risk question

"What is the likelihood that the introduction of a consignment of Atlantic salmon eggs into a hatchery results in the introduction and establishment of Rs within 180 days of hatching". The farm-level model is used to explore the scenario of a farm with a history of freedom from Rs, no broodstock screening for Rs, but where the disease has been introduced but remains undetected. This scenario is reflected in the choice of parameters, a low prevalence is assumed, no clinical disease and therefore it is assumed that few of the fish are heavily infected.

Data requirements, availability and risk estimation

Prevalence of infection (P1,2)

Where BKD is endemic, the prevalence of Rs vary greatly with environmental conditions. In this scenario, a low prevalence (2%) has been chosen to reflect recent introduction of the bacterium.

Probability infected female produces internally infected eggs (during ooegenesis or in coelom) (P3)

The probability of internal infection will be positively associated with the level of infection. A high level of infection will result in ovarian infection and infection of the eggs during oogenesis or in the coelom. Evelyn et al. (1984) found that vertical transmission took place when 4 x 10^9 Rs cell/ml were present in the coelomic fluid, at which level the fluid became cloudy. More recent work (Lee & Evelyn, 1989) has shown that smolts derived from eggs that were not deliberately exposed to Rs, but which were obtained from ovarian fluid naturally infected with as few as 28 to 113 Rs cells/ml, can also become infected.

Table 3. Input values for farm-level model

P	PART 1	input	min	most likely	max
1	prevalence in females	0.022	0.002	0.02	0.05
2	prevalence in males	0.022	0.002	0.02	0.05
3	probability infected female produces internally infected eggs (during ooegenesis or in coelomic fluid)	0.118	0.01	0.1	0.3
4	Probability that infected female produces externally contaminated eggs	0.118	0.01	0.1	0.3
5	probability infected male produces externally cointaminated milt	0.010	0	0.01	0.02
6	probability that eggs become internally infected at fertilisation (requires external contamination from milt or ovarian / coelomic fluid)	0.300	0.1	0.3	0.5
7	probability that disinfection is 100% effective (pathogen completely removed from eggs)	0.717	0.50	0.75	0.80
	PART 2				
7	internalised pathogen survives incubation	1	1	1	1
9	external pathogen survives incubation	0.15	0.05	0.15	0.25
10	batch with internally infected eggs is retained	1	1	1	1
11	batch with externally infected eggs is retained	1	1	1	1
	probability that disinfection is 100% effective (pathogen completely removed from eggs)	0.717	0.50	0.75	0.80
12	internalised pathogen survives incubation	0.978	0.95	0.98	1
	external pathogen survives incubation	15	10	15	20
	PART 3				
13	probability that disinfection is 100% effective (pathogen completely removed from eggs)	0.717	0.50	0.75	0.80
14	internal infection survives to hatching	1	1	1	1
15	external infection survives to hatching	1	1	1	1
16	internal infection results in disease and shedding and a disease outbreak	1	1	1	1
17	external infection results in disease and shedding and a disease outbreak	1	1	1	1
	males per mating	2	1	2	10
	batches per consignment	25	3	25	1

The probability of clinical disease appears to increase with prevalence. The estimates chosen reflect the low prevalence and absence of clinical disease.

Probability infected male produces externally infected milt (P4)
No data exists on which to estimate the likelihood of milt infection. The same parameters as for P 3 were used.

Probability that eggs become internally infected at fertilisation (requires external contamination from milt or coelomic fluid
Work by Evelyn et al (1986b) attempted to increase infection rates by fertilising eggs whilst immersed in contaminated coelomic fluid and by using very high levels of challenge. However, contrary to expectations fertilisation did not appear to aid entry of the bacterium into the egg. They concluded that the male salmonid does not play a significant role in vertical transmission. Based on this information very low values for the probability of internal infection occurring at fertilisation has been used.

Probability that infected female produces externally infected eggs
No good data exists on which to base this assessment. It is known that Rs can infect the kidneys whilst the coelomic fluid is free of bacteria. The uncertainty is reflected in the wide range of values used.

Probability that disinfection is 100% effective (pathogen completely removed from eggs)
Iodine treatment (250 or 500 mg/l for 15-20 minutes) is effective at eliminating Rs, however, there is evidence that very low levels of bacteria may survive on the surface of the eggs, presumably protected within cell aggregrates (Evelyn et al., 1984; Evelyn et al., 1986a). The evidence suggests that Rs contamination can be reduced to a very low level by disinfection but complete elimination is not certain.

