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Evidence Project Final Report

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Project identification

1. Defra Project code: F1190
2. Project title: Risks associated with imports of commodities and domestic commodity trade
3. Contractor organisation(s): Centre for Environment, Fisheries and Aquaculture Science, Weymouth Laboratory, Barrack Road, Weymouth, Dorset, DT4 8UB
4. Total Defra project costs (agreed fixed price): £ 422,668
5. Project: start date 01/04/2009
   end date 31/03/2013

6. It is Defra's intention to publish this form.

   Please confirm your agreement to do so.....**YES** ☒ **NO** ☐

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   In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

   (b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The overall objective of this project was to improve our understanding of the disease threats associated with the import of aquatic animal commodities to support policies to minimise the risk of pathogen entry via the commodity trade.

To achieve this, the project was structured into the following objectives

1. Identify hazards associated with import of aquatic animal products.
2. Review the risk of spread of pathogens through processing and sale.
3. Development of recommendations for bio-containment in processing plants.
4. Literature review on pathogen concentration in aquatic animal tissues.
5. Undertake experimental research to provide information in support of risk assessments.

Through delivery on these objectives, the project provided data on

- what products are being imported
- where they are going after passing the border
- how much pathogen these products may be carrying
- what risks may arise from further processing of product in processing facilities in England and Wales (E&W)
- and how risks associated with processing may be reduced.

Objective 1. Identify hazards associated with imports of aquatic animal products.

In order to assess whether exotic pathogens may be introduced into E&W from infected sources with commercial trade, it is essential to have data on the size of the import trade in susceptible species from infected areas. In the past, we have used Her Majesty's Revenue and Customs (HMRC) data, which are available publically online. However, these were found to be at a too summarised level for the purposes of Aquatic animal (AA) import risk assessments. Therefore, alternative data sources were investigated: Animals and animal products imported from third countries are subject to Border Inspection Controls. The trade flows are captured by TRACES (Trade Control and Expert System), a web-based veterinarian certification tool controlling the import and export of live animals and animal products to and from the European Union. The information kept by TRACES was identified as useful, but not complete enough in order to generate statistics of relevant commodities imported.

On arrival at the Border Inspection Posts (BIP), a consignment needs to be accompanied by a health certificate. These capture product state (raw or cooked) and species. However, health certificates are paper records and not transcribed into an electronic database. BIP data are limited to data on third country

imports, therefore not covering imports from EU member states.
HMRC hold data for EU imports. However, these rely on self declaration by the importers and only importers bringing in commodities above a value of £ 600,000.- per year are required to provide data, which is likely to lead to a misrepresentation of EU imports of AA. Other databases, such as FishStatPlus (FAO) or Eurostat rely on information provided by HMRC.
In conclusion: accessibility of reliable import data captured at a level suitable for risk assessments remains a problem. Suggestions for improvements are provided.

Criteria to assess the safety of aquatic animal (AA) commodities irrespective of the disease status of source populations were developed in collaboration with international scientists. This was done as part of an ad hoc Group called by the World Organisation for Animal Health, OIE, on the Safety of products derived from aquatic animals. The developed criteria are based on the amount of viable pathogen likely to be still present in the imported commodity, its eventual use and the amount of waste that is likely to be generated from the product. The ad hoc Group assessed all aquatic animal products listed in Articles X.X.3 and X.X.11./12. of all disease chapters of the OIE Aquatic code, based on data available in the peer reviewed scientific literature. This led to a revision of the lists of aquatic animal products that can be traded internationally irrespective of the disease status of the source populations.

Processing of AA products was recognised as one of the most critical steps where pathogen release may occur.

Objective 2. Review the risk of spread of pathogens through processing and sale.
Visits were undertaken to rainbow trout (RBT) farms in E&W to obtain information about on farm processing. In 2012, 28 farms had processing facilities on site. Most of these sourced product from UK sources or used only own stock. Where product is processed from external sources, a potential for bringing exotic pathogens on site exists. The risk of pathogen release was considered to be higher via liquid waste compared to solid waste. AA processing on fish farms carries a particular potential risk for pathogen establishment, due to the presence of high density AA populations often in the immediate vicinity of where processing takes place. Risk of pathogen spread via processing of domestically grown fish in the early stages following an exotic pathogen introduction was also assessed and it was concluded that on farm processing represents one potential pathway (amongst others) in the early phase of an epidemic.

Objective 3. Development of recommendations for bio-containment in processing plants
EU legislation on AA diseases (EU Directive 2006/88) provides that AA subject to disease control measures may be processed, if not displaying clinical signs of disease. However, this activity should not jeopardise the health status of farmed and wild AA and must only be undertaken in duly authorised processing establishments. Processing of AA generates liquid waste, which will be treated to a greater or lesser extent, before being returned into the aquatic environment. Since AA live in the aquatic environment, there is potential that pathogens released with liquid waste will infect farmed or wild AA. There are currently no facilities authorised in E&W permitted to process AA subject to control measures. To inform authorisation requirements for such facilities, the published literature was reviewed to identify physical or chemical methods suitable for the inactivation of fish pathogens in liquid waste. Ozone and heat treatment are in principal useful methods due to their potentially short treatment time. Conditions where an alkaline or acid pH treatment is used are likely to be less applicable due to the extended treatment times required. The available published literature on the efficacy of treatment methods for the relevant pathogens was very limited. Further work is required to provide specific recommendations for practical applications.

Objective 4. Literature review on pathogen concentration in aquatic animal tissues.
Literature reviews were undertaken to collate the published information on pathogen load, minimal infectious dose and the effect of some typical storage conditions, i.e. chilling and freezing for those diseases listed under Council Directive 2006/88.
The review highlighted that substantial quantities of viable virus can be found in fish tissues that would make up waste during processing (e.g. skin, head tissues) emphasising the potential risks associated with waste disposal. Freezing tends to preserve virus viability, which is highly relevant, given that the majority of fish product is imported frozen or chilled and could therefore potentially carry viable virus.
Published information for most of the molluscan parasites is very limited. However the information available suggests that if released, some of the parasites can survive for time periods which may be sufficient to reach a new host, provided the release is in the vicinity of potential host populations.
Amongst the crustacean pathogens, viral loads in imported product could be substantial. Of greatest concern were the potentially high loads of white spot syndrome virus that may be found in product imported for human consumption and the fact that such products may be used as angling bait.

Objective 5. Undertake experimental research relating to the risks associated with introduction and

spread of exotic pathogens to provide information in support of RA.
Experimental work was undertaken to determine the minimal infectious dose for viral haemorrhagic septicaemia virus (VHSV) in rainbow trout. The lowest concentration where infection was induced was at a Tissue Culture Infectious Dose 50 per ml of 0.8. This represents a relatively low virus quantity compared to infectious doses described for some other fish viruses. This means that small amounts of virus could induce infection. Market size fish appeared to be more susceptible to develop disease and die compared to small and medium-size fish exposed to similar challenge doses, which contrasts with previously published data.
In another study, where we investigated the effect of cold storage on VHSV titres in various RBT tissues, we found that both chilled and frozen product can still contain considerable virus loads, including tissues that would make up waste tissue.
In further studies, we investigated the effect of storage on viability of white spot syndrome virus (WSSV) in warm water shrimp tissues. Frozen infected shrimp were defrosted and stored for variable time periods at room temperature. Viable virus titres declined gradually over 24h at room temperature, but virus levels were still sufficient to induce infection in penaeid shrimp after 24 h. The data suggest that WSSV is likely to be still viable if infected warm water shrimp were used as angling bait.

The data obtained through this project significantly improved our understanding of the disease threats associated with imports of aquatic animal commodities and provide a sound basis upon which to develop risk mitigation strategies. A number of data gaps were identified, which could be addressed in future studies.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Exchange).

Introduction/ Background:

Countries or zones declared free from a disease will allow import of live aquatic animals (AA) of the susceptible species for aquaculture purposes only from other countries or zones of an equivalent disease status. Pathogens spread, however, despite the presence of such trade restrictions. Routes may be illegal live-AA movements, movements of AA incubating the disease or reared under environmental conditions where the disease was not apparent, or non-live AA movements. Trade of animal products, often called commodity trade, is a recognised risk for the spread of terrestrial animal diseases e.g. foot and mouth disease and classical swine fever. Commodity trade is also recognised as a possible route for spread of AA pathogens: pilchard herpesvirus is thought to have been introduced to Australia through the import of frozen pilchards for tuna feed; the myxosporidian parasite Myxobolus cerebralis is believed to have been introduced into North America with frozen fish; and WSSV is likely to have reached the US in frozen shrimp.
England and Wales experienced a number of introductions of exotic pathogens, where imports of fish products for human consumption and further processing have been or were likely to be associated with the introduction of the pathogens (e.g. outbreaks of viral haemorrhagic septicaemia (VHS) (2006) and sleeping disease (2002) in rainbow trout farms).

The overall objective of this project was to improve our understanding of the disease threats associated with the imports of AA commodities to inform future policies to minimise the risk of pathogen entry and release via the commodity trade.

To achieve this, the project was structured into the following objectives
1. Identify hazards associated with imports of AA products.
2. Review the risk of spread of pathogens through processing and sale.
3. Development of recommendations for bio-containment in processing plants.
4. Literature review on pathogen concentration in aquatic animal tissues.
5. Undertake experimental research to provide information in support of risk assessments.

Through delivery on these objectives, the project provided data on
- what products are being imported
- where they are going after passing the border
- how much pathogen these products may be carrying
- what risks may arise from further processing of product in processing facilities in E&W
- and how risks associated with processing may be reduced.

Objective 1. Identify hazards associated with imports of aquatic animal products

1.a Identification of data sources on commodity imports

Information on imports of AA into the UK is required to undertake import risk assessments (IRA). In order to assess whether pathogen may be introduced into E&W from infected sources, the size of the import trade in susceptible species from infected areas provides essential information. In the past difficulties were encountered in obtaining suitable data on volume of import of live animals and aquatic animal products imported for human consumption for IRA of various aquatic animal pathogens.
Data relating to import trade required when undertaking a risk assessment include:

In the case of live animal imports for aquaculture or human consumption
- Numbers of aquatic animals imported into the UK of a certain species (e.g. Common carp)
- Country of origin of these imports
- Destination within the UK

In the case of import of products for human consumption:
- Quantities (weight) of imported commodity
- Country of origin
- Degree of processing
- Condition of product (chilled, frozen)
- Destination within the UK
- Distribution chains within the UK

It was therefore explored, which data sources may be available for future IRAs.

There are a number of government bodies that capture import data for different purposes. The main 2 areas where data capture occurs are 1. for human and animal health purposes, and 2. for tax purposes. In previous import risk assessments, we have used data made available by Her Majesty's Revenue and Customs (HMRC). However, these were found to be at a too summarised level to be useful for crustacea and molluscs IRA.

Data capture for human or animal health purposes
Animals and animal products imported from third countries are subject to Border Inspection Controls. The trade flows are captured by TRACES (Trade Control and Expert System), a web-based veterinarian certification tool controlling the import and export of live animals and animal products to and from the European Union. The information kept by TRACES was identified as useful, but not complete enough in order to generate statistics of relevant commodities imported.

Border Inspection Posts (BIPs) were contacted to explore what information they hold that may complement information not captured by TRACES or HMRC data respectively. The data held by the BIPs are the most comprehensive of all. Most importantly, the health certificates or packing lists accompanying the consignments hold information on whether the product is raw or cooked, and of the species (information not available through other sources). A limitation of BIP data is that the BIPs only inspect live animals or products imported from third countries, therefore not covering imports from EU member states.

Data capture for tax purposes
Goods imported from third countries have to be declared to customs on arrival. These data are recorded and are made freely available in summarised format on the internet by HMRC. This data source has been used for previous import risk assessments. Data are reported using so called CN (Combined Nomenclature) codes.
The Combined nomenclature (CN) is a method for designating goods and merchandise which was established to meet the requirements both of the Common Customs Tariff and of the external trade statistics of the Community. The CN is also used in intra-Community trade statistics.
The CN is comprised of the Harmonized System (HS) nomenclature with further Community subdivisions. The Harmonized system is run by the World Customs Organisation (WCO). This systematic list of commodities forms the basis for international trade negotiations, and is applied by most trading nations. Each CN subdivision has an eight digit code number, the CN code, followed by a description.
Each CN subheading has an eight digit code number: the first six digits relate to the headings and subheadings of the harmonized system (HS) nomenclature; the seventh and eighth digits identify the CN subheadings.
Member States may insert further subdivisions after the CN subheadings for national statistical purposes, and after the Taric (online customs tariff database) subheadings for other national purposes. Taric subheadings extend the CN code subheadings by adding a further 2 digits (bringing the number of digits to a total of 10) and are currently used only for some commodities (e.g. TARIC code 0302 11 80 10: = 0302 Fish, fresh or chilled, excluding fish fillets and other fish meat of heading 0304; 0302 11 - Salmonidae, excluding livers and roes - - Trout (Salmo trutta, Oncorhynchus mykiss, Oncorhynchus clarki, Oncorhynchus aguabonita, Oncorhynchus gilae, Oncorhynchus apache and Oncorhynchus chrysogaster); 0302 11 80 Other; 0302 11 80 10 Of the species Oncorhynchus mykiss).

<u>CN codes for Aquatic animal products and live aquatic animals</u>
There are nine main 4 digit HS headings and in the order of four hundred 8 digit CN codes that cover the majority of aquatic animals or their products. The HS codes covering the majority of CN subheadings are shown in

Table 1.
Within each group, further subheadings exist.
Within the live fish group (0301), there are 2 main subgroups: Ornamental (CN code 030110) and “other live fish”. “Other live fish” (CN code 03019) is broken down further by groups of species or individual species (Trout, Eels, Carp, Bluefin tunas, Southern bluefin tunas) and “other” live fish. The purpose of import (human consumption or aquaculture) is not captured.
Amongst the non-viable fish categories (0302 – 0305), the system provides a reasonable breakdown with regards to the degree of processing. However, several fish species are usually collectively recorded under one CN code, which therefore does not allow analysis of datasets by species.
The classification of crustaceans and molluscs is highly summarised. There is a high degree of subsuming products of different degrees of processing and from various species within one 8 digit CN code. This has made the analysis of trade data based on HMRC data difficult for these animal groups in the past.
Further product groups captured under the CN codes are for example fish waste, dead aquatic animals unfit for human consumption, fish oil, extracts and juices from aquatic animals, and fish feed.

Table 1: HS codes of the most traded commodities of aquatic animals or their products

HS code	Description
0301	Live fish
0302	Fish, fresh or chilled, excluding fish fillets and other fish meat of heading 0304
0303	Fish, frozen, excluding fish fillets and other fish meat of heading 0304
0304	Fish fillets and other fish meat (whether or not minced), fresh, chilled or frozen
0305	Fish, dried, salted or in brine; smoked fish, whether or not cooked before or during the smoking process; flours, meals and pellets of fish, fit for human consumption
0306	Crustaceans, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; crustaceans, in shell, cooked by steaming or by boiling in water, whether or not chilled, frozen, dried, salted or in brine; flours, meals and pellets of crustaceans, fit for human consumption
0307	Molluscs, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; aquatic invertebrates other than crustaceans and molluscs, live, fresh, chilled, frozen, dried, salted or in brine; flours, meals and pellets of aquatic invertebrates other than crustaceans, fit for human consumption
1604	Prepared or preserved fish; caviar and caviar substitutes prepared from fish eggs
1605	Crustaceans, molluscs and other aquatic invertebrates, prepared or preserved

EU arrivals

HMRC also holds data for EU imports. However, HMRC is relying here on self declarations by the importers and only importers bringing in above a value of £ 600,000.- of commodities (from 1 January 2010, UK Tradeinfo team, pers comm.) are requested to provide a self-declaration. Therefore, EU internal trade data are less accurate compared to third country imports. Representatives of the aquaculture industry in E&W commented that this threshold would probably exclude many AA processors.
Other databases, such as FishStatPlus (FAO) or Eurostat rely on information fed in by HMRC. FishStatPlus also uses information provided by the industry and makes adjustments to harmonise data if 2 countries provide different figures for their mutual trade. Therefore, it appears to be advisable to use the original data to analyse trade data for imports into the UK.

Developing a database of BIP data

It emerged from the work described above that the greatest level of detail on the degree of processing and species imported, was captured in BIP records. Since this information cannot be retrieved from HMRC data and the absence of detail was found to place a high level of uncertainty on risk assessments undertaken to date, a database covering AA commodity import data for a 12 month period was constructed under a defra funded student project (F1172). This database is now used for IRAs and has been used to analyse commodity trade in species susceptible to Perkinsus marinus and Microcytos mackini (see risk assessments reported below).

Conclusions and recommendations

There are no easily accessible data sources that provide the required level of detail on quantity of trade for commodities potentially carrying AA pathogens. This may be improved if changes in the CN code system were made or if the UK made more use of the option of using subheadings to capture more information about imported commodities. However, such changes would have resource implications (extra time for capturing more detail of imported AA products) and may therefore not be practical at present.
A significant problem for understanding exposure risk is the limited data capture of intra-community (EU) trade. The majority of imports in live AA for human consumption and a substantial proportion of trade in non-viable raw AA products that may be carrying AA pathogens of concern are from intra-community trade. Because this trade per individual business is often at a relatively small scale, it will not require reporting to HMRC. This results in a significant lack of data capture of this trade and makes it very difficult to assess risks associated with such trade. Options for data capture should be explored.