Pathogen survives from fertilisation, through incubation to hatching
Laboratory experiments have demonstrated that Rs can survive in sediment / faecal material for up to 21 days (Austin & Rayment, 1985); however, the bacterium was at no time found in the water overlying the sediment. Whilst Rs can survive in filtered-sterilised river water for up to 28 days, in unsterile river water the Rs count declines dramatically after 48 hours (Austin & Rayment, 1985). Rs is probably unable to compete with the normal water-borne organisms. The evidence suggests that Rs has a low probability of surviving on the surface of eggs through to hatching, even if disinfection is not practised, and there is reasonable chance that the consignment will be free of Rs before hatching. There is no data on which to assess the survival of Rs in the egg. However, it known that Rs can survive and multiply inside phagocytic leucocytes (Gutenberger et al., 1997). A high probability of survival is assumed.

Retention of egg batches
Eggs are quality graded and dead unfertilised eggs are picked out at the eyed stage. A batch of eggs may be discarded if it contains a high level of unfertilised or dead eggs. There is no evidence that internal or external infection affects either male or female fertility or the subsequent mortality of eggs that are successfully fertilised. The same probability of retention was used for uninfected, internally and externally infected batches.

Infection results in disease and shedding and a disease outbreak
No published data exists on which to base an estimate of this probability. Therefore, in this analysis it has been assumed that infection results in a disease outbreak.

Number of males per mating
The number of males used to fertilise a batch of eggs will depend on the breeding strategy employed by individual hatcheries (this may range from single pair crosses to the use of pooled milt from several males and will be influenced by a number of factors including: sperm quality, milt storage practices and whether the eggs are destined for production stock or future broodstock).

Number of batches per consignment
The number of batches used to complete a consignment will be a function of consignment size and the fecundity of the female broodfish. A consignment may vary from 10,000 to a million or more eggs depending on the customer (ongrower) and purpose of use. Fecundity is related to fish size and strain. The number of eggs produced by individual females could range from 3000 for a small (ca. 4kg) 2-sea winter hen to 20,000 for a 15kg+ 3 or 4 sea- winter hen.

Risk estimation

A stochastic model using the @risk (Palisade) software for BKD has been constructed based on the scenario tree illustrated in Figure 1. The parameter estimates were based on a review of the literature (summarised above) and discussions at a workshop organised by the Fish Egg Trade consortium and held in Aarhus on the 15 August 2005.

The model was run 1000 times using latin hypercube sampling. Regression tornado graphs were used to identify the inputs to which the output was most sensitive. Egg disinfection can take place at three points in the model. The model was run without disinfection and with varying levels of disinfection: i) three times ii) only before sale iii) before sale and on arrival at new site.

Results

Table 4. Probability of disease transmission via the purchase of an egg consignment – results from the simulation

Name	model	Min	Mean	Max	5%	95%
internal infection	DIS0[1]	0.0032	0.0904	0.4991	0.0147	0.2215
external infection	DIS0[1]	0.0031	0.0845	0.6923	0.0124	0.2476
internal & external infection	DIS0[1]	0.0080	0.1749	1.1071	0.0313	0.4182
internal infection	DIS1[2]	0.0039	0.0903	0.4816	0.0155	0.2224
external infection	DIS1[2]	0.0005	0.0247	0.2141	0.0033	0.0754
internal & external infection	DIS1[2]	0.0056	0.1151	0.5484	0.0213	0.2800
internal infection	DIS2[3]	0.0035	0.0908	0.4744	0.0164	0.2415
external infection	DIS2[3]	0.0009	0.0252	0.1854	0.0034	0.0804
internal & external infection	DIS2[3]	0.0046	0.1160	0.5359	0.0215	0.2917
internal infection	DIS3[4]	0.0034	0.0914	0.4548	0.0144	0.2306
external infection	DIS3[4]	0.0002	0.0075	0.1231	0.0009	0.0254
internal & external infection	DIS3[4]	0.0042	0.0990	0.4864	0.0169	0.2429

[1]no disinfection, [2]eggs disinfected before sale only, [3]eggs disinfected before sale and after purchase [4]eggs disinfected after fertilisation, before sale and after purchase

The mean risk of transmission via internal infection is, as expected similar for all three situations regarding disinfection (p~0.09 or 9%). The overall mean probability of vertical transmission resulting in disease when disinfection is practised (DIS3) is only slightly higher than for internal transmission (0.099). When disinfection takes place at all possible points (DIS3) the probability of vertical transmission via external infection is very low compared with internal infection (0.0075 compared with 0.0914), which is the main route of transmission. However, if disinfection is not practised (DIS0), external infection and internal transmission are approximately equally important. All the estimates of transmission have wide confidence limits.