1.b Description of types of commodities and 1.d Categorisation of commodity import by associated risk

This objective was addressed through collaborative work of the project manager with a group of international aquatic animal health scientists. The World Organisation for Animal Health, OIE, had called

an ad hoc Group on the Safety of products derived from aquatic animals and invited the project manager of this project as an expert.
The ad hoc Group developed criteria that could be used to assess the safety of aquatic commodities irrespective of the disease status of source populations, which were adopted by the World Assembly of Delegates (Chapter 5.3 of the Aquatic Code).
The criteria defining the safety of aquatic animal products are in short:

- Whether the pathogen is likely to be absent in the tissues imported even if the tissues are derived from an infected animal (e.g. the pathogen may not be found in fish skin)
- If the tissues imported are likely to initially carry the pathogen, whether the type of processing the product has been subject to is likely to inactivate the pathogen

If one of the above applies, the risk of pathogen introduction is considered negligible.

Furthermore, it was considered that products imported for human consumption carry a low risk, if

- They are prepared and packaged for retail trade **and**

either

- include only small amounts of waste tissues generated by the consumer

Or

- The pathogen is not normally found in the waste tissues generated by the consumer.

Following the adoption of the criteria by the OIE World Assembly of Delegates, the ad hoc Group assessed the aquatic animal products listed in Articles X.X.3 and X.X.11./12. of all disease chapters of the Aquatic code.
The assessments of commodities were based on data available in the peer reviewed scientific literature. The process followed the basic principles for undertaking an import risk assessment, in that the assessment process was based on scientific evidence and the information upon which the decisions for the assessments of individual products were made is transparent. The revised lists of aquatic animal products were adopted by the World Assembly of Delegates in May 2011. This lead to a revision of all disease chapters in the Aquatic Code and the changes are implemented in the current version of the Aquatic Code. The individual assessments will shortly be made available on the OIE website.
The pathogens listed by OIE (which also includes the pathogens listed under the Aquatic Animal Health Directive 2006/88) varied with regards to their tenacity towards processing procedures, for example temperature treatments: Most viruses are well preserved by freezing, whereas the fungal-like pathogen Aphanomyces invadans would be expected to be inactivated. Similarly, there are differences in the sensitivity of disease agents towards heat treatment or drying. The only type of treatment that is considered to safely inactivate any of the pathogens listed in Aquatic Animal Health Directive 2006/88 is heat sterilisation (121°C for at least 3.6 min in a hermetically sealed container). Therefore, it is not possible to provide a simple classification of products that applies across all notifiable disease agents and each commodity had to be assessed using pathogen specific information.
The knowledge generated through literature reviews undertaken under objective 4 and the experimental work within this project (objective 5) were highly relevant for the work of the ad hoc group.
The revised list of commodities considered 'safe' could lead to significant changes in international trade. An example of an important change is that eviscerated fish are no longer considered as safe by default, since significant amounts of potentially pathogen contaminated waste is generated during further processing.
The criteria for commodity assessments and the revised code chapters for the individual diseases are available at http://www.oie.int/international-standard-setting/aquatic-code/access-online/. The work of the ad hoc group was recently published in a peer reviewed journal (Oidtmann et al. 2013).

1.c Pathways and destinations of imported commodities

In 2007, the combined value of imported seafood was near 2 billion £. Following arrival, the commodities are initially handled by import agents, who organise transport to fish markets, directly to fish processors or to wholesale distributors. Following processing or sale at fish auctions, the product may go back to wholesale. The 2 main sectors eventually receiving the product are 1. catering and institutions out of homes (including fish & chips shops, schools, and restaurants) and 2. Retail to homes. Waste that is potentially carrying viable pathogen is generated mainly at the processing stage.
The following 3 main types of processors exist: primary, secondary and mixed. Primary processing includes cutting, filleting, picking, peeling, washing, chilling, packing, heading and gutting; Secondary processing includes brining, smoking, cooking, freezing, canning, deboning, breading, battering, vacuum and controlled packaging, production of ready meals. "Mixed" processors are processors who carry out processes from both of these categories. Primary processors are therefore the stage where most raw AA waste is generated.
In 2008, there were a total of 479 seafood processing plants (counted were only processing plants where 50% or more of the turnover was generated from processing of AA; number does not include salmon processors) in the UK (Brown, 2009). Of these about 280 were located in England and Wales, and the

region with the highest density of processing plants was the Humberside region (about 90 processing plants). The proportions of the various processor types were 46% primary, 12 % secondary, and 42% mixed.
In 2008 there were 18 salmon processing plants in England, Wales and Ireland. This compares to 53 in Scotland. Of all salmon processing plants in the UK, 23% undertook primary processing, 23% secondary processing and 54% mixed.
The majority of commodities processed in UK processing plants are imported, followed by domestic landings, and a far smaller proportion sourced from domestic aquaculture. There is a geographical focus of processing plants in the Humberside area. These mainly source their products through imports and mainly raw. The area with the greatest volume of shellfish processing was South/Midlands/Wales. Except for the South West of England, all areas were using direct imports as the main supply source.
The destinations of processed products include: Restaurants, Retail Freezer Centres, Retail Supermarkets, Food Service, Food service Pubs, Fish Flyers, Food Service Institutional, Retail Market Stalls, Wholesale Distributors, Wholesale Merchants, Processors, exporters and others (Brown, 2009).
It was found to be extremely difficult to obtain data on the distribution chain of specific commodities without investing substantial time, which was not within the scope of this project.

Objective 2. Review the risk of spread of pathogens through processing and sale.

The outbreak of VHS in a rainbow trout farm in the greater Ouse catchment in 2006 highlighted processing of aquatic animal products as one of the potential pathways of introduction of exotic pathogens. Processing facilities were present on an upstream rainbow trout farm and a nearby smokery, located upstream near the river. The source of introduction could not be fully established, but introduction via AA products was considered a likely pathway.
There are currently in excess of 400 AA processing facilities in E&W (Cefas data). Quantities of AA commodities imported into the UK in 2012 from third countries and EU MS combined are in the order of 500,000 tonnes of product with a value of 1.6 billion pounds (HMRC data, based on commodities starting with CN code 03). The total amount of AA purchased by AA processors amounted to 570,000 tonnes (Brown, 2009; data for 2008), which is made up by both domestic landings, animals produced in aquaculture and imported product. If AA products were purchased by the processing industry in proportion to their availability on the market, approximately 60% (or approximately 335,000 t) of processed product is from imports. In the main, processing takes place in dedicated processing facilities away from fish farms. However, there are (as of 2012) 28 fish farms that carry out processing on farm, including two of which process AA products from non UK sources.
We investigated current processing practices on rainbow trout farms in E&W, developed a database of establishments handling fresh fishery products, assessed bio-containment at processing sites away from fish farms, and assessed the risk of pathogen spread during the early stage of an exotic pathogen introduction due to processing.

On farm processing
As of December 2012, there were just over 300 authorised fish farms in E&W of which 28 had aquatic animal processing facilities on site. A study investigating on site processing practices in E&W was undertaken to allow a better estimation of the risks associated with this activity. During statutory visits, Cefas Fish Health Inspectorate obtained information from fish farmers about their processing activities. Twenty-one farms undertaking processing on site participated in the study. Two of these farms processed AA product from outside of the UK.
Risk of pathogen introduction onto fish farms arises where AA for processing are sourced from external sources (i.e. not grown on site). Farms sourcing AA products from outside of the UK are of particular concern: E&W currently enjoy a very high health status for fish diseases (i.e. are free from most of the notifiable pathogens). However, VHS and IHN are endemic in several European countries. Where a fish farm sources fish from outside E&W, there is a risk that such fish carry notifiable fish viruses. There are no requirements with regards to the aquatic animal disease health status of such animals, as long as the animals are eviscerated and did not show signs of disease. As a result, infected fish can be brought onto fish farm sites for further processing (e.g. filleting or smoking). Processing on farms is mainly of raw product and the types of processing undertaken generate mainly waste that is in a raw state (not heat treated). As a result, the solid and liquid waste may contain viable pathogen.
Solid waste disposal is generally well regulated. As long as processors store the solid waste safely (e.g. avoiding access by vermin) the risks associated with solid waste are considered extremely low. However, in 2 cases, solid waste went to exceptional destinations (lobster pot bait and into a private sewage treatment facility, which discharged into the adjacent river). These exceptional routes are of concern because potentially pathogen carrying solid waste is introduced into the aquatic environment, leading to potential exposure of susceptible species to these pathogens.
Processing of AA generates liquid waste (e.g. washing water runoff from cleaning fish carcasses), which will be treated to a greater or lesser extent, before being returned to the aquatic environment. Since AA

live in the aquatic environment, there is potential that pathogens released with liquid waste will infect farmed or wild AA. There are currently no requirements to inactivate AA pathogens with liquid waste released from processing facilities (unless these facilities are for the purpose of processing AA subject to disease control measures). Two farms discharged liquid waste into the nearby river – either with or without treatment. This may have limited or no disease implications as long as the processing units only source from the own site. However, processors can choose where to source fish from and therefore this risk may change considerably if such sites were processing fish from non-UK sources. The other destinations of liquid waste are in the main septic tank or public sewer. Insufficient data are available at present to assess whether pathogen would be sufficiently inactivated by such treatment.
AA processing on fish farms carries a particular potential risk for pathogen establishment, due to the presence of high density AA populations often in the immediate vicinity of where processing takes place (Oidtmann et al. 2011d).
Given the species processed and the farms where processing takes place, the main pathogens of concern are VHSV and IHNV.
Information obtained under this and other objectives of this project was used for an import risk assessment of the likelihood of introduction and establishment of VHSV genotype 1a in E&W via the processing of imported rainbow trout carcases from continental Europe (Pearce et al, in press).