Figure 4. Distribution of probability of vertical transmission via internal infection (DIS3)

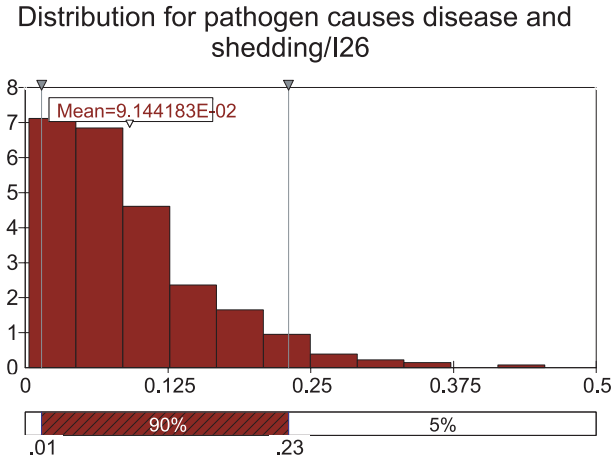


Figure 5. Distribution of probability of vertical transmission via external infection (DIS3)

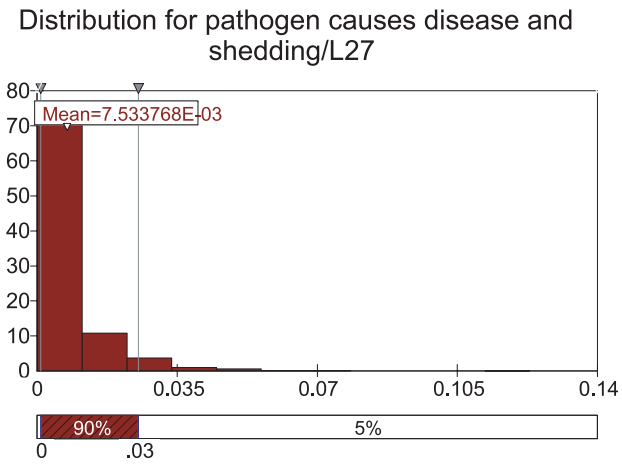


Figure 6. Distribution of probability of vertical transmission via internal & external infection (DIS3)

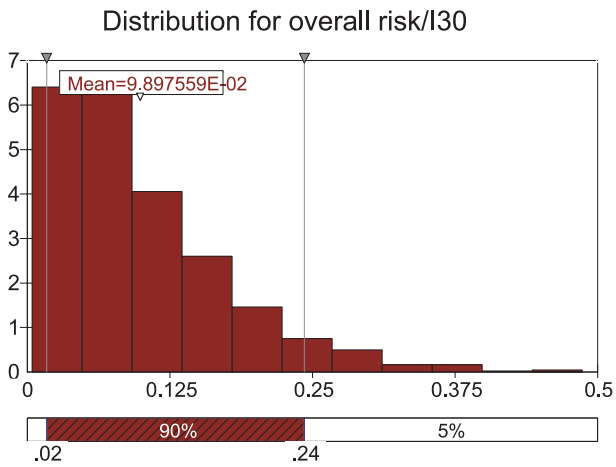
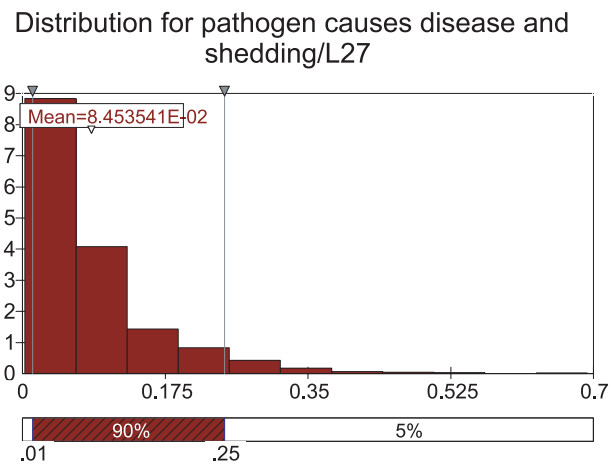


Figure 7. Distribution of probability of vertical transmission via external infection when no disinfection is practised (DIS0)



Tornado analysis

Only three factors significantly influenced the probability of internal transmission: the number of egg batches in a consignment, the probability that an infected female produces internally infected eggs, and the prevalence of infection in females. A much greater number of factors significantly influence external infection (see Table 6), of which the number of egg batches in a consignment and number of males per mating were the most important factor. However, the total variation explained in the tornado analysis was only 0.64, compared with 0.86 for internal infection.

Unsurprisingly, the main factors influencing the vertical transmission via internal and external egg infection were the same as for internal infection.

Table 5. Tornado regression analysis for probability of vertical transmission via internal egg infection (DIS3)

input parameter	regression statistic
number of egg batches that make up a consignment	0.578
probability infected female produces internally infected egg	0.500
prevalence in females	0.466

R-Squared= 0.86

Table 6. Tornado regression analysis for probability of vertical transmission via external egg infection (DIS3)

input parameter	regression statistic
number of males per mating	0.474
number of egg batches that make up a consignment	0.422
probability infected male produces externally infected milt	0.231
external pathogen survives incubation	0.216
prevalence in males	0.209

R-Squared= 0.64

Table 7. Tornado regression analysis for probability of vertical transmission via internal and external egg infection (DIS3)

input parameter	regression statistic
number of egg batches that make up a consignment	0.599
probability infected female produces internally infected egg	0.471
prevalence in females	0.458

R-Squared= 0.86

Discussion

If egg disinfection is not practised, internal and external routes of transmission are approximately equal. Disinfection greatly reduces the risk of external transmission in model DIS3 to the extent that it is comparatively unimportant compared with internal transmission. All the estimates of transmission have wide confidence limits that reflect the range of values used. The number of egg batches in a consignment was an important factor determining the risk of both internal and external transmission. The risk of introducing disease is reduced if eggs are sourced from farms with broodstock that produce large numbers of eggs and thus eggs from fewer fish

are used to make up a consignment. Prevalence in females and the probability that infected females produce infected eggs were the other two factors significantly influencing internal vertical transmission when no testing of individual broodfish is being practiced. The tornado analysis also revealed the importance of males as a source of infection for external transmission (number of males used per mating, prevalence of infection in males and probability that males produce infected milt were all important). External survival during incubation was also an important factor, even though disinfection was practiced.

The results of this analysis have wide confidence and the results should therefore be treated with caution. However, the results highlight the advantage of systematic disease monitoring and surveillance over time to assure that low-prevalence infection will likely be detected. The results can also be used to identify research that is needed to improve our estimates of the parameters significantly influencing vertical transmission of Rs.

Fish level model

Risk question

What is the likelihood that the use of eggs from Atlantic salmon *(Salmo salar)* broodfish which have tested negative for *Renibacterium salmoninarum*, fertilised with milt from a test negative male fish, will result in an outbreak of bacterial kidney disease in the fry/fingerling and disease spread in the hatchery? In this scenario, the infection status of the population may be unknown, or disease may be rarely observed in the source population.

Data requirements, availability and risk estimation

The epidemiology of BKD varies greatly between species and production systems depending on a number of management and environmental factors. The fish-level model requires data that will vary between production systems and farms and is therefore best used at the site or population level. The parameter estimates used are given in Table 8. Since most of the parameter estimates were based on incomplete data and expert opinion, pert distribution were used.

Probability of a selected female fish testing negative(P1)
The prevalence of Rs infection varies greatly depending on numerous factors. Rainbow trout appear relatively resistant to BKD and the prevalence in broodstock is often low. Chinook salmon are more susceptible and prevalences are often high. The prevalence on a particular site will depend on environmental conditions (i.e. water quality and temperature, stocking density) and management, in particular testing and segregation of broodstock. A most likely prevalence of 30% was selected (10 and 50% minimum and maximum values, respectively) to reflect a situation where testing has been introduced in an effort to control an endemic infection.