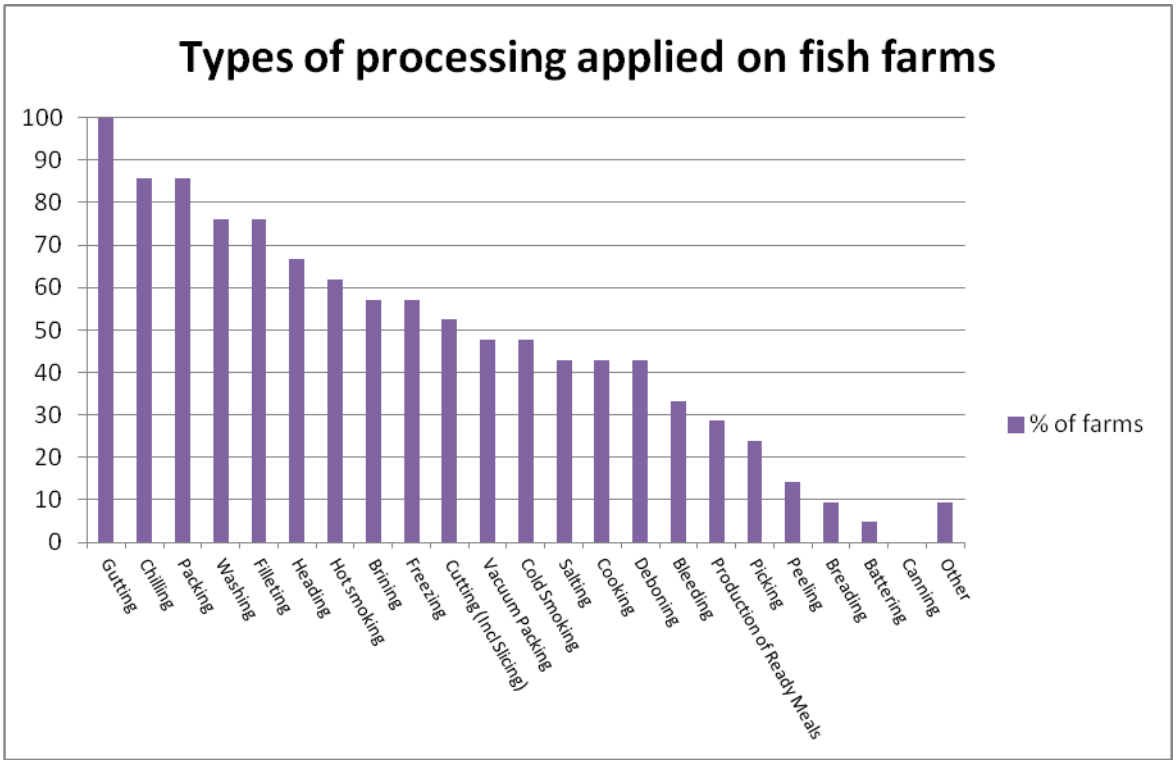

Figure 1: Types of processing undertaken by processors located on fish farms. Results are shown in %. Data are based on 21 on farm processors.

It is important to keep awareness high amongst farmers that operate on-farm facilities. It might be advisable to include the provision of information about the sources of aquatic animals for processing as part of the farm authorisation with farmers notifying the CA should they start sourcing AA products which are likely to have been grown / harvested outside England and Wales. An alternative might be that fish farmers commit themselves to sourcing from safe sources only (industry self-regulation). Farms sourcing AA from non-UK sources are a relevant potential pathway for introduction of exotic pathogens. The origin of AA used for processing in on-farm facilities should be monitored.

Risk of pathogen spread via processing of domestically grown fish in the early stages following an exotic pathogen introduction
In the event of an introduction of an exotic pathogen or the emergence of a new disease, fish will initially continue to be processed on farm or off site until the disease has been identified and control measures put in place. It is important to understand where domestically grown fish are processed and assess whether pathogen release into the aquatic environment may occur from such sites, since this presents a potential pathway for pathogen release in the early stage of a disease outbreak. Contact tracing following the identification of a notifiable pathogen traditionally focused on live fish movements to other fish farms or for restocking; historically the potential release of pathogen via processing waste has received little attention.
A significant part of the domestic (E&W) annual rainbow trout (RBT) production is processed in Scotland. Based on data from 2011 and 2012, six on-farm processing facilities processed RBT from other RBT

farms in E&W. Two of these sites process approximately 1300 t pa combined (representing about 20% of all rainbow trout production in E&W). The quantities processed on other farm sites were small in comparison (2-16 t pa). Through web-searches and information provided in industry directories, a further 24 RBT processing facilities not located on farms were identified. However, the number is likely to be higher, since there is no obligation to provide information about the fish species processed.
Farms receiving fish from other farms for processing may hold the fish live prior to processing. Bringing fish on site live for processing generally carries a higher likelihood of pathogen spread compared to transfer of dead fish due to the fact that pathogen can continue to amplify in the fish host and be released into the rearing water and transport stress increasing the chance of development of clinical disease.
Based on the presently available information, the risk of processing on farm sites is clearly higher compared to the risk associated with processing in farm-independent processing facilities. On site processing takes place in close vicinity to high density susceptible fish populations (i.e. the fish reared on site). Therefore, exposure of a susceptible fish population on site to pathogen is far more likely. Risk of introduction increases with the number of sources from which fish are processed on site. Once established in the farmed fish population, further spread from this site depends on connectivity with open water courses and movements on/off site.
However, it would be useful to obtain a more comprehensive picture of where processing of domestically grown RBT or other salmonid species takes place.

Identification of processors (not on-farm sites) in E&W
Substantial difficulties were encountered during the lifetime of the project in identifying processors of aquatic animals. Eventually, a database was established covering processors listed on the FSA database and the Fish Industry Directory. The information captured includes full business address, GIS location, and – where the information was easily accessible during the websearch – information about species processed was also recorded.
This database will be used in another Defra funded project (FC1203) to plot location of processors of AA products relative to open water courses and the sewage network in the form of regional case studies and should allow to further assess the potential for pathogen release and exposure.

Objective 3. Development of recommendations for bio-containment in processing plants.

The economic consequences of an introduction of a notifiable aquatic animal pathogen onto a farm can be severe. On detection, control measures are applied, which often require the destruction of the stock on site. Therefore, the financial impact of the control measures for the farmer could be reduced, if the farmed stock not showing signs of clinical disease was slaughtered and processed for human consumption. None of the notifiable aquatic animal diseases are zoonotic; therefore, there is no health risk per se for the consumer arising from the detected aquatic animal pathogen.
Directive 2006/88/EC provides that in the case of confirmation of notifiable diseases in aquaculture animals, harvesting and further processing can be allowed from farms put under official controls, if such aquaculture animals are processed in authorised processing plants. The slaughter and processing must be undertaken in a way which does not jeopardise the health status of farmed and wild aquatic animals. This includes the discharge of effluents.
We assessed selected physical or chemical treatment methods for their efficiency in inactivating the fish viruses listed under Directive 2006/88 with specific focus on the treatment of liquid waste. This was done by reviewing the peer reviewed literature and use of grey literature. Due to the dearth of information for the listed viruses, infectious pancreatic necrosis virus (IPNV), which is not listed in the EU directive, but is known to be a resilient virus, was also included in the review. Information was often not available for inactivation of the virus in liquid waste. Therefore also published data on the effect of treatment of water or solid waste was reviewed. Methods effective for solid waste were expected to also be effective on liquid waste (following evidence that physical and chemical treatment methods in a high humidity / wet matrix tend to be more efficient; e.g. moist vs. dry heat sterilisation). An evaluation of the environmental impact or costs of alternative treatment methods were not within the scope of the review.
UV: A fluence of a minimum of 80 J m^{-2} should inactivate VHSV, IHNV and ISAV, but not IPNV, in waste water from a fish processing plant where the liquid waste is not very turbid. The advantage of the method is that no harmful by-products are produced, but the process requires relatively clear liquids to be successful. Adequate penetration of UV into solid wastes and very turbid liquid waste is unlikely.
Alkaline pH: pH 12-14 for a minimum of 24 h should inactivate ISAV (a minimum of 48 h for IPNV) in a fish homogenate. The method is effective on solid wastes but would require a contact time of up to 2 days. Disposal of the treated material could be problematic.
Acid pH: pH 3.5 – 4.3 (obtained with organic acids) for a minimum of 24 h should inactivate IHNV and ISAV in fish silage or a fish homogenate. IPNV is only likely to be inactivated at ≤ pH 1.5 for 24 h or longer. There are no data on the inactivation of the viruses in processing plant waste at low pH obtained

using inorganic acids. However, from studies using other matrices, inorganic acids should inactivate the viruses faster than in material acidified with organic acids. Like with alkaline pH treatment, a long contact time is required.
Heat: ≥55°C for at least 10 min should inactivate IHNV and ISAV in fish homogenates. The Community Reference Laboratory for fish diseases uses 120°C for 2 min for aquarium water, and EFSA (2011) has concluded that heating a fish homogenate at ≥85°C for at least 25 min should inactivate fish pathogens. Relatively short contact times are required, even for treatment of solid waste.
Ozone: Data are only available for inactivation of ISAV in diluted tissue homogenates, and those results are only qualitative. In such a matrix ISAV was inactivated in 4 - 10 min at 8 mg min L^{-1} ozone.