Probability that test negative fish is actually infected(P2)
The probability of detecting the pathogen will depend on the test used. Screening programmes have examined ovarian fluid for the presence of Rs using indirect fluorescent antibody techniques (IFAT) (Lee & Gordon, 1987). The techniques has been made considerably more sensitive by adding a membrane filtration step (Elliott & Barila, 1987). More recently highly sensitive molecular methods have been developed (e.g. nested RT-PCR). Information on test specificity and sensitivity for the currently available tests is not available, and the values selected are based on expert opinion, the range between the minimum and maximum values reflect uncertainty around the most likely value.

Probability that infected females produce infected eggs (true intra-ovum infection) (P3a)
Rs could become internalised in the ovum during oogenesis (i.e. in the ovary), in the coelom, or post spawning. Evelyn et al (1984) found that vertical transmission took place when 4 x 10^9 Rs cell/ml were present in the coelomic fluid, at which level the fluid became cloudy. More recent work (Lee & Evelyn, 1989) has shown that smolts derived from eggs that were not deliberately exposed to Rs, but which were obtained from ovarian fluid naturally infected with as few as 28 to 113 Rs cells/ml, can also become infected. This finding suggests that infection may transfer to the ovum directly from ovarian tissue before ovulation.

The values chosen are in part determined by the scenario "few heavily infected fish", and the wide range between the minimum and maximum values reflect both our uncertainty and true underlying variability.

Probability that infected males produce infected eggs (true intra-ovum infection) (P3b)
Published work indicated that use of infected milt did not lead to intra-ovum infections with Rs (Evelyn et al 1986b). Therefore, in this model P3b has been set to 0.

Probability that milt is infected (P4)
There are no data on contamination of milt from Rs infected males. The uncertainity about the probability of an infected male producing Rs contaminated milt is reflected in the wide range of values used (0.02 to 0.3). The mean value chosen for P4 was 0,07.

Probability of surface contamination (P5a & 5b)
The probability of surface contamination of eggs from infected fish (P5a) is probably moderate but little data exists on which to base an accurate estimate which is reflected in the range between the minimum and maximum values. It is assumed that use of infected milt will result in external infection of the eggs (P=1).

Probability that disinfection of eyed eggs does not take place or is ineffective (P6)
Iodophor compounds are known to be extremely effective at inactivating Rs (Elliott et al., 1991). However, there is evidence (Evelyn et al., 1984; Evelyn et al., 1986a) that low numbers of Rs may survive disinfection.

Probability that eggs hatch (P7 & 8)
There are no data on the influence of Rs infection on the likelihood of hatching. Eggs which die during incubation turn cloudy and are likely to be removed; however, it is assumed that this process will not result in an infected batch becoming Rs free. It is also assumed that the infection status of the batch (internal or external) does not influence whether the batch is discarded or retained.

Probability of pathogen spread from fry / fingerlings from infected or surface-contaminated eggs (P9 & 10)
The probability of clinical disease will depend on environmental factors, including water quality and stocking density. Horizontal transmission of BKD is well established and potentially epidemiologically important (Balfry et al., 1996) and shedding is highly likely to result in horizontal transmission.

It is assumed that there is a high probability that clinical disease, pathogen spreading and spread from infected smolts will occur leading to an outbreak.

Table 8. Parameter estimates for the fish level *Renibacterium salmoninarum* assessment