Based on the information obtained, a method (chemical or physical treatment) suitable for use in the UK could be selected in consultation with stakeholders. Further research would be required to determine the parameters necessary for inactivation of VHSV, IHNV, ISAV, EHNV and KHV in fish processing waste since existing data are rather sparse.
Alternatively, as heat treatment of processing waste appears to be a good contender as a suitable method, a risk assessment of that method could be done, utilising information on sterilisation/ pasteurisation methods.

Although the main objective of the review was to assess physical and chemical treatment methods for their potential application to liquid waste, the review also provided useful information on the efficacy of solid waste treatments. UV and ozone treatment are largely methods restricted in their application to liquid waste treatment, heat and treatment with alkaline or acid pH can be in principal applied to both liquid and solid waste.

Objective 4. Literature review on pathogen concentration in aquatic animal tissues.

The potential risk of pathogen release from imported aquatic animal products depends on a range of factors: the pathogen load in the product, the treatments that the products have been subjected to prior to import and the expected amount of waste generated from the product and the pathogen load in such waste.
Aquatic animal products imported for human consumption may be imported in a wide range of processing stages: live, raw, cooked, pasteurised, frozen, chilled, dried to just name a few.
Each of the pathogens listed under Directive 2006/88 has specific biological characteristics, including how well they withstand exposure to different physicochemical treatments. Furthermore, the pathogen load in infected susceptible hosts as well as the minimal infectious dose may vary depending on species.
Literature reviews were undertaken to collate the published information with regards to pathogen load, minimal infectious dose and the effect of some typical storage conditions, i.e. chilling and freezing. The information will be used for future risk assessments and can be updated as new information becomes available. The reviews covered all diseases listed under Directive 2006/88.

4.a Review of pathogen load in fish
In general, there was very limited published information on pathogen load in tissues imported for human consumption, such as muscle tissue. Studies that did investigate pathogen loads in tissues often focussed on internal organs.
For some of the diseases (e.g. epizootic haematopoietic necrosis (EHN), infectious haematopoietic necrosis, infectious (IHN)), there was qualitative information as to whether or not pathogen can be found in certain tissues, but no quantitative data. No information on whether the pathogen occurs in muscle tissue was available for IHN, KHVD, EHN and SVC. *A. invadans* and ISAV are known to occur in muscle tissue; but no data were found on pathogen load.
The pathogens for which the most comprehensive data were available, are IHNV and VHSV. The viruses cause diseases mainly in salmonid species, and have occurred in Europe for several decades. However, even for those diseases, there was no or very little information on pathogen load in muscle tissue in the published literature. A recent Cefas study has substantially contributed data on pathogen load for VHS (Oidtmann et al. 2011a).
Virus titres generally appeared to be highest in mortalities followed by clinically affected fish. Data on pathogen titres in non-clinical fish are only available for a limited number of diseases (e.g. IHN, VHS). Virus titres for IHN and VHS ranged from 10^1 to 10^{10} $TCID_{50}$ or PFU g $tissue^{-1}$. The maximum titres found in SVC affected fish were in the order of 10^8 $TCID_{50}$ g^{-1}. The highest virus titres were usually observed in internal organs. In survivors, virus titres gradually decline until eventually virus can no longer be detected. In all of the viral diseases reviewed, virus was found in both brain and gills. The viral titres in these tissues were not available for all viral diseases, but where known could be substantial, ranging from just above the level of detection to 10^9 $TCID_{50}$ g^{-1}.

An important finding is that in cases where researchers tested virus presence in skin or mucous, relevant levels of virus were detected (IHNV, KHV). This applies not only for the clinical disease stage but also during incubation and in survivors. The skin appears to be one of the main target tissues for KHV replication. The results from the diseases where skin or mucous was tested would suggest that skin or mucous is likely to harbour pathogen also in the other diseases.
The review on viability of pathogens following freezing or when stored at cold temperatures shows that freezing appears to reduce virus titres. For the diseases where data were available, the drop in titre was usually in the order of 2-3 log10. The length of freeze storage appeared to have little effect and virus was still detectable several months after freezing. Storage at around or just above 0°C lead to a decline in virus titres, resulting eventually in undetectable levels after 3 days up to several weeks.
No data were available for the effect of freezing on *A. invadans*. However, data from a closely related Aphanomyces species (*A. astaci*) would suggest that storage at -20°C eventually kills the pathogen after a currently unknown time period.
The length of survival of *A. invadans* if tissues were stored at around or just above 0°C is difficult to predict. However, it is expected that the pathogen is likely to survive for several days.
The review highlighted that substantial quantities of viable virus can be found in fish tissues that would make up waste during processing (e.g. skin, head tissues) emphasising the potential risks associated with waste disposal. Freezing tends to preserve virus viability, which is highly relevant, given that the majority of fish product is imported frozen or chilled and could therefore potentially carry viable virus. The review also highlighted existing data gaps with regards to the effect of freezing and cold storage on viable VHSV titres. Work to address this data gap was undertaken under objective 5 of this project.

4.b Reviews of pathogen load in crustacean tissues
White spot disease
A wide range of crustacean species are susceptible to White spot syndrome virus (WSSV). These include the main species of tropical decapod crustacea farmed for human consumption (warm water shrimp) and several decapod species found in England and Wales. Data on pathogen load were available for several of the major penaeid shrimp species farmed for aquaculture and for one crab species. Most data are based on experimental infection, but some data were available for farmed or wild shrimp. Due to the unavailability of immortal cell lines to determine viral load of viable virus, quantitative PCR was the main method used for quantification. The viral loads measured in shrimp at the onset of mortality events were extremely high (in the order of 10^9 to 10^{10} copy numbers/gram of tissue). In a farm setting, the onset of increased mortalities will often trigger emergency harvests. Therefore, shrimp obtained from emergency harvests are likely to carry substantial concentrations of viral particles.
Viral load did not vary greatly with tissue type. The WSSV load in wild crustaceans, farmed crustaceans not undergoing a mortality event, or survivors of a mortality event were significantly lower (usually by multiple logs).
The minimal infectious dose (MID) for oral infection of naıve penaeid shrimp appears to be in the order of 100 copies detected by real-time PCR (Oidtmann & Stentiford, 2011). Copy numbers that can be found per mg of tissue in shrimp from experimental studies imitating emergency harvest exceed by several log10 the MID identified for tropical shrimp by oral route.
The majority of panaeid shrimp are imported into England and Wales frozen. There are currently no studies that have investigated the drop in viable virus titres following freezing or cold storage. However, transmission of WSSV from previously frozen infected shrimp to naive shrimp has been demonstrated in experimental studies. In summary, the literature review has demonstrated that WSSV load in emergency harvested shrimp can be substantial and that the virus can survive freezing, although the effect on decrease of viability still remains to be investigated. The review was published in a peer reviewed journal (Oidtmann and Stentiford, 2011).

Taura Syndrome (TS)
Natural infections with TS virus (TSV) mainly occur in penaeid prawns of commercial importance. A number of tropical panaeid shrimp species have also been shown to be susceptible by experimental challenge. In addition, some crab species are susceptible to infection without developing clinical disease and therefore may act as carriers of infection. To date, TSV virus has not been described in cold-water species. Although infection associated with mortalities mainly affects juvenile shrimp, infection can also be found in adults. Shrimp can remain carriers of TSV for prolonged periods.
The published literature on TSV pathogen load in crustacea is still relatively limited. The data mainly rely on quantitative PCR assays. Viral loads in emergency harvested shrimp may be very high (between 10^8 and 10^{10} copy numbers/g of tissue). Virus levels are still relatively high in survivors, meaning that the pathogen load in survivors may be sufficient to induce transmission. Equally, high amounts of virus may be present in other crustacean species exposed to TSV without the animals developing disease, as has been demonstrated in crabs.
Data on the viability of TSV following freezing are limited and are mainly reported for -80°C. However, in general, freezing is used to preserve virus (keep it viable).
Although transmission of TSV has largely been associated with the transport of live shrimp, the data

clearly suggest that commodity shrimp would present a possible pathway for pathogen transfer as has been previously suggested.
Given the current limited susceptible species range of TSV, the disease is not considered as much of a threat as white spot syndrome virus, for which the susceptible species range is known to be broad and includes species native to European waters. It is currently unknown, which wild and farmed species in the UK may be susceptible to TSV and whether the virus is able to replicate at ambient temperatures prevailing in UK waters. European Member States with susceptible marine species including farmed and wild penaeid shrimp (e.g. Penaeus japonicus, P. kerathurus, P. semisulcatus, Metapenaeus spp.) are likely to be at higher risk from introduction and establishment of TS than states bordered by marine waters in Northern Europe and landlocked states (containing only freshwater hosts) in Central and Eastern Europe.