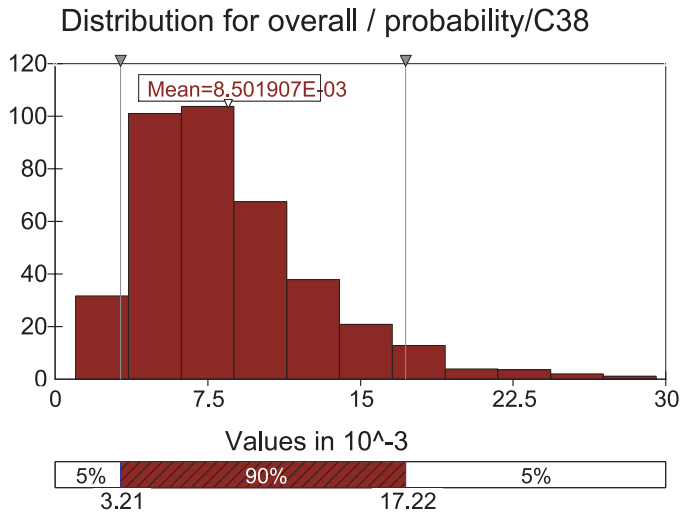
P	Description	Factors affecting probability		
female brood stock)				
		most likely	minimum	maximum
1	prevalence of infection	0.3	0.1	0.5
2	test sensitivity	0.8	0.7	0.95
3a	probability that infected fish produces infected eggs (true intra-ovum infection)	0.15	0.1	0.5
3b	probability that use of externally infected milt results in internal infection	0	0	0
4	probability that milt is infected (output from Figure 3)	0,07	0.02	0.3
5a	probability of external infection of eggs	0.1	0.02	0.3
5b	probability of external infection of eggs from use of externally infected milt	1	1	1
6	probability that disinfection of eyed eggs does not take place or is ineffective	0.02	0.01	0.05
7	probability that infected eggs are retained and hatch	1	1	1
8	probability that eggs with surface contamination are retained and hatch	1	1	1
9	probability of pathogen spread from fry / fingerlings hatched from infected eggs	0.7	0.6	0.95
10	probability of pathogen spread from fry hatched from surface contaminated eggs	0.7	0.6	0.95
male brood stock (Figure 3)				
1	prevalence of infection (probability of a selected male fish being negative)	0.3	0.1	0.5
2	test sensitivity (probability that positive fish is test-positive)	0.8	0.7	0.95
3	probability of surface contamination	0.1	0.02	0.3
4	probability that the milt is viable and used	1	1	1

Results

A stochastic model using the @risk (Palisade) software for BKD was constructed based on the scenario trees illustrated in Figure 2. The model was run 1000 times using latin square sampling.

The parameter estimates were based on a review of the literature (summarised above) and discussions at a workshop organised by the Fish Egg Trade consortium and held in Aarhus on the 15 August 2005.

Figure 8. Distribution of the probability of a BKD outbreak due to vertical transmission



If no testing took place the risk of an outbreak of BKD was 4.6% per henfish (equivalent to one outbreak per 22 fish), with 95% confidence intervals of 1.4% to 11.6%.

In the testing scenario, if 1000 female broodfish were tested, 700 would be found to be test negative and therefore used in the breeding programme, of which 37 would be false negatives (i.e. infected with Rs). Using the data from Table 8, the risk of contracting an outbreak of BKD despite testing was estimated 0.85% (equivalent to one outbreak per 118 fish tested), with 95% confidence intervals of 0.10% to 2.95% (Figure 8). The risk of transmission via external contamination was insignificant compared to internal vertical transmission.

Tornado analysis

The output was most sensitive to the probability that an infected fish produces infected eggs, the prevalence of infection in female fish, and the test sensitivity (Table 9).

Table 9. Tornado analysis for overall likelihood of an outbreak per henfish tested

Input variable	Regression statistics
probability that infected fish produces infected eggs	0.629
prevalence of Rs infection	0.485
test sensitivity	-0.457
probability of pathogen spread from fry / fingerlings from infected eggs	0.156
R-Squared	0.924

Discussion

Many of the variables will vary between species, production systems and sites. The application of the fish-level model, therefore, is most appropriate for the assessment of Rs transmission within a single farm or from one or a group of infected populations. It is clear that data will not exist for a number of variables however, expert opinion could effectively address the deficiencies in the published literature. Sensitivity analysis from a quantitative model could identify where research could most effectively be directed. The results of this model clearly demonstrate the significant decrease in the risk of vertical transmission that can be achieved through broodstock testing, even facing a "worst-case" scenario with 30% prevalence of infection and only a poor test.

There is evidence that the probability of intra-ovum vertical transmission is positively correlated to the concentration of Rs in the ceolomic / ovarian fluid (Evelyn et al., 1986b). From a risk assessment view point, this association might best explored using the NAS-NRC model (Anon, 1983), originally developed to set maximum limits of chemical substances in the environment, food, etc. Such assessment was, however, considered beyond the scope of this report and would require the generation of new and original data.

General discussion

The benefits of risk assessment

Risk assessment provides a rigorous method for examining vertical disease transmission. Developing the "risk question" forces a careful consideration and description of the hazard. Creating a scenario tree ensures that all the steps necessary for the hazard to occur are identified. Breaking down vertical transmission into a series of logical steps, allows the information required to assess the probability of each step to be identified. This work provides a framework to assess the data required to undertake a risk analysis for a particular pathogen and host. Some adaptation of the scenario trees may be necessary to account for particular circumstances.

Quantitative versus qualitative

Development of the risk question and a scenario tree is common to both qualitative and quantitative risk assessment. It is recommended that a qualitative risk assessment precedes a quantitative assessment so that all the available data is identified and reviewed (Vose, 2001). In choosing which approach to adopt, the first question to ask is whether qualitative results will produce sufficient information for decision making; ultimately risk assessment is a no more than a decision making tool. For example, risk assessments for the vertical transmission of fish pathogens may be undertaken to evaluate whether to screen broodstock. If the outputs of a qualitative analysis are not thought to be adequate, a quantitative assessment may be undertaken if the resources and data allow. For many vertically transmitted fish pathogens the data may be insufficient for a thorough quantitative risk assessment. BKD is one of the best studied vertically transmitted pathogens however, there was little or no data for a number of variables. Uncertainty around input parameter estimates can be modelled with wide confidence limits in a stochastic model, and it can be argued that the results, which will also have wide confidence limits, are of little more use than quantitative estimates. However, a stochastic model provides the opportunity for sensitivity analysis to identify the most important input parameters. Therefore, the results of a sensitivity analysis can be used to direct future research.

Consequence assessment

Consequence analysis has not been attempted in this study. The consequences of vertical transmission can be characterised as increased mortality and therefore economic loss. The scale of the consequences will depend on the extent of disease spread. If the purchase of infected eyed eggs leads to introduction of a pathogen into a country or region previously free of the disease, the consequences, both direct through mortality and indirect through loss of trade, could be very high. Spread within an infected region to an uninfected farm may have important consequences for the particular farm but of little importance at a regional level.

Conclusion and recommendations

Risk assessment has most extensively been used to investigate the international spread of animal pathogens via the movement of animal commodities. The method recommended by the OIE, based on the Covello-Merkhofer approach was followed in the current study. The farm–level model addresses a commodity (i.e. fish eggs) based disease transmission question. The same scenario tree could be used to examine spread of vertically transmitted pathogens between regions or countries; however, at these levels input parameters will be less accurate and thus will need to be modelled with wider confidence limits. The fish level model addresses a different type of question, namely the effect of broodstock testing for risk management. In this paper the OIE guidelines were used, however, the NAS-NRC model (Anon, 1983) may be more appropriate for a quantitative analysis of the likelihood of vertical transmission with level of infection, which is effectively a dose-response association.

The work described in this report illustrates that quantitative risk assessment can be used to investigate the vertical transmission of fish pathogens and provides generic scenario trees which serve as a useful starting point for future risk assessments. The data requirements for a risk assessment of vertical transmission are clearly documented. The risk assessments discussed in this paper are most appropriate when used not just for a specific pathogen and host, but a particular farm or production system, since many of the parameter estimates vary greatly in different situations. Risk assessment is a tool for decision making and the usefulness of the methodology will be properly tested when it is used to support decision making in the management of vertically transmitted fish diseases.

On the basis of this report the following recommendations for risk assessments can be made:

- the hazard and the scope of the assessment should be described by a risk question
- the denominator for the study should be defined (i.e. a batch or consignment of eggs)
- a scenario tree should be constructed illustrating the steps necessary for the hazard to occur
- risk assessment should be used to prioritise future research into vertical transmission.

Recommendations for future research

In conclusion, we believe that future research on quantitative risk assessment for vertically transmissible fish diseases should focus on the following priorities:

- Quantification of the "internal infection ratios" of eggs or embryos, given relevant levels and courses of infection in parental fish.
- Quantification of sensitivity and specificity for relevant broodfish diagnostic techniques (again given relevant levels and courses of infection).
- Survival kinetics for internalised infection of fertilised eggs and externally contaminated eggs.
- Scenario trees for BKD and IPN in salmonids should be developed covering random mixing of eggs during incubation, and assessments covering the transmission scenario for nodavirus infections of relevant marine aquaculture species should be performed.

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