Yellowhead disease (YHD)
Several tropical shrimp species have been shown to be susceptible to viruses in the Yellowhead complex; however, with the exception of P. japonicus, no temperate water species have been specifically tested for susceptibility.
Copy numbers of virus in survivors of an YHD outbreak are in the order of 10^4 copies per ng RNA (determined by quantitative PCR) and are therefore at levels similar to those found in TSV infected shrimp. The virus remains viable following freezing, which has been repeatedly demonstrated since passage trials to naïve hosts utilise material previously stored frozen. However, studies investigating the potential drop in viable virus titres as a result of freezing or cold storage are missing. Several studies have demonstrated the presence of viable Yellow Head Virus in imported commodity crustaceans by demonstrating transmission from such material to naïve susceptible species.
Since studies on the susceptible species range of YHD are currently largely limited to tropical shrimp species, it is currently difficult to assess whether the import of commodity shrimp carrying viable virus presents a threat for UK crustacean species.

Of the 3 pathogens listed by EU Directive 2006/88, WSSV is clearly of greatest concern due to its wide susceptible species range, which includes species native to E&W. Several pathways for pathogen introduction have been identified (Oidtmann et al. 2012). The most direct pathway would be via use of infected shrimp as angling bait. The viral load would be likely to be sufficient to cause infection in a native species, if a native crustacean species ate infected shrimp tissue – especially if the shrimp were from an emergency harvest. To assess the viability of WSSV following freezing and thawing, in vivo trials were undertaken, which are reported under objective 5.

4.c. Reviews of pathogen load in mollusc tissues
With the publication of Council Directive 2006/88, five mollusc diseases have become notifiable. At the time of publication of the Directive, three of the mollusc diseases were listed as exotic to the EU and two as non-exotic. Since then, one of the formerly exotic diseases (B. exitiosa) has been detected in four European countries, including England. Another mollusc disease (infection with Oyster Herpes virus), which had not been listed in the Directive, has emerged in 4 European countries and was detected in 2010 in England. Although the route of introduction into England remains to be resolved for both cases, introduction via live shellfish imported for human consumption is clearly a possible route, demonstrating the risks for transboundary disease spread via international trade.

Although studies have been published reporting on the tissue types in which the various parasites have been found, data on parasite load are very limited. The only study identified to report on pathogen load is for Perkinsus marinus: Following experimental exposure of oysters to P. marinus, individual hosts were found to contain between 10^3 and 10^6 parasites per gram of wet weight of oyster tissue.

Data on the minimal infectious dose are largely missing for the listed diseases. One study exists on B. exitiosa which mentions that exposure of oysters to 100 parasite cells via injection leads to infection. Another study estimates that between 10 and 100 Perkinsus marinus cells are required to instigate a new infection in a naïve host.

Based on Border Inspection Post data, the majority of mollusc products imported from third countries are imported raw and frozen. Imports from European countries are mainly live and chilled. Although temperatures used for chilling may be below the temperatures found in the natural habitats of the imported susceptible species, water temperatures in areas where some of the pathogens naturally occur can be 10°C or lower; therefore, chilling of live animals would be expected to have little effect on the viability of the parasites. The limited information that is available (some publications have investigated viability of some of the relevant parasites at 4°C), supports that they tolerate chilling at least for limited time periods. Data on the effect of freezing are available for a parasite closely related to M. refringens, called M. sydneyi. Following freezing for 220 days, spore viability was reduced to 5.8% when frozen at -20°C and

3.5% when frozen at -70°C respectively. After being frozen for 7 days, viability was reduced to around 20%. It is probable that similar results would be obtained for M. refringens. In vitro experiments show that freezing of meronts of P. marinus cells for 30 minutes at -80°C and -20°C lead to a reduction in the percentage of viable cells from 93.6% to 54% and 92.7% respectively. No data on the effect of freezing are available for Bonamia spp. or Microcytos mackini.

Given that the majority of molluscs imported from other EU MS are imported live and chilled, there is a clear route by which viable parasites infecting mollusc tissues can be imported. The literature reviews also provide information on the viability of the parasites outside of the mollusc host. Although the information for most of the parasites is very limited, it still suggests that if released, some of the parasites can survive for time periods which may be sufficient to reach a new host, provided the release is in the vicinity of potential host populations.

The recent outbreaks of previously exotic mollusc diseases in England (B. exitiosa and new variant oyster herpes virus) have clearly highlighted the risks associated with commodity trade.

Further studies on the effect of freezing and storage for some of the reviewed pathogens would be useful to assist with future import risk assessments.

Import risk assessment for molluscan pathogens

Qualitative risk assessments were carried out to investigate the likelihood of introduction and establishment of *Perkinsus marinus* and *Mikrocytos mackini* in England and Wales due to import of live molluscs for human consumption and for aquaculture purposes. The guidelines for Import Risk Analysis published by the World Organisation for Animal Health (OIE) were followed (http://www.oie.int/en/international-standard-setting/aquatic-code/). Trade pathways for the import of live molluscs for human consumption and aquaculture purposes were identified and illustrated. The steps necessary for the introduction and establishment of *P. marinus and M. mackini* via those trade pathways were identified and individually assessed against a list of defined data requirements. The assessment was first undertaken at the level of an individual consignment and in a second step extrapolated taking into account the current level of trade in the susceptible species.
P. marinus and *M. mackini* are listed in current European Community aquatic animal health legislation (2006/88/EC) as being exotic to Europe and detection has never been confirmed in this region.
The main route for potential exposure of susceptible species in coastal waters in the UK to *P. marinus* or *M. mackini* was identified to be waste disposal generated by end-consumers or waste that might be released from transport vessels into the marine environment. The risk associated with this route was assessed as being extremely low.
There was a considerable level of uncertainty associated with several of the steps assessed. This relates for example to information on transmission parameters (e.g. minimal infectious dose, the survival of the pathogen in the marine environment).

<u>Results specific to *Perkinsus marinus*</u>
The trade data on imports for human consumption into the UK show that since 2009 there have been no imports of susceptible species from any of the countries (USA, Mexico, Brazil, Cuba, Puerto Rico, Venezuela, French Polynesia and Hawaii) where this pathogen is either confirmed or suspected to be present. From the USA, up until 2009 the quantities imported were relatively small (usually considerably less than 50 tonnes per annum). From the other countries there has been no recorded trade (data available from 1996 onwards).
There is no evidence to suggest that the native and commercially exploited listed susceptible species in the UK would be severely affected if they became infected with *P. marinus*.
The overall trade in imports of molluscs for aquaculture purposes (deposit) is extremely small and therefore easily monitored and controlled. Currently there is no trade from outside the EU in any of the species known to be susceptible to *P. marinus*. Historically, there have been imports of a susceptible species (*Crassostrea gigas*) for aquaculture for a limited period from an area free of the disease but present elsewhere in the known infected country (USA). This trade was closely monitored and sampling at the time and subsequently indicate that no diseases were introduced.
There are several crustacean vector species for *P. marinus*. Trade of live animals in this group from known infected countries is minimal. It appears that mollusc species have accidentally been left out of the listed vector species – both in Directive 2006/88/EC as well as in a report on the subject by EFSA. There is currently no trade in live molluscs for aquaculture from known infected countries. However, trade needs to be monitored.
Imports of live bivalve molluscs into the EU are currently not allowed from the USA but negotiations may lead to a resumption of trade in susceptible species for human consumption from this source. Any

significant increases in trade, together with any changes in trade for aquaculture purposes, may trigger a review of the risk assessment.

Results specific to *Mikrocytos mackini*

The trade data on imports for human consumption into the UK show that since 2009 there have been no imports of susceptible species from the two countries (Canada, USA) with this pathogen. Up to 2009 the quantities imported were relatively small (usually considerably less than 50 tonnes per annum).

Currently there are no imports from outside the EU in any of the species known to be susceptible to *M. mackini.* Historically, there have been imports of a susceptible species (*Crassostrea gigas*) for aquaculture for a limited period from a disease-free compartment in an infected area. This trade was closely monitored and sampling at the time and subsequently indicate that no diseases were introduced.

In September 2009 imports of live bivalve molluscs into the EU were approved from Canada. They are currently not allowed from the USA but negotiations may lead to a resumption of trade in susceptible species for human consumption from this source. Any significant increases in trade, together with any changes in trade for aquaculture purposes, may trigger a review of the risk assessment.

Conclusions

There is no need for immediate action to mitigate against potential risks of introduction of *P. marinus* or *M. mackini.* However, due to the limited information available to assess some of the steps in the biological pathway, the risk assessment carries a high level of uncertainty.

Other potential routes of introductions were identified, such as introduction of the pathogen via ballast water discharge, and translocation of susceptible species through pathways other than commercial trade in the susceptible species, (e.g. hull fouling, co-transport of susceptible species in consignments of non-susceptible species, illegal movements of molluscs from infected areas into disease free areas). These are potentially relevant, but were outside the scope of the risk assessments.

Objective 5. Undertake experimental research relating to the risks associated with introduction and spread of exotic pathogens to provide information in support of RA.

Determining the minimal infectious dose for viral haemorrhagic septicaemia virus (VHSV) in rainbow trout

For this project, we undertook experimental work to determine the minimal infectious dose (MID) of VHSV for rainbow trout of different sizes: fry, 6.5g; medium size, ca. 80g; and market size, 285g. All fish had been hatched from eggs from a single supplier, and grown on in the Cefas lab facilities. The fish were exposed in groups of 30 to one of three concentrations of VHSV via bath challenge, plus control groups. There were two groups (replicate tanks) per challenge. The isolate used was the English 2006 VHSV, which is considered to be highly virulent. Challenge doses, measured as Tissue Culture Infectious Dose 50 per ml (TCID50 ml-1), were 0.1, 1 and 10 for fry, 1, 10 and 100 for medium size fish, and around 0.8, 8 and 86 for market size fish.

Over the 6 weeks observation period, only one of the fry groups had mortalities. Twenty eight of 30 fish died in one of the two groups challenged with the highest dose. All fry from any of the other groups sampled after 3 and 6 weeks tested negative by tissue culture.

In the medium size fish, mortalities occurred in both groups at the highest challenge, in one group at the low challenge, but none in any of the other groups. In groups where mortalities occurred, more than 15 fish had died after 3 weeks. After 6 weeks, most fish had died in both high dose groups (23 and 27 respectively), and 25 in one group exposed to the low challenge dose. Virus was detected in some, but not all, of the survivors.

In the market size fish, mortalities were observed in all groups (low to highest concentration). Fifteen or more fish had died after 3 weeks in both high challenge groups and one of the medium challenge groups, and 12 had died in the other medium challenge group. In one of low challenge groups 2 fish had died and 12 out of 13 fish tested positive. In the other low challenge group, no mortalities had occurred after 3 weeks and none of the 15 fish sampled after 3 weeks tested positive (Figure 2).

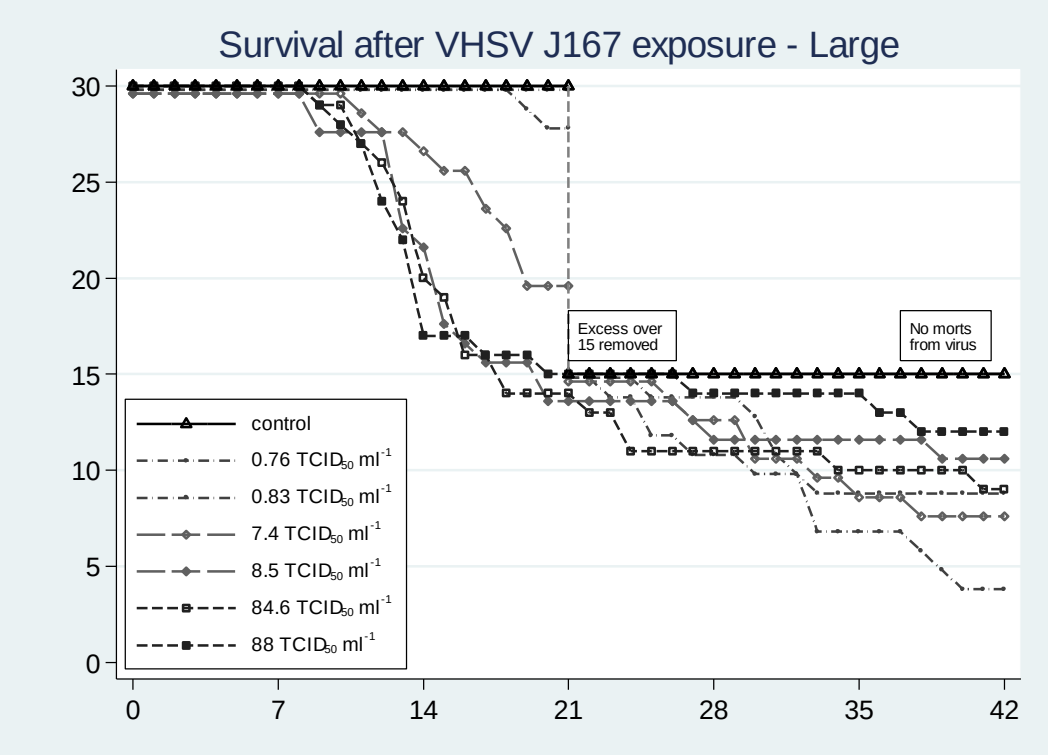

Figure 2. Number of large rainbow trout surviving following immersion challenge with VHSV. On day 21, fish were removed from any tank with more than 15 survivors, leaving a maximum of 15 fish in each group for the next 21 days.

The results from the challenges were surprising and inconsistent: It appears that market size fish were more susceptible to develop disease and die compared to small and medium-size fish exposed to similar challenge doses. This contrasts with previously published data, where fry are normally considered to be the most susceptible fish size with the highest mortality rates (OIE diagnostic manual). Furthermore, in the medium size fish group, the results were not monotonic with dose, i.e. fish died in one of the low challenge group, but not in the groups exposed to the middle virus concentration. In groups where no mortality occurred, virus was not detected. Taken together, it appears that at low virus concentrations the initial infection of individual fish is a stochastic process (i.e. subject to chance). Further work needs to be undertaken to substantiate these findings.

Effect of storage and freezing on VHSV in rainbow trout tissues

In a previous study, we established virus concentrations per gram of tissue present in infected market-size rainbow trout at different stages of infection: before development of clinical signs; in clinically affected fish; mortalities; and survivors immediately after killing by schedule 1 method (Oidtmann et al. 2011a). The virus concentrations were found to be substantial and sufficient to infect rainbow trout fry immersed in tissue homogenates from such fish (Oidtmann et al 2011b).

Rainbow trout carcasses are imported either chilled or frozen. No data at all are available on the loss of infectivity due to storage or freezing in tissues imported for human consumption, such as muscle tissue, head tissues or skin in any species susceptible to VHSV.

Market size rainbow trout were challenged with VHSV by immersion. When first mortalities occurred in a group, fish not showing clinical signs were removed, killed by a schedule 1 method (concussion of the brain by striking the cranium followed by exsanguination) and brain, gills, muscle, skin, mucous, and internal organs samples collected. The tissue/mucous samples were apportioned and one portion submitted to virus titration without additional storage. The remaining portions were stored under defined conditions imitating commercial conditions until processed for titration. Twenty fish were sampled in this way.

In order to assess whether storage in portions rather than of whole fish carcases affects the virus titres measured, a further 20 fish were processed in a different way. The fish were killed by the schedule 1 method described above and gutted. 1 g of muscle tissue was sampled and titrated without additional storage. The fish carcases were then packed individually into plastic bags and frozen. After defined time periods, the carcasses were thawed. The thawed run-off water was collected from the freezer bag and titrated. 1 g of muscle tissue was sampled and submitted to titration. The results are shown in Table 1.

Table 1: Summary statistics of TCID50 (virus titration) per 1mg of tissue from rainbow trout challenged

with VHSV and sampled before onset of clinical signs. Data presented as log 10 of arithmetic mean (n=20) of TCID50 measured after different time intervals following storage compared to no additional storage.

	Mucous	Brain	Muscle	Skin	Gill	Liver/head kid./spleen	Carcase muscle
No additional storage	3.74	3.71	3.14	3.25	4.96	6.22	3.46
ICE 48h	3.93	4.29	3.39	3.33	5.35	6.43	
-20°C 1 month	3.02	2.47	2.00	0.38	2.53	3.86	

The results show that virus titres observed after storage of tissues over 48 h on ice were slightly higher than after 24 h in transport medium. After 1 month of storage at -20°C, a drop in titre by 0.71 to 2.43 log10 was observed. The most substantial drops were observed in skin, gill and internal organs. After 1 month at -20°C, the highest titres were still present in internal organs, followed by mucous, gills and brain. The virus titres observed were still substantial. Mucous, skin and head tissues (including gills and brain) are typical waste generated during processing and would therefore carry a potential risk of releasing VHSV into the environment.
The study demonstrates that both chilled and frozen product can still contain considerable virus loads. The data will form an essential element for future import risk assessments for aquatic animal products and will feed into recommendations for requirements waste treatment in fish processing plants.

Development of methods for detection and quantification of VHSV in water samples

VHSV challenges undertaken under another Defra funded project (F1188 C3384) to determine the effect of stocking density on transmission of VHSV to rainbow trout were used as an opportunity to explore methods for the quantification of VHSV in water samples. The ability to quantify VHSV in water samples would be useful for multiple purposes in the future: e.g. to assess the actual viral concentration in the water column during bath challenge (traditionally, a set amount of virus is added to a defined volume of water and the virus concentration is determined in a sample of the inoculum), to measure quantities of virus released from infected fish, or to measure the quantities of virus released during processing of infected fish.

Samples were taken from 4 tanks (each containing thirty 15 cm rainbow trout in 65 l water) in which the fish were challenged at a calculated (based on amounts of virus added to the water) virus concentration of either 1x101 Tissue culture infectious dose 50 (TCID50) ml-1,or 1x102 TCID50 ml-1. Water samples were collected 1 h following adding of the virus suspension to the tanks (tank flow was turned off immediately before adding the virus suspension) and reinstated after 4 h. Positive controls were made up of clean tank water samples, spiked with VHSV shortly before use for the various titration methods in the lab. Methods generally used for the detection of viable virus quantities in fish tissues were employed to measure the amount of virus present in the water samples (tissue culture titration and plaque assay).

The reading of the TCID50 ml-1 titration returned some unusual results. The number of wells showing cytopathic effect declined with increasing dilution - fewer wells provided a positive reading at low dilution compared to higher dilutions (where fewer viruses should be present). However the association was not linear. This could be caused by a number of reasons: interference effects or inadequate mixing of the virus dilutions. For the plaque assay cell monolayers were inoculated with 200ul adsorption volumes of water samples diluted in virus culture medium. The adsorption volume is removed after 1 hour and the monolayers covered with an agarose/culture medium overlay. In this way the cytopathic effect produced by virus in the sample form visible plaques on the monolayers.
The work described above was a first test to assess the suitability of methods for the detection of virus in water samples. Because it is relevant to discriminate between viable and non-viable virus, methods were employed that are able to detect viable virus. PCR based methods cannot discriminate between viable and non-viable virus and are therefore not considered the first choice for the purpose given. Further work is required to develop suitable methods for the detection of VHSV in water samples. Some of the difficulties experienced may be the result of toxicity of suspended solids in the water with tissue culture cells. These may possibly be resolved through use of alternative filtration methods. However, filtration is associated with potential virus loss which needs to be avoided. If interference problems when using tissue culture methods cannot be overcome, one may have to reconsider using quantitative PCR.
Some work was also done to investigate methods for virus concentration in the water samples. Centrifugal filter devices for concentration and purification of biological samples were used. The results were not satisfactory to date and suitable methods have to be explored further. Potential alternative methods to be explored are ultracentrifugation, polyethylene-glycol precipitation and tangential flow.

Effect of storage on viability of white spot syndrome virus in warm water shrimp tissues

An import risk assessment on the risks of introduction and establishment of WSSV in E&W via import of

non-viable commodities for human consumption concluded that one of the most likely routes of release of WSSV into the environment is via diversion of shrimp imported for human consumption as angling bait.

Given the potentially widespread exposure of domestic crustacean populations to WSSV infected material, the question arose, why WSSV infection had not yet been found in wild crustacean populations. A possible explanation was considered to be a decline in virus levels as a result of freezing, thawing and storage. To further assess the risk that may arise from the use of prawns as angling bait, we investigated the effect of storage of WSSV infected muscle tissue at ambient temperature on viable virus titres. Shrimp are often purchased frozen and anglers are likely to take shrimp to the angling site without chilling devices. The shrimp are therefore likely to be exposed to ambient temperature, which may lead to a decline in viable virus numbers.

Since tissue cultures are currently not available to grow and titrate WSSV, the decline in viable virus titres had to be investigated by bioassay. An array of preparatory work was required in the run-up of the main experiments: Shrimp needed to be grown from larvae to near harvest size (for human consumption) in the Cefas laboratory. Husbandry problems needed to be overcome and suitable rearing systems developed, leading to some delays before suitable experimental animals were available. Penaeid shrimp had not previously been used at Cefas for per os challenges; experience needed to be developed to decide on experimental setup. Variations in the amount of feed offered and the setup of containers were tested to inform the main trial. The pilot studies provided some unexpected results in that onset of infection or mortalities was later than would have been expected from published literature. Those animals that had been successfully infected per os, showed little variation in virus concentration across the length of the abdominal muscle, suggesting that subsections obtained from one animal should provide similar viral loads. One objective of the pre-trials had been to investigate when best to sample the donor animals, since it was intended to generate animals with a medium level of viral load.

However, as mentioned above, cumulative mortalities did not progress as expected and very few infected animals were generated. In both pilot and main per os challenges, experimental animals were kept in individual containers with separate water supply, allowing each animal to count as a separate challenge.

In the main study, white leg shrimp (Litopenaeus vannamei) were challenged per os with WSSV infected muscle tissue. To generate infected muscle tissue, 20 white leg shrimp had been WSSV challenged by injection, and removed from their tanks when severely moribund or dead; they were expected to carry very high levels of WSSV. The donor animals were stored at -70°C until further use.

Each of the 20 donor animals was removed from the freezer and immediately dissected. Subsections of the abdominal muscle were either used for feeding, or sampled to determine WSSV copy numbers by PCR. Tissue prepared for feeding was offered to a receptor animal immediately, or stored for 4, 8 or 24 h at room temperature (20°C). One donor animal provided feed tissue for 4 receptor animals (one per time point). Each receptor animal (20 animals per time point, 4 time points = 80 challenged animals in total; all animals kept in individual pots with separate water supply) was offered ca. 400 mg of infected shrimp muscle tissue. Another 10 animals (controls) were offered muscle tissue from non-infected donor animals. Feed take-up was recorded. Over the 3 week observation period, feed consumption, moults and mortalities were recorded.

Mortalities were highest in the group receiving muscle tissue that had not been stored (n=13), and gradually declined with the length of storage of the muscle tissue. Mortalities (n=5) were still observed in those animals receiving tissue stored for 24h. WSSV was confirmed by PCR in all animals that died.

The results indicate that a decline in viable virus appears to occur as a result of storage; however, virus levels were still sufficient to induce infection after 24h. Litopenaeus vannamei had initially been chosen as the species to undertake these challenges with, based on its reportedly very high susceptibility to WSSV. 100% mortality had been expected amongst the animals receiving fresh muscle tissue (0h).

Conclusions

The results of the project have highlighted the risk of introduction of exotic notifiable pathogens via imports of aquatic animal products, e.g. for human consumption. Imported products could carry sufficient virus quantities to induce infection in susceptible animals (e.g. susceptible animals exposed to infected tissue through the use of AA product as bait for angling or trapping). Processing on fish farms was also found to be an important potential route for pathogen introduction. The risk associated with off farm processing depends on the quantities of pathogen released and subsequent waste treatment. Liquid waste was found to be of particular concern, since it will be returned to the aquatic environment (after varying levels of treatment), where surviving pathogens could potentially infect farmed or wild AA. There are currently no specific requirements to inactivate AA pathogens in liquid waste released from processing facilities, unless these facilities are specifically for the purpose of processing AA subject to disease control measures.

Availability of data suitable for import risk assessments remains a problem. The project has managed to

obtain data for third country imports. However, suitable data on imports from EU MS are not available. This is of concern since imports from other EU countries are particularly relevant to assess the risks for exposure to salmonid species pathogens (VHS, IHN, ISA) and mollusc pathogens (OHV, Bonamia). Assessments of the likelihood of introduction and establishment of *Mikrocytos mackini* and *Perkinsus marinus* conclude that the likelihood of introduction of these pathogens is currently extremely low, because there is little or no trade from infected countries at present. However, the risk may increase significantly if import trade increases.
The project has provided further evidence that supports the initial notion that commodity import for human consumption is one of the most likely routes of introduction of exotic pathogens into England and Wales.

Future work

Future studies should aim to address the most relevant remaining data gaps. In particular, work that will allow to better assess pathogen release from processing is recommended. This includes obtaining more information about the efficacy of sewerage treatment methods to inactivate AA pathogens, developing a better understanding of the coverage of sewage treatment network across E&W relative to AA processing and investigating how much pathogen may be released during processing. To develop methods that could mitigate against AA pathogen release from processing, waste water treatment methods that can inactivate AA pathogens should be explored further. Data are also still required on the minimal infectious dose for many of the notifiable pathogens, which are essential when evaluating whether release of pathogen (e.g. from processing) may lead to infection in susceptible animals.
Some of the potential routes of pathogen release involve the general public (e.g. angling bait). Increasing public awareness for the risk of introducing exotic organisms (non-native animals, plants or pathogens) will be essential in the future to complement mitigation measures applied by industry.

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References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Peer reviewed publications

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11. OIE (2012) Aquatic Animal Health Code. Availble at http://www.oie.int/international-standard-setting/aquatic-code